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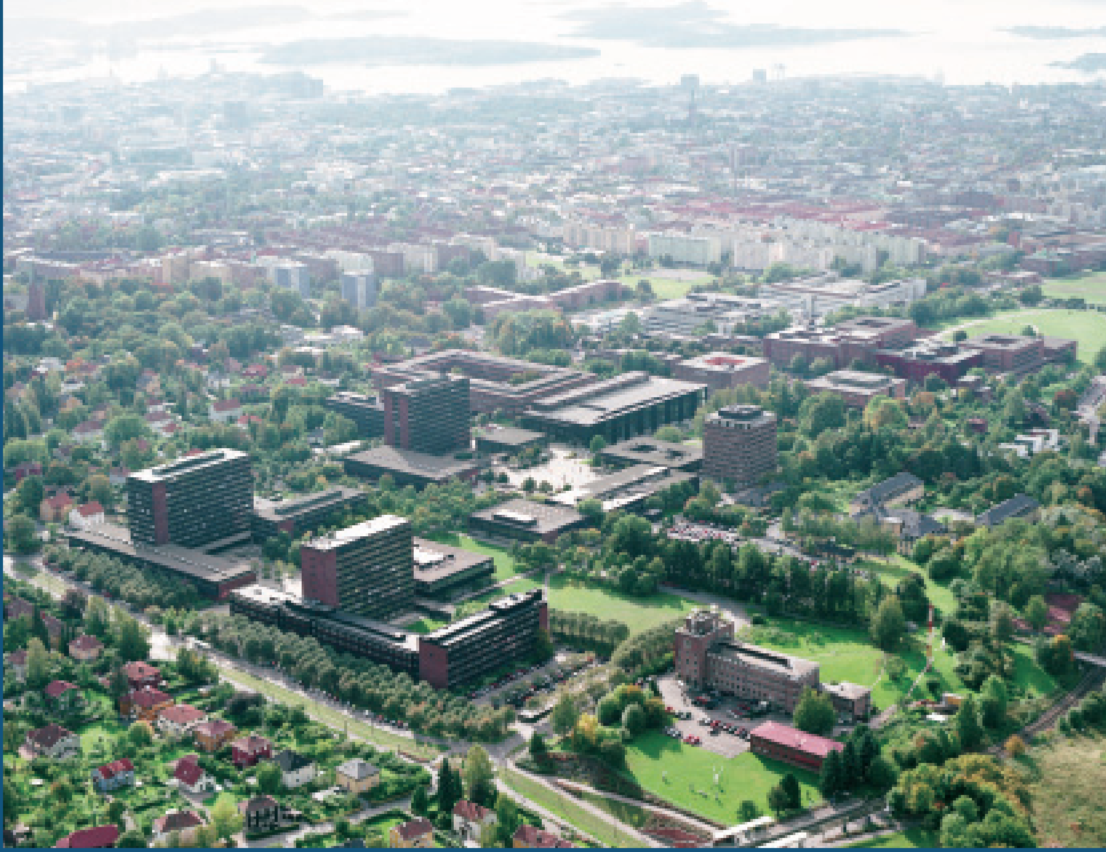

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1 - Character evolution in the Lasiosphaeriaceae sensu lato

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There are a limited number of phylogenetically informative morphological characters for delimiting taxa above the species level in many pyrenomycetes, mostly due to their small stature and simplistic form. Ascospore morphology has been used extensively throughout the filamentous ascomycetes for distinguishing families and genera and many genera in the Lasiosphaeriaceae have been segregated using ascospore morphology. However, other morphological characters, such as ascomatal wall characters, have been suggested, but not tested, for delimiting genera in this family. For example, several genera possess unique bombardioid walls in which the middle wall layer is gelatinized and several species of *Lasiosphaeria* and *Cercophora* possess identical three-layered walls in which the outer layer is composed of hyphal strands. To test the utility of these morphological characters for predicting evolutionary relationships, sequences of the 28S nuclear ribosomal large-subunit (LSU) gene were generated for taxa within the Lasiosphaeriaceae and selected outgroups. Sequences from two nuclear protein-coding genes, B-tubulin and RPB2, were also generated to corroborate the LSU data. Maximum parsimony and Bayesian analyses of separate and combined data sets suggest that ascospore characters are extremely homoplastic and not useful for delimiting genera in the Lasiosphaeriaceae. Ascomatal wall characters are, however, sometimes phylogenetically informative and could be used for segregating taxa.

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2 - Developing phylogenies for integrating mitotic fungi in the Hypocreales and Diaporthales

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Recent comprehensive studies of the Hypocreales and Diaporthales using both morphological and molecular characters present the opportunity to integrate the mitotic fungi and to evaluate character evolution of both teleomorphic and anamorphic states. The majority of plant-associated fungi are mitotic species without any known sexual state and limited morphology. Using molecular sequence data it is possible to integrate the mitotic fungi into the Hypocreales and Diaporthales and to demonstrate that a vast number of mitotic fungi are derived from within these sexual state lineages. In the Hypocreales, the mitotic fungi tend to be hyphomycetous with the exception of the Clavicipitaceae while in the Diaporthales they are primarily coelomycetous. Despite considerable effort, some mitotic species cannot be closely linked with sexual states or even families and thus appear to represent divergent lineages. The character evolution within the Hypocreales and Diaporthales appear similar with fruiting bodies ranging from solitary perithecia to compound stromata, often characteristic of specific families. Ascospore morphology is also characteristic of specific families although exceptions exist in each family. Biologically the Diaporthales appear to be confined to plant substrata often as virulent plant pathogens in temperate regions while the Hypocreales fill much broader niches attacking insects and other fungi as well as plant substrata with their greatest diversity in tropical regions.

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3 - Remembering and dismembering *Stilbella*: A classical hyphomycete genus

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The 275 described taxa of *Stilbum* and *Stilbella* were reduced to 45 species in seven genera in my 1985 monograph. Cladistic analysis of LSU rDNA sequences provides new insight into the phylogeny and taxonomy of these fungi. *Stilbella*-like anamorphs occur in four families of Hypocreales (Bionectriaceae, Hypocreaceae, Nectriaceae, Clavicipitaceae), in the Phyllachorales, Microascales and Leotiales. The type, *S. fimetaria*, is related to the cleistothecial *Emericellopsis* (Bionectriaceae). Previous delimitations of *Tubercularia* (teleomorphs: *Nectria* ss, Nectriaceae), *Gliocladium* (teleomorphs: *Sphaerostilbella*, Hypocreaceae), *Rhizostilbella* (teleomorph: *Corallomycetella*, Nectriaceae) and *Polycephalomyces* (teleomorphs: *Byssostilbe*, Clavicipitaceae) are supported by LSU data. The recently described *Gracilistilbella* (teleomorphs: *Stilbocrea*), is a monophyletic group within the Bionectriaceae. *Volutella* (teleomorphs *Cosmospora*, Nectriaceae), previously restricted to setose sporodochial species, is emended to include synnematous species. *Stilbella annulata* is related to the Phyllachorales. The phylogenetic distribution of these anamorphs suggests that synnemata are either plesiomorphic in the Hypocreales and sporadically expressed, or that they have arisen repeatedly. A strictly redefined *Stilbella* includes only two species, but implies recognition of many monotypic genera. This once large form taxon is a useful metaphor for patterns in anamorph taxonomy revealed by molecular data.

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4 - Ecology and evolution in the Onygenales: an overview based on molecular and morphological characters

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Among the filamentous ascomycetes, few groups have received as much attention in molecular phylogenetic studies as the members of the Onygenales, an order that includes the most important fungal pathogens of mammals and the only fungi capable of degrading keratin. Parasitic Onygenales are thought to be derived from saprobic ancestors, but the origins of parasitism within the Arthrodermataceae and Onygenaceae, two families of keratinolytic Onygenales, remains obscure. Phylogenies based on analyses of conserved gene regions have demonstrated that parasitic species do not form a separate lineage within the Onygenales, but these sequences lack sufficient numbers of phylogenetically informative characters to resolve the branching order among recently diverged taxa or evaluate the evolution of ecological and morphological characters hypothesized to be correlated with the origins of parasitism. This study, which addresses the question of how the evolution of specific characters may be correlated to the evolution of parasitism in the Onygenales, is based on the analyses of molecular (mitSSU, partial nucLSU gene sequences) and morphological characters for nearly 50 members of this order. Emphasis has been placed on the inclusion of saprobic representatives of the Arthrodermataceae and Onygenaceae, and members of the Gymnoascaceae (the closest non-keratinolytic relatives of these families) have been included to determine the direction of the evolution of characters of interest.

5 - Evolution of anamorph form in the Clavicipitaceae

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Members of the Clavicipitaceae exhibit a diverse array of life histories, and a diverse array of anamorph forms. Most members are symbiotic with plants, fungi, or arthropods. Among the arthropod pathogenic members, classification has so far been based upon the scant characters of the sexual stromata, and the resulting systems developed by Kobayasi and other authors prove to be difficult to apply. Increasingly, we know that they do not reflect relationships as revealed by molecular evidence. Asexual forms have seldom been considered key characters for classification, and indeed, anamorph connections are as yet unknown for about 50% of teleomorph species. Yet molecular evidence suggests that anamorph genera (for example, *Harposporium*, *Hirsutella*, and a subset of *Paecilomyces* species) may each comprise monophyletic groups correlated with distinctive teleomorphic characters. The functional significance of differing forms in the life histories of these organisms has barely been studied, although biocontrol experience suggests that anamorphs play important roles in pathogenicity. This study addresses the evolution of anamorph form, particularly among the arthropod pathogenic species of the Clavicipitaceae, and highlights the potential role of anamorph morphology and ecological factors in improving classification systems in the Clavicipitaceae.

6 - Well supported, major groups among the Euascomycetes

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A data set with 1555 sequences of SSU rDNA has been phylogenetically analyzed. Cladistic methods using parsimony jackknifing for establishing group frequencies and the parsimony ratchet algorithm for finding most parsimonious trees was utilised. Until recently attempting to find all most parsimonious trees in large data sets, has been impractical, given current computational limitations. The parsimony ratchet method for rapid parsimony analysis of large data sets was found very efficient in searching and finding most parsimonious trees in the current large data set. The results show that the Glomales are sister group to the Dikaryomycetes. The Ascomycetes include the Euascomycetes and are both monophyletic. Within the Euascomycetes the inoperculate Euascomycetes are monophyletic except for the Orbiliomycetes which are included in an operculate, pezizalean sister group. *Geoglossum* is the sister group to the rest of the inoperculate euascomycetes. The Sordariomycetes, Dothideomycetes, Chaetothyriomycetes and Eurotiomycetes are each highly supported as monophyletic whereas the Leotiomycetes and Lecanoromycetes are both shown to be paraphyletic. In the consensus ratchet tree the Dothideomycetes, Chaetothyriomycetes, Eurotiomycetes and the paraphyletic assemblage Lecanoromycetes form the sister group to the Sordariomycetes and the paraphyletic assemblage Leotiomycetes, but that topology receives no jackknife support.

7 - Changes in systematics of heterobasidiomycetous fungi during 30 years

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Systematics and hypotheses about their evolution have been revolutionized within the last few decades. Main impacts for new views came from (a) the detection of new key species, (b) the application of new techniques to study sofar unknown characters, (c) the reconstruction of life-histories in nature and/or in culture, (d) the understanding of fungus-host interrelationships, and (e) the combination of old and new datasets for comparative interpretations. (a) I will present comments on species originally hidden outside the Basidiomycetes, but now representing key taxa in the heterobasidiomycetous groups, and new species with considerable importance for basidiomycetous phylogeny. (b) Clarification of monophyla referred in major parts to ultrastructural characters. A surprisingly high support for new systematic arrangements came from molecular studies. (c) There was a renaissance for ontogenetic studies in culture. Thus, life-history experiments provided new insights in most of heterobasidiomyxcetous taxa, especially in smuts, tremelloid fungi and yeasts. (d) Coevolutionary processes play an important role in diverse heterobasidiomycetous taxa. Therefore, systematics of hosts have to be included in fungal character sets. (e) Fortunately, most phylogentic interpretations are now based on several datasets, suggesting possibilities for improved evolutionary hypotheses.

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8 - Ultrastructure of heterobasidiomycetous fungi

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Significant ultrastructural characteristics of heterobasidiomycetes, such as nuclear and spindle pole body behaviour, septal pore apparatus, colacosomes, symplechosomes as well as myco- and phytoparasitic interactions will be discussed with special regard to their role as phylogenetic markers.

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9 - Heterobasidiomycetous yeasts: diversity, phylogeny and classification

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Basidiomycetous yeasts form a remarkably diverse group of fungi and are distributed throughout the three main lineages of the Basidiomycota: Urediniomycetes, Ustilaginomycetes and Hymenomycetes. Traditionally, the yeast stages of plant or mycoparasitic basidiomycetes and the unicellular forms of fruitbody-producing taxa have been excluded from the yeast domain. When these non-conventional basidiomycetous yeasts are cultivated in laboratory media, the usual growth form is unicellular and corresponds to the haploid and/or saprophytic phases. Non-conventional dimorphic basidiomycetes rarely develop their complete life cycle in culture, contrary to typical basidiomycetous yeasts. This artificial separation of taxa, mainly for historical reasons, prevents an integrated assessment of the diversity of these groups and the implementation of a natural classification system. The objectives of this presentation are two-fold: (i) to advocate a new concept of 'basidiomycetous yeasts' encompassing all dimorphic basidiomycetes, regardless of their life cycle and (ii) to present and discuss new developments concerning the systematics of asexual (*Rhodotorula*) and sexual (*Leucosporidium*, *Mastigobasidium*, *Rhodosporidium* and *Sporidiobolus*) taxa of the sub-class Microbotryomycetidae. The polyphyletic nature of *Rhodotorula* and the relationship between *Leucosporidium* / *Mastigobasidium* and the Microbotryales will be addressed.

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10 - Molecular phylogeny of rusts

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Sequence data from nuclear large subunit ribosomal DNA was used to infer phylogenetic relationships of selected genera of the rust fungi and to test the monophyly of these genera. Neighbor joining analysis and a Bayesian method of phylogenetic inference using Monte Carlo Markov chains confirm that (1) *Puccinia*, *Uromyces*, *Cumminsiella* and *Endophyllum* have a common origin. (2) The autoecious Rosaceae-rusts *Phragmidium*, *Kuehneola*, *Triphragmium*, and *Trachyspora* are a monophyletic group, representing Phragmidiaceae. (3) The gasteroid genus *Ochropsora* is closely related to *Tranzschelia*. The monophyly of the following genera could be confirmed: *Chrysomyxa*, *Coleosporium*, *Cronartium*, *Gymnosporangium*, *Melampsora*, *Phragmidium*, and *Tranzschelia*, whereas the genera *Puccinia*, *Pucciniastrum*, *Thekopsora* and *Uromyces* are polyphyletic. The molecular phylogenetic hypotheses are compared to morphology-based systematics with special emphasis on spermogonial and teliospore morphology, and the usefulness of these characters on different systematic levels is discussed.

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11 - Evolution in smuts

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Many changes in the view of smut phylogeny have been discussed within the last decade. An overview of the main lineages of that plant parasitic group is presented. Based on an integrated phylogeny hypotheses of the major traits of evolutionary mechanisms will be discussed. New morphological, ecological, and coevolutionary aspects of smut evolution are in focus of present studies and will be highlighted.

12 - An integrating approach to ecology, morphology, and systematics of smut fungi

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Systematic relationships are studied in order to understand evolution, which is the result of the interaction of the organisms with environmental factors. The latter decide whether a combination of morphological characteristics contributes or not to the fitness and evolutionary success of the respective individual. These are aspects which apparently are important for the survival of certain species of plant parasitic smut fungi: Host species with high frequency of individuals growing on sites exposed to wind or water; Development of sori in nutrient rich parts of the host plant; Exposure of masses of teliospores to the respective vector of dispersal for a long period of time; Protection of diaspores against desiccation, intense light, and feeding when young and during dispersal; Adaptations to different vectors of dispersal. The integration of ecological, morphological, and systematic aspects is illustrated by *Cintractia amazonica* Syd. & P. Syd. in the relationship of *Cintractia* s. l. and *Tilletia* spp. as well as *Erratomyces patelii* (Pavgi & Thirum.) M. Piepenbr. & R. Bauer versus *Entyloma* spp.

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13 - Phylogeny of morphological and molecular studies in the genera *Tremella* and *Sirobasidium*

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Comparison with morphological and molecular phylogenies, six groups in the genera *Tremella* and *Sirobasidium* are concluded. They are: 1. Aurantia group: heterogeneously mixed with basidiomycetaceous host hyphae up to the subhymenium, e.g. *T. aurantia*, *T. encephala*; 2. Foliacea group: basidiocarps foliose and brown, subhymenium with numerous anastomoses, haustoria rarely, e.g. *T. foliacea* sensu lato, *T. giraffa*; 3. Fuciformis group: heterogeneously associated with *Hypoxylon* basidiocarps, e.g. *T. flava*, *T. fuciformis*; 4. Indecorata group: heterogeneously associated with pyrenomycetaceous perithecia, e.g. *T. indecorata*, *T. moriformis*, *T. nivalis*; 5. Mesenterica group: basidiocarps gyrose to cerebriform, yellowish, basidia oval, hyphidia existing, subhymenial structure loose, e.g. *T. mesenterica*, *T. taiwanensis*, *T. tropica*; 6.Sirobasidium group: chained basidia, basidiomata habitually associated with ascomata or stromata of ascomycetes, e.g. *S. magnum*, *S. intermediae*. The results of LSU phylogenies compared with morphological data for the *Tremella* grouping strongly suggest that using large subunit (LSU) rDNA sequences are potentially useful in taxonomic, ecological, and evolutionary population-level studies. Moreover, molecular phylogeny implies that the genus *Sirobasidium* developed from that of *Tremella* evolutionally.

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14 - Auriculariales and related taxa

M. Weiß[1*], M.-A. Selosse[2], K.-H. Rexer[3] & F. Oberwinkler[1]

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We present current hypotheses of phylogenetic relationships within heterobasidiomycetous Hymenomycetes with particular reference to Auriculariales. Molecular phylogenetic estimations are discussed in correlation to morphological and ecological data.

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15 - Tulasnelloid and other rhizoctonia-forming fungi

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Tulasnella, *Ceratobasidium*, *Oliveonia*, and other genera of rhizoctonia-forming fungi are of phylogenetic interest since they lie somewhere on the boundary between the holo- and heterobasidiomycetes. This pivotal position appears to be supported by micromorphological research, ultrastructure research, and molecular research. However, the precise disposition and relationships of these fungi have yet to be satisfactorily resolved. Morphologically, the Tulasnellales appear to be a well-defined and coherent group based on their unique basidia. But there is an unusual plasticity of forms (including spores, basidia, and conidial structures) within the group and sequencing data has proved difficult to interpret. The Ceratobasidiales present equal but different problems, particularly in defining genera and species. These problems are made more critical by the economic importance of plant pathogenic and orchid endomycorrhizal rhizoctonias. For several species, any change in nomenclature (such as synonymization) resulting from systematic research may be rejected, raising interesting questions about the relationship between taxonomists and the consumers of their output.

16 - Systemic phenomena in pine pathosystems

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In many herbaceous host-pathogen systems, localized infections by pathogens or beneficial microorganisms, or localized treatment with certain chemicals, induce whole-plant resistance to subsequent pathogenic infections. This phenomenon is termed Systemic Induced Resistance (SIR). This presentation will illustrate similar phenotypes and associated putative defense responses in three conifer pathosystems: 1) pitch canker of Monterey pine, caused by *Fusarium circinatum*; 2) *Heterobasidion annosum* root and butt rot of ponderosa pine; and 3) *Sphaeropsis sapinea* tip blight and canker of Austrian pine. An SIR phenotype was recently described in Monterey pine (Bonello et al. 2001. Systemic induced resistance in Monterey pine. Forest Pathology 31:99-106), while systemic changes in defensive secondary metabolism were observed in mature ponderosa pines (in preparation). Working with potted Austrian pine we have now observed a unique phenomenon in which expression of SIR, or the opposite phenotype of systemic induced susceptibility (SIS), depend on whether the challenge infections occur on the stem or on the shoot tips. We are now attempting to correlate this organ specific SIR/SIS phenomenon with the expression of specific putative defense responses of the host. The significance of these results will be discussed along with future lines of investigation.

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17 - Longevity and plant defence: Host-pathogen interactions in short- and long-lived tissues of trees

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Relative to many herbaceous plants, trees are subject to a wide range of diseases during their long life cycles. Different diseases may be classed as persistent, once established present within the host until the tree dies, or periodic, attacking short-lived tissues when the environmental conditions favour pathogen development. This presentation is focused on two broad categories of disease which affect overall tree growth and productivity: (1) fine root diseases and (2) secondary root diseases. The main host species discussed will be *Pinus sylvestris* and *Picea sitchensis*. In mature trees and under normal forest conditions, disease of fine roots are most likely to be periodic, killing limited sections of the fine root system, except where prevailing microclimatic conditions favour disease. Diseases of secondary roots, however, are often persistent, colonising woody tissues, but remaining restricted to bark or heartwood tissues unless conditions favour pathogen development. This presentation considers the interactions between tree roots and pathogenic fungi in terms of host defence and the various external factors influencing disease development.

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18 - Pathogenicity of the *Heterobasidion annosum* complex

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The outcome of an interaction between a pathogen and its host is dependent on the genotype of the individuals involved as well as environmental conditions. We have taking both a genetic and a genomic approach to address the question of what genetic factors are of importance for pathogenicity and host specialisation in the tree pathogen *Heterobasidion annosum*. In North America *H. annosum* is present as two intersterile groups corresponding to biological species with different host preferences; the S-group which has spruce, fir and hemlock as its main hosts and the P-group which has pines as its main hosts. In the genomic approach we have sequenced 1148 randomly picked cDNA clones from a library constructed from mRNA of *H. annosum* challenged with Scots pine roots. Contig analysis identified 339 unique contig sequences out of which 247 were assigned a putative function deduced from sequence similarity with proteins in public databases. In the genetic approach we are currently preparing a genetic linkage map using AFLP markers using a mapping population of 97 progeny isolates from a cross between a P and a S homokaryon. Virulence on pine has been analysed for the 97 progeny isolates. Further studies of SP hybrids of *H. annosum* have revealed an importance of the mitochondria for virulence in pine.

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19 - Phytophthora in forest trees: host-parasite interactions

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Phytophthora is well known as a genus of agricultural pathogens, often destructive when soil conditions favor zoospore spread. *Phytophthora* species are also widespread in many forests, but little is known about their interactions with potential hosts. We focus this analysis on three host tree-pathogen systems that illustrate a range of pathogenic behaviors; by reviewing what is known, we hope to focus attention on critical gaps in our understanding of forest

Phytophthoras. *P. cinnamomi* is the most studied of the three, with a broad host range and global distribution. It is introduced to Australia, where it causes ecological destruction in some forest communities, but has negligible impact in others. In some hosts at least, it moves in the xylem well beyond the margin of phloem necrosis. *P. lateralis*, by contrast, exhibits essentially host specific pathogenicity in forests of the western United States. Native *Chamaecyparis lawsoniana* are aggressively attacked and killed as the pathogen colonizes the phloem in a broad advancing front that moves up the roots into the stem. *P. quercina* is associated with oak decline in Europe, but appears to be native, or at least of long standing in these forests. It is confined to the fine roots of susceptible oaks, but induces symptoms far beyond the colonized tissues. We review what is known and describe ongoing research into the infection biology and pathogenesis of these contrasting species, and host responses to them.

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20 - Induction of defense structures in conifers: Norway spruce as a model system and a brief phylogenetic study

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Norway spruce was used as a model system to study anatomical and chemical defense responses to *Ceratocystis polonica* and attack by its bark-beetle vector *Ips typographus*. Fungal inoculation into the phloem generated responses within 3 wk, including increase in polyphenolic parenchyma cell (PP cell) size and staining, wound periderm initiation, and traumatic resin duct (TD) formation. Fungi were not seen in samples 3 wk after phloem inoculation, but were in some samples 6-9 wk after inoculation. Inoculations into the cambium resulted in partial (3 wk) or complete (6, 9 wk) fungal colonization. This indicates that PP cells have defenses capable of inhibiting fungal growth. Samples taken near bark-beetle galleries had similar anatomical responses, validating the inoculation approach. In an attempt to determine signals involved in these reactions, we found methyl jasmonate (MJ) induced similar anatomical and chemical responses. A single MJ treatment induced swelling of PP cells, increase in their phenolic contents, and formation of additional PP cells and TDs. Treatment enhanced resin flow and increased resistance to C. polonica. MJ application to the oldest internode of 2-yr-old saplings also induced TD formation, and, more surprisingly, TDs were formed in the untreated internode. MJ was found to have similar effects at upregulating defense responses in other conifer trees, although surprising differences were found in some taxa.

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21 - An overview of wound response and antimicrobial defence in eucalypts

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With the expanding use of regrowth eucalypt forests and plantations for wood production, the profitability of processing operations (particularly sawmilling) will increasingly depend on the maximum recovery of high quality products from relatively small logs. The quality and value of eucalypt logs can be significantly affected by the incidence, location and extent of discoloration and defect resulting from kino veins, decay fungi and insect attack. Our group is the first internationally to carry out detailed studies of eucalypt wood defence. We seek to compare two important Australian plantation species (*E. nitens* and *E. globulus*) with other tree species. Our studies in the area of eucalypt defence initially focused on the formation of a reaction zone (antimicrobial defence barrier between healthy and decayed sapwood). Our most recent studies have also investigated the new tissue formed immediately adjacent to the wound site which hitherto has been given relatively little importance. Both reaction zone and especially wound tissue have been found to contain a surprising concoction of tannins, flavonoids and terpenes. The nature and significance of these extractives and the tissue changes observed in response to wounding and infection in eucalypts will be examined in the broader context of tree defence strategies.

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22 - Clonality in wood-inhabiting fungi on different spatial scales

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This presentation focuses on clonal spread in wood-inhabiting fungi and its impact on population structures over local and large geographic areas. The work is based on genetic and spatial data and reveals strikingly different modes and consequences of asexual propagation in a number of species. The following species are discussed: 1) *Heterobasidion annosum*, 2) *Rhizina undulata*, 3) *Stereum sanguinolentum*, 4) *Amylostereum chailletii*, 5) *A. areolatum* and 6) *Phlebiopsis gigantea*. Clonality in those species arises as a result of: a) mycelial growth and colonisation of spatially separated resource units (trees) over discrete territories (species 1 and 2); b) airborne

spread of non-outcrossing spores (species 2 and 3); c) vectoring of vegetative mycelium by woodwasps and wood material (species 4 and 5); d) use of single fungal strain as a large-scale biocontrol agent (species 6). The results show that fungal clones at one extreme are small and local; at the other extreme, clones of the wood-inhabiting fungi are capable of moving between widely separated locations and have a pronounced impact on population structure of a given species on trans- or even inter-continental scales. Persistence of such clones in nature varies from several years up to several decades. They are mainly characteristic for forest areas under anthropogenic impact, with the presence of stumps, wounds on living trees, burned areas and exotic species.

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23 - Sexuality and genetic variation in three boreal Polypores possessing divergent niche requirements

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The population structure and sexuality of Fennoscandian populations of three model species, the vulnerable *Phellinus nigrolimitatus* and *Fomitopsis rosea* and the common pioneer wood-fungus *Trichaptum abietinum*, was investigated. Molecular support for a heterothallic mating system in *P. nigrolimitatus* was found. Molecular data and mating studies demonstrated that *T. abietinum* and *F. rosea* have a heterothallic mating system. The observed genotype distributions in the geographic populations of the model species were mostly in accordance with Hardy-Weinberg expectations. Our results suggest that the mating populations are large and that panmictic conditions exist. Although *P. nigrolimitatus* and *F. rosea* probably have experienced decline in population sizes as a result of negative impacts from forestry, no distinct heterozygote deficits ascribed to inbreeding could apparently be detected. The molecular markers revealed little genetic differentiation among geographic populations of the three model species, indicating high gene flow and good dispersal ability. The geographic populations may be regarded as subpopulations sharing the same gene pool. A high number of mating types were present in one *T. abietinum* and two *F. rosea* populations, a feature that may indicate high genetic diversity. A high number of *T. abietinum* and *F. rosea* genets occurred on single logs. In *P. nigrolimitatus* fewer but larger genets that often produced more than one basidiocarp occurred.

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24 - Genetic structure of *Daldinia loculata* populations

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The genetic structure of the postfire ascomycete *Daldinia loculata* was studied on both a local and a continental scale. We were able to show endophytic establishment of *D. loculata* by isolating mycelia from non-decayed wood of birches. The distribution of genets was studied at one Swedish forest site, and in all trees studied we found only one, or occassionally two, unique genets per stem. The relatively large genet in the stem apparently was one of the parents of the offspring of the stromata attached to the tree, while each stroma had one or several unique second parents. Low levels of genetic differentiation among Eurasian subpopulations were confirmed by analysing the allelic distribution nuclear gene loci of 1 Russian and 6 Fennoscandian subpopulations. Taken together, these results suggests that *D. loculata* consists of a long-lived background Eurasian population of latent mycelia in non-burned forests, established by ascospores dispersed from scattered burned forest sites. When the trees die from a forest fire, the latently established genets starts to decay the wood, and the sexual reproduction is triggered. In order to investigate the spatial distribution and origin of the parents of the stromata, all vegetative genets and offspring of the attached stromata were sampled at one burned forest site. The sequence of highly variable nuclear gene loci were analysed from each mycelium and the high level of unique genets found indicates ascospore origin of all mycelia.

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25 - Population genetics and dispersal biology of endangered lichen species: some implications for lichen conservation biology

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Global populations of endangered lichen-forming fungi are often highly fragmented and local populations are reduced to critical size, e.g. in *Erioderma pedicellatum*. Especially in heterothallic species such as *Lobaria pulmonaria* this process is likely to reduce the survival probability of local populations if genetic diversity is reduced. For instance, in this species genetically depauperate populations seem to be unable to reproduce sexually and therefore propagation is limited to vegetative, symbiotic dispersal units. However, the low range of these relatively large dispersal units limits the local populations to rather low average population densities. The study of the dispersal and post-dispersal processes is therefore of prime importance to identify critical distances among habitats, especially in managed forests. PCR-based techniques were recently developed and allow the detection of single symbiotic propagules. Further, sets of markers with high levels of genetic variation will become available and will allow to detect rare genotypes which depend on conservation measures such as population augmentation.

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26 - Genetic heterogeneity in *Phialocephala fortinii* populations along a latitudinal transect

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Dark septate root endophytes (DSE) commonly inhabit roots of vascular plants in cold-stressed environments. One species of DSE, *Phialocephala fortinii*, displays very little host specificity and, when inoculated on some plants, a variety of effects can be observed including an increase in dry weight of the host plant, a decrease in dry weight of the host plant, or no observed effect. The apparent strict asexual mode of reproduction would indicate that there should be minimal variation in this fungus within a population, with distinct clones occupying distinct habitats. To assess whether genetic variation within *P. fortinii* is correlated to latitude and how this variation is structured within and among populations, *P. fortinii* was isolated from *Salix* root fragments collected from 40 x 40 m plots along a latitudinal transect from 49°N to 78°N. Genetic heterogeneity of isolated *P. fortinii* strains was evaluated by amplified fragment length polymorphism (AFLP) analysis and comparisons were made within populations and between populations. Clones were not detected within any population, and at all latitudes a single, distinct individual was found inhabiting each collected *Salix* root fragment. Genetically distinct populations were detected only in arctic latitudes.

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27 - Mechanisms and factors implicated in genet distribution and in dynamics of populations of ectomycorrhizal basidiomycetes

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Among ectomycorrhizal fungi -root symbionts of woody plants-, Basidiomycetes are the most diversified (with about 4500 described species) and studied ones. In natural environments, roots of woody plants are potentially connected to an assemblage of several fungal species each represented by more or less different individuals. To understand the functioning and ecological roles of ectomycorrhizal fungi, a frequent approach is to focus on local populations of these fungi to determine their genetic diversity and its distribution. This to subsequently infer how biotic and abiotic processes and determinants lead or not to establishment of fungal individuals, to their persistence as growing mycelia and, finally, to temporal changes in fungal populations. Contrasting with the strongly discussed but more often conserved sporophores sampling method, the constant development of molecular markers in the last twelve years has largely contributed to great advances in this approach. Accurate characterizations of populations have revealed large variations of demographic strategies (often defined as the relative parts of sexual reproduction and of vegetative growth in structuring a population) among species as well as between populations sampled in different environments of a done species. Advances and comments concerning this approach are illustrated with examples from works using *Tricholoma populinum*, *Tricholoma scalpturatum*, *Hebeloma cylindrosporum* and *Suillus variegatus* as models.

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28 - Global patterns of genetic variation in *Schizophyllum commune*

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Some mushroom species can be noticeably common and have wide geographic distributions. One such species is the wood decaying split-gill mushroom *Schizophyllum commune*. Our initial study addressed the global population structure of *S. commune* and asked whether long distance spore dispersal could possibly explain the fungus' distribution and occurrence. Earlier studies by John Raper demonstrated that the global population is completely interbreeding, and more surprisingly that the many mating-types are randomly distributed with regard to climate and geography. Using both allozyme and DNA markers, we demonstrated that *S. commune* is in fact divisible into three highly divergent geographic lineages: South America, North America, and the Eastern Hemisphere. Evidence for even smaller scale population substructure has been observed among isolated populations in the Caribbean. Through a selective spore-trapping method, we found that spores of *S. commune* are extremely common in the air flora, yet extant populations are genetically subdivided, perhaps arguing for a role in natural selection in maintenance of population differentiation. Finally, direct studies of the mating-type genes themselves suggest an explanation for Raper's observation of mating-type distribution is the prolonged maintenance of all possible mating-types within each geographic lineage through strong balancing selection.

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29 - Chemical organization of the *Aspergillus fumigatus* cell wall and a revisited role for GPI-anchor proteins in cell wall organization

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Recent structural analysis of covalent linkages between the different constitutive polysaccharides of the structural alkali-insoluble skeleton of the cell wall of *Aspergillus*

fumigatus have shown that branching of the beta, 1-3 glucan through beta, 1-6 linkages is the first event in the construction of the cell wall followed by linkages of the branched glucan with chitin, galactomannan and beta 1-3,1-4 glucan. In contrast to yeast, no proteins covalently bound to cell wall polysaccharides that could play a morphogenetic role in cell wall construction, have been identified in *A. fumigatus*. Accordingly, branching enzymes responsible for the linkages between polysaccharides represent essential enzymes for the construction of the cell wall. During the search of such glycosyltransferases, we identified a beta 1-3 glucanosyltransferase playing an important role in cell wall biogenesis. This enzyme that is responsible for elongation of beta1-3 glucans, was glycosylphosphatidyl inositol (GPI) anchored to the plasma membrane. Comparative proteomic and genomic approaches have identified 5 GPI-protein families common to both *A. fumigatus* and yeast. None of the genes encoding putative polysaccharide-bound proteins in yeast have been found in *A. fumigatus*. Disruption of all the GPI-genes has been undertaken in *A. fumigatus*. Up to now, data suggest that the *A. fumigatus* GPI-proteins have enzymatic functions involved in the biosynthesis of fungal cell wall.

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30 - The regulation of chitin synthesis in yeast and pathogenic fungi: A common theme?

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Chitin synthesis is a process maintained across the fungal kingdom that, thanks to the power of genetic manipulation of yeast cells, is now beginning to be understood. Chitin synthesis is based on the regulation of distinct Chitin Synthases (CS) isoenzymes, whose number ranges from 1 in *Schizosaccharomyces pombe* to 7 in some filamentous fungi such as *Aspergillus fumigatus*. This high diversity makes it difficult to find a unique model of regulation. Sequence analysis, together with the functional data reported, strongly support the notion that all fungi contains two highly divergent CS families, one of each crucial for cell division and the other involved in bulk chitin synthesis. The first family is likely to be controlled at cell cycle level, whereas the second one would depend on the function of several genes whose function has been studied in the yeast *Saccharomyces*. We review the current knowledge about the function of such genes and whether these genes may be conserved in other fungi. Finally, we discuss our recent results in the characterization of some of these genes in *A. fumigatus* and *C. albicans*.

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31 - Alpha and beta (1-3) glucan synthesis in *Aspergillus fumigatus*

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Alpha and beta (1-3)glucan are the main components of the cell wall of *A. fumigatus*. Like in other fungi, beta (1-3)glucan synthesis in *A. fumigatus* is under the control of FKS and RHO1 that are essential genes. Two genes AGS1 and AGS2 encoding two putative alpha (1-3)glucan synthases have been now characterized in *A. fumigatus*. The predicted Ags1 and Ags2 proteins have an estimated molecular size of 273 kDa. Ags1p and Ags2p have three major hydrophobic regions separating two hydrophilic domains. The first hydrophilic domain is homologous to bacterial alpha amylases, and the second to bacterial glycogen synthases. AGS1 and AGS2 were disrupted in *A. fumigatus*. The ags1 and ags2 mutants have similar phenotype. Growth and conidiation are both reduced. Morphologically, abnormal sporulating structures were seen in liquid culture during early growth, whereas the wild type strain (WT) only produces mycelium. The ags1 mutant is more sensitive to the cell wall inhibitors Nikkomycin and Congo Red than the WT and ags2 strains. No difference was seen in the carbohydrate composition of the alkali insoluble and soluble fractions of the cell wall of ags2 and WT strains. In contrast, a reduction in the alpha (1-3)glucan content of the alkali soluble fraction of the ags1 was observed. These results were in agreement with the immunolocalization of Ags1p and Ags2p since Ags1p was localized at the cell wall whereas Ags2p was found in the Golgi apparatus.

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32 - Beta-Glucan synthesis in *S. cerevisiae*: a genome-wide synthetic lethal analysis of participating gene families

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We used the yeast deletion set to find synthetic lethal and sick interactions of genes involved in beta-glucan synthesis, to define networks of participating genes. We used deletion mutant arrays (Tong et al., 2001, Science 294: 2364) to make double mutants in query glucan genes. We examined two gene families: FKS1, GSC2, FKS3 and KRE6 and SKN1. For FKS1, encoding a beta-1,3-glucan synthase, we find synthetic interactions with 82 genes, principally in areas of wall maintenance, cell stress, protein degradation, lipid and fatty acid metabolism and transcription. For GSC2 and FKS3, we find 20 and 83 interactions respectively. Surprisingly, there is little overlap between the sets for the three FKS genes, a total of 175 interactions involving 165 genes, reflecting the distinct biological contexts in which these related proteins function.

KRE6 encodes a glucanase-like protein required for beta-1,6-glucan synthesis, and is synthetically lethal with the related SKN1. KRE6 interacts synthetically with 87 genes, and SKN1 with 39. Again there is little overlap between the sets, with a total of 126 interactions among 121 genes. These genes are mainly in areas of cell wall maintenance, cell stress, protein degradation, protein modification, lipid and fatty acid metabolism and vesicle transport. The KRE6/SKN1 set show limited overlap with the FKS family set, consistent with their distinct functions, with a total for both families of 301 interactions among 286 different genes.

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33 - The PMTs: an evolutionarily conserved family of protein O-mannosyltransferases

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Protein O-mannosylation is an essential protein modification in yeast. Furthermore, it is indispensable for cell morphology and cell wall integrity [1]. Yet, how O-mannosylation affects cell wall architecture is still obscure. In yeasts and fungi O-mannosylation is initiated at the endoplasmic reticulum by an evolutionarily conserved family of protein O-mannosyltransferases, the PMTs. Phylogenetic analyses revealed, that this family can be divided into Pmt1p, Pmt2p and Pmt4p like subfamilies, including transferases closely related to Pmt1p, Pmt2p and Pmt4p, respectively. Here we present the molecular characterization of the PMT subfamilies of *S. cerevisiae* showing that Pmts are integral membrane proteins with seven membrane spanning domains which form specific high molecular weight complexes in vivo. Further, characterizing pmt mutants we found that their phenotype closely resembles mutants of the cell wall integrity signaling (PKC1-) pathway which monitors cell wall stability [2]. Analysis of the PKC1-pathway revealed that O-mannosylation is essential for induction of the this signal transduction cascade upon external stresses. Biochemical analysis of the highly O-mannosylated upstream receptors of the cell wall integrity pathway showed that abated O-mannosylation affects their maturation, stability and function. References [1] Strahl-Bolsinger S et al. (1999) Biochim. Biophys. Acta 1426: 297-307 [2] Heinisch JJ et al. (1999) Mol Microbiol 32: 671-680.

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34 - Regulation of glycosylation and pH in the yeast Golgi

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In yeast, glycoproteins are heavily modified by mannose in the Golgi. The donor for mannosylation is GDP-mannose. Use by lumenal mannosyltransferase requires that GDP-mannose be transported from the cytosol to the Golgi by a specific transporter. Once the mannose is donated to protein, GDP is converted to GMP by nucleoside diphosphatases. GMP is an antiporter whose export from the Golgi is coupled to the lumenal import of GDP-mannose. Substrate provision thus involves a cycle in which mannosylation acts as a sink to continuously generate the antiporter. As a byproduct of this cycle, hydrolysis of GDP to GMP generates a huge amount of phosphate that must be removed to prevent an overly acidic lumenal pH. Existence of a phosphate transporter is hypothesized to be critical for pH homeostasis in the Golgi, as mannosylation in the Golgi accounts for the vast majority of cellular GDP hydrolysis. We present evidence that ERD1 plays a key role in regulating lumenal Golgi pH. Erd1p is Golgi localized and is homologous to other phosphate transporters. erd1 mutants suffer from a number of Golgi defects, including glycosylation and ER protein retention. erd1 mutants are EGTA sensitive,show pH- and PO4-dependent growth defects and are suppressed by ERS1, a gene that functions to regulate vacuolar pH. Taken together our results support the model that Erd1p regulates the removal of lumenal phosphate that is generated through the consumption of nucleotide sugars during glycosylation in the Golgi.

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35 - Environmental stimuli suppress the *Neurospora crassa cot-1* phenotype

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The*Neurospora crassa* colonial temperature sensitive-1 (*cot-1*) gene encodes a Ser/Thr protein kinase required for proper hyphal elongation. The temperature-sensitive *cot-1* strain exhibits normal spreading radial growth at or below 25 °C, but mutant colonies grow slowly with extensively branched hyphae at or above 32 °C. Antibodies raised against COT1 detect a 67-kDa polypeptide that is absent in extracts obtained from *cot-1* grown at restrictive conditions. The *cot-1* hyperbranching phenotype is accompanied by an increase in proton efflux (as determined by rate of medium acidification) and a 50-75% reduction in relative intracellular sodium content (determined by X-ray microanalysis). Ammending the growth medium with ion pump inhibitors (DES, Amiloride or Ouabain), NaCl (0.5-1.5M) or sorbitol (1-1.5M) remedies the ionic imbalance and suppresses the *cot-1* phenotype to various degrees. The COT1 67kDa polypeptide was detected in extragenic suppressors of *cot-1* (exhibiting partial or full suppression), but not in *cot-1* cultures suppressed by the tested environmental stimuli. Based on these results we suggest that impaired COT1 function confers changes in cellular ionic homeostasis and that suppression of the *cot-1* phenotype by genetic alterations versus changes in environmental conditions may involve different pathways.

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36 - The status of names and records of Australian macrofungi

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In the *Catalogue and Bibliography of Australian Fungi*, all names applied to Australian fungi are arranged under accepted names, with a comprehensive listing of literature records. Treatments for basidiomycete macrofungi are complete, and data from the first volume is accessible on-line. Some macrofungi are recorded from Australia only as names on herbarium specimens. Such records will soon be accessible through the development of on-line specimen databases. Mapping schemes are generating large numbers of unvouchered sight records for selected macrofungi. Information from different types of records (literature, herbarium and sight) needs to be integrated and assessed. It is apparent that the take up of names and the quality of records for Australian macrofungi varies considerably. The take up of names refers to how widely and with what confidence names are applied. Quality is measured by such things as the amount of descriptive data supporting the record and whether there are vouchers. Most species based on Australian types have been disposed among modern genera, but often are known only from the type. For species based on extra-Australian types, numerous Australian records appear to be misapplied. A scheme will be outlined for coding the status and reliability of names and records. Filters can be applied according to the various purposes for which data might be used (such as nomenclature, conservation status, distribution, quarantine, identification or regional censuses).

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37 - Distribution of Southern Hemisphere inoperculate discomycetes, the influence of Gondwanaland

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Many authors have attempted to explain present-day species-level distributions of Southern Hemisphere fungi with reference to ancient continents and geology. We suggest that present-day species-level distributions are more likely to reflect the present-day proximity of landmasses, or anthropogenic factors, than they are to reflect the ancient geology of Gondwanaland. The question of the influence of Gondwanaland on the distribution of Southern Hemisphere fungi needs to be addressed by considering phylogenies at higher taxonomic levels. Some examples are provided, including *Cyttaria* and *Torrendiella*. The extent to which general fungal distribution is still influenced by the events of 20-80 million years ago will be known only after many more robust phylogenies are available for groups of Southern Hemisphere fungi at about the level of genus or family.

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38 - Coprophilous ascomycetes of Australia

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Coprophilous ascomycetes of Australia This short narration is a summary of approximately three years research into the coprophilous ascomycetes of Australia. The culmination of this project is a recently completed fully illustrated manual documenting these fungi. The project was funded by the government financed Australian Biological Resources Study (ABRS). A total of 180 species of ascomycete fungi were identified 20 of which (=11%) represent hitherto undescribed species. The most frequently encountered genera include: *Podospora* (31 species), *Sporormiella* (19 species), *Chaetomium* (16 species) and *Ascobolus* (13 species). This presentation will include illustrations and discussion of a few of the most noteworthy taxa. The completed publication will also include the hitherto unpublished research of the late Harry Dade who in his retirement years also worked on Australian coprophilous fungi. The performing of this research also highlighted the urgency of upgrading of keys and the great need for more direct observations of living fungi on their natural substrates.

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39 - Saprobic and mycorrhizal ammonia fungi of the Southern Hemisphere

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Saprobic ammonia fungi, such as *Ascobolus denudatus*, *Peziza moravecii*, *Tephrocybe tesquorum* and *Coprinopsis phlyctidospora* were observed both in New Zealand and Australia. Morphological characters of collections of each fungus from New Zealand and Australia broadly fit within the species concepts of the same ammonia fungi from the Northern Hemisphere. The genetic variation among the *C. phlyctidospora* collections that were obtained from the Netherlands, Japan, New Zealand and Australia was examined by analyses based on the ITS rDNA sequences and by inter- and intracompatibility of the isolates from Japan and those from New Zealand and Australia. *C. phlyctidospora* is a species complex and individuals currently recognized as *C. phlyctidospora* in the Northern Hemisphere and those in the Southern Hemisphere are

distinct species. Mycorrhizal ammonia fungi obtained in the Southern Hemisphere were *Hebeloma* spp. and *Laccaria* spp. Many collections of the *Hebeloma* spp. obtained from New Zealand conformed to the morphological species concept of *H. aminophilum* in Australia. Data from their ITS rDNA sequences supported the above identification. *H. vinosophyllum*, a species only recorded from Japan, is a Northern Hemisphere counterpart species to *H. aminophilum*. These results suggest that some ammonia fungi in the Southern Hemisphere have closely similar counterpart species in the Northern Hemisphere.

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40 - Southern Hemisphere truffles - friends or relatives?

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Current research utilising molecular data is focussing attention on the evolution of and relationships between and within previously stable higher taxonomic groupings. Preliminary data is causing many researchers to take a closer look at morphological characters traditionally thought to delimit genera, families and even orders. The truffles or sequestrate fungi are a polyphyletic, diverse group of macrofungi, well represented in Australia and New Zealand. Where do they fit into the changing world of fungal relationships? Three examples will be discussed, highlighting some of the challenges at different taxonomic levels facing researchers. A new genus of truffle based on a single species is described and its position within the Agaricales confirmed. The recently described genus *Amarrendia* is thought to be the simplified 'end-point' of a morphological continuum including the agaricoid *Amanita* and the secotioid *Torrendia*. Relationships to other Amanitaceae are examined using molecular and morphological data. In Australasia the truffle-like Russulales are more diverse than previously thought, with some 40 new species recently described. Analyses of morphological characters provide some support of truffle generic boundaries, while molecular work suggests multiple origins of truffles within *Russula* and *Lactarius*. However, it is possible that there are both lineages which contain both agaricoid and truffle species, as well as lineages with strictly truffle morphology.

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41 - Corticiaceae of Patagonia: species' richness, rarity and distributional patterns

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The Corticiaceae (Aphyllophorales, Basidiomycota) of southern Argentina are reviewed, with emphasis on Tierra del Fuego. A total of 156 species were recorded of which 20% are endemic, 3% austral and 77% were either cosmopolitan or with another distributional pattern. Within the endemic species we found the new genera and species *Nothocorticium patagonicum* and *Rhizochaete brunnea*, and species in *Hyphodontia* (8), *Dendrothele* (5), *Athelopsis* (2), *Hymenochaete* (2), *Aleurodiscus* (3), *Amyloathelia* (1), *Ceraceomyces* (1), *Fibricium* (1), *Hypochniciellum* (1), *Leptosporomyces* (1), *Sistotrema* (2), *Tubulicrinis* (2), *Tulasnella* (1) and *Vararia* (1). An assessment of rarity of these taxa is presented, based on their abundance, distributional area and niche specificity. Of the five austral taxa, three are very rare, being recorded only once (*Dendrothele biapiculata*, *Epithelopsis fulva* and *Hypochniciellum oblongisporum*). Some endemic taxa appear to be closely related to Australian/New Zealand taxa, sharing distinctive characters within their genera. For example, *Aleurodiscus antarcticus*, *A. triviale* (Argentina) and *A. parmuliformis* (New Zealand) have smooth basidiospores and skeletocystidia and, morphologically seem intermediate between *Aleurodiscus* and *Stereum*. Species in *Hyphodontia* are also discussed as well as noteworthy species from other genera. Our knowledge of these fungi is far from complete since several forest types with distinctive environments remain unexplored.

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42 - Rust fungi (Uredinales) and smut fungi (Ustilaginales) in New Zealand

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This paper examines the relationship between rust fungi and smut fungi in New Zealand and other parts of the world. New Zealand is a land of immigrants, and much of the rust and smut mycota is also introduced. Early Polynesian migrants may have introduced some rust fungi to the northern, subtropical Kermadec Islands, but European introductions of host plants, together with contaminating fungal spores, are the principal source of introduced rusts and smuts. Despite strict quarantine enforcement new rusts and smuts are continuing to be introduced. Recently introduced plant material may have introduced gladiolus rust and a dock rust from South Africa. A major source of new arrivals is wind-blown rust spores from Australia. The native rust and smut mycota has close affinities to the Australian mycota. Of 125 native rusts, 89 (71%) are considered to be endemic while 33 (26%) also occur in Australia; of 30 native smuts, 14 (47%) are endemic and 12 (40%) are also found in Australia. Some indigenous rusts and smuts are known only in outlying islands e.g., *Puccinia embergeriae* (Chatham Is), *Uredo inflata* (Auckland Is), *Restiosporium dissimile* (Chatham Is). *Puccinia oreoboli* is restricted to the New Zealand subantarctic islands and to the highlands of Papua New Guinea while the smut, *Microbotryum nivale*, is known on an isolated mountain in Central Otago, and in Arctic Europe and Greenland. Only two indigenous rusts and three smuts are known to have South American affinities.

43 - Witches' broom and frosty pods: The unexpected relatedness of two major cacao pathogens

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The agaric *Crinipellis perniciosa* is a hemibiotrophic pathogen which causes witches' broom disease of cacao and has recently decimated the Brazilian cacao industry. In addition to the pathogenic C-biotype, other biotypes are found associated with unrelated plant taxa, notably lianas (L-biotype) and solanaceous hosts (S-biotype). The C- and S- biotypes are non-outcrossing and form broom symptoms on hosts whereas the L-biotype is outcrossing and asymptomatic. Genetic relatedness within and between biotypes can provide information about the past spread of the disease and the likely future direction of evolution of the pathogen. Phylogenetic analysis of several regions of the rRNA locus (ITS, partial IGS, partial 28S) revealed near identity between all C- and S-biotype isolates from diverse locations. L-biotype isolates formed a discrete but distinct clade. Surprisingly, analysis of sequences from *Moniliophthora roreri*, an imperfect basidiomycete pathogen of cacao in the New World revealed a very close relationship with *C. perniciosa* (closer than the morphologically very similar *C. eggersii*). Similarities in host infection between *C. perniciosa* and *M. roreri* were noted by Harry Evans but it is difficult to reconcile the gross morphological differences. Pairings between monokaryons of *C. perniciosa* and *M. roreri* (both lacking clamps) gave rise to a clamped dikaryotic mycelium suggestive of a hybridisation event. The nature and implications of these findings are discussed.

44 - Conservation of fungi in the Southern Hemisphere - Challenges and progress

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Fungal conservation in all parts of the Southern Hemisphere lags well behind organisms of larger size, capable of movement, or coloured green. Other challenges are the inadequate inventories and knowledge of distribution of fungi, the few professional and amateur mycologists, and the simplistic world-view confined to 'plants and animals'. The authors are not aware of any Southern Hemisphere government Red Data Lists for Fungi using IUCN criteria. But in Australasia and other southern regions, some progress is evident. In New Zealand, the conservation status of fungi is being considered for the first time at national level with iconic threatened fungi including species of *Claustula*, *Ganoderma*, and *Puccinia*. In Australia, distribution of selected species of fungi is being mapped in the nationally coordinated programme 'Fungimap', and two reserves have been established based in part on the presence of threatened fungi including *Hypocreopsis* and *Hygrocybeae*. Both countries have checklists of all recorded fungi in preparation. In southern South America corticioid fungi have been assessed for rarity. The conservation status of many South Australian macrofungi has also been assessed, although incomplete knowledge of distribution often limits definitive conclusions. Examples are given of threatened fungi in different regions and a summary presented of fungal conservation hemisphere-wide.

45 - How alpine are 'alpine' fungi?

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Several of the 'alpine' fungi from alpine tundra have been observed in lowlands but in association with *Salix* species; others can change the host genus in lower elevations. Vice versa 'lowland' fungi are occasionally observed in alpine tundra but seemingly without being able to establish permanent populations. Factors, which enable alpine fungi to survive the stress conditions of the alpine tundra, are either passive (growth respectively fructification in especially protected habitats or only during favourable periods or the ability of basidiomata to revive) or active (reduction of water content, increase of carbohydrate storage, special proteins and enzymes, membrane lipids etc.).

46 - *Cortinarius favrei*: an example for phylogenetic, morphological, and ecological species concepts in alpine fungi

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Extensive analysis of closely related species from alpine, subalpine and montane habitats should facilitate the investigation of ecological, morphological and phylogenetic species concepts in ectomycorrhizal mushrooms. *Cortinarius favrei* is one of the most frequent alpine *Cortinarius* spp. associated with dwarf willow (e.g. *Salix herbacea*, *S. polaris*) and *Dryas*. The morphologically very similar *C. absarokensis* occurs with shrubby willow in subalpine habitats. In contrast, *C. septentrionalis*, *C. trivialis* and *C. vernicosus* are closely related species occurring in boreal habitats in association with mostly deciduous trees. For this study, numerous collections of *C. favrei* and of *C. absarokensis* from circumpolar localities between the West-coast of the USA and the Russian Far-East were morphologically compared to each other and to closely related taxa of subgenus *Myxacium* section *Myxacium*. Moreover, nuclear DNA sequence data from the internal transcribed spacers (ITS1 and ITS2) were generated and analyzed for the study group. Based on these extensive molecular and morphological data, phylogenetic, morphological, and ecological species concepts in alpine fungi are discussed.

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47 - Basidiomycetes of Greenland

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Based on c. 10.000 collections of basidiomycetes from the Greenland Herbarium in Copenhagen (C) a checklist including 843 species has been established (in press). A quantitative analysis of the composition of the list regarding mode of nutrition and taxonomic structure is presented and compared to that from a temperate, northern European region. The development of special arctic elements in the funga as well as different ways of adaptation to the cold environment is shown and some fungal taxa acting as pioneers in young landscapes are pointed out. Examples of the major distributional types of basidiomycetes in Greenland are presented as well as typical representatives of an arctic(-alpine) and subarctic(-subalpine) circumpolar distribution based on the authors collections from Alaska, Siberia, Svalbard, Iceland and the Alps as well as records from the literature. Finally, the biodiversity of the Greenland funga is compared to that of the well-investigated Greenland flora.

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48 - Mycoknowledge related to Svalbard (Spitsbergen)

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Svalbard is today an easily accessible arctic region with well equipped research stations and housing facilities. Never the less, the mycological exploration has been casual and our knowledge is fragmentary. Only the lichen flora, comprising almost 600 recognised species, may be considered well studied. During the almost 170 years from S. C. Sommerfelt published the first account on fungi from Svalbard, up till today, only about 600 non-lichenised fungi have been recognised. They belong to all four divisions of the Mycota, but only a few taxonomic groups have been systematically sampled and studied, e.g. ascomycetes of the genera *Lamprospora* and *Pleospora*, gasteromycetes of the genera *Calvatia* and *Bovista*, agarics of the genera, *Arrhenia* and *Galerina*. An account on micromycetes on vascular plants exists (197 species) and a checklist of Svalbard's pyrenomycetes ss. lat. (129 species). Some ecological groups have been studied in detail, e.g. lichenicolous fungi (60 species) and dryadicolous fungi (34 species). Furthermore, soil fungi have been isolated from peat soils and mycorrhiza and root-associated fungi have been studied in a number of vascular plants. A taxonomic catalogue to Svalbard plants, fungi, algae and cyanobacteria was published in 1996 with the intention also of giving a broad impression of occurrence and ecology of the species. For most of the fungi this is very tentative.

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49 - Fungal diversity in arctic Lapland and the Scandinavian mountains

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In Fennoscandia (North Europe) the climatic timberline is 1200 m asl. in the central mountain massif of S. Norway, and decreases gradually towards the north and coastal areas. It reaches the sea level on the N. coast of Finnmark (71°N). The area above the timberline covers large parts (32%) of Norway. In Sweden the arctic (oroarctic) mountain areas are situated near the Norwegian border. In Finland the arctic vegetation covers only small areas in the northernmost Lapland. Records concerning Basidiomycetes from the arctic zone are sporadic. Some areas, particularly around the research stations, have been objects for more intensive studies and collecting. The following areas where relatively plentiful data is available are included in the presentation: 1) S. Norway, Hardangervidda (Finse), Jotunheimen, Rondane, Dovrefjell 2) N. Sweden, Norrbotten (Abisko) 3) NW. Finnish Lapland (Kilpisjärvi), the adjacent areas in Troms, Norway 4) NE. Finnish Lapland (Kevo, the fjells of Utsjoki and Inari), the adjacent areas in Finnmark, Norway About 400 identified fungus taxa (Basidiomycetes except wood-inhabiting Aphyllophorales) have been dealt with, and ecological aspects are discussed. There is a considerable reduction (60-80%) in the number of fungus species in the arctic zone as compared with that in the subarctic birch forests. The proportion of mycorrhizal species is highest (ca. 60%) in low arctic continental areas versus the low - middle arctic, slightly oceanic areas (45%).

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50 - Alpine macrofungi of North America (Rocky Mountains)

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Remarkably, macrofungi of the North American alpine zone have remained largely unknown until our recent intensive efforts in the Rocky Mountains revealed a diverse mycoflora. While Agaricales are well known for many arctic-alpine regions, only a few were reported above treeline in NA. To date, we have discovered over 150 species of Agaricales from the Canadian border south into the Rocky Mountains, and above treeline (3300 m at 45°N, 3600 m at 38°N). Most are typical arctic-alpine fungi at their furthest southern extent in NA. Predominant ectomycorrhizal plants are *Salix reticulata*, *S. arctica*, *S. planifolia*, *S. glauca*, *Dryas octopetala*, and *Betula glandulosa* (rare). Mycorrhizal taxa include *Amanita* cf *nivalis*, *A. greenlandica*, *Russula nana*, *R. norvegica*, *R. delica*, *Lactarius nanus*, *L. glyciosmus*, *L. repraesentaneous*, *L. salicis-reticulatae*, *Leccinum rotundifolia* (rare), *Laccaria bicolor*, *L. pumila*, *L. montana*, *Entoloma alpicola*, *Cortinarius absarokensis*, *C. favrei*, *C. hinnuleus*, *Hebeloma* spp., and over 28 *Inocybe* species. Bryophilous taxa include *Omphalina*, *Rickenella*, *Galerina*, *Marasmius epidryas*, *Arrhenia auriscalpium*, and *A.* (*Phaeotellus*) *acerosus*. Terrestrial saprophytes include *Cystoderma*, *Lepiota*, *Melanoleuca*, *Calocybe*, and *Collybia*. Most are known from other arctic-alpine habitats, some are alpine-subalpine, and a few appear to be new. Species distributions differ, with some restricted to northern or southern RM regions.

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51 - Basidiomycetes in arctic tundra in North Amercia

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Approximately 140 species of Agaricales and Aphyllophorales in the Basidiomycetes have been described and reported from arctic tundra in North America. Of these about 30% are ectomycorrhizal associates of 14 species and two varieties of *Salix*, in addition to *Dryas integrifolia*, *Arctostaphylos rubra*, and *Cassiope tetragona*. Chief among the ectomycorrhizal associates are *Cortinarius*, *Inocybe*, *Hebeloma*, *Laccaria*, *Lactarius*, and *Russula*. Three species of basidiolichens in the genus *Botrydina* (*Omphalina*) are widely distributed and common. More than 32 genera of decomposers contain about 100 species. *Galerina*, *Phaeogalera*, and *Leptoglossum* have 22 species which are decomposers of pleurocarpous Bryophytes. Over 60 species of decomposers belong to the Coprinaceae, Tricholomataceae, Strophariaceae, and Hygrophoraceae. Distribution of tundra species appears to be circumpolar with restricted endemism. The mycoflora is discussed in relation to the Alaska North Slope tundra, and the arcto-alpine habitats in the mountainous regions of northern North America.

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52 - Arctic-alpine agarics and boletes (Basidiomycota) past, present and future

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By definition, arctic-alpine agarics and boletes are ectomycorrhizal, saprobic or parasitic mycota which closely interact-coexist with autochthonous associations of lichens, mosses and angiosperms exposed to the harsh ecological conditions above the upper timberline in alpine habitats or in tundra of Subarctica and Subantarctica. The first records on arctic-alpine macromycetes have been published by Hooker (1811, Iceland), Greville (1822, Scotland), Sommerfeldt (1833, Svalbard) and Heer (1936, Switzerland). In general, comprehensive data about taxonomy, ecology and distribution of arctic-alpine macrofungi are still scarce and limited to few localities of small geographic range in the Alps (Austria, Italy, France, Switzerland), Pyrenees (Spain), Rocky Mts. (Colorado), and several sites located in high circumpolar latitudes (Alaska, Fennoscandia, Faerøer, Greenland, Iceland, Russia, Svalbard). Taxonomically and ecologically the moss-, lichen- and peat-associated taxa of *Galerina* are today by far the best known group of arctic-alpine agarics. Due to lack of data, the following localities are still white or grey spots on the arctic-alpine map viz. the Caucasus, the Himalayas, northern Japan (Hokkaido), and the high mountain ranges in the equatorial belt of South America (páramos), SE-Asia and Africa. Mycological fieldwork in these regions is urgent.

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53 - From microscopes to molecules: a journey through time

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The earliest references to marine fungi were made in 1846. In 1907 there were only 16 known species of marine fungi. It was not until the 1940's that any substantial interest in marine fungi was generated. The Halosphaeriaceae was the first family of pyrenomycetous marine ascomycetes to be proposed. The importance of morphological characters used to classify the family however were disputed, which led to inconsistencies in the listing of representative genera. Our understanding of the taxonomic importance of these characters has increased with the availability of new techniques. Here we look back on the journey through time from light microscope to electron microscopes to molecular phylogeny using genera in the Halosphaeriaceae as examples of initial concepts and current perceptions.

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54 - Mangrove mycology in South East Asia: Who, what...and what else ?

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One of the earliest records of marine fungi in south east Asia was that of Jones (1968), who reported *Lulworthia floridana* and *Halosphaeria quadric-cornuta* on drift wood collected in Singapore. Interest in marine mycology in the region was nurtured at IMC3 in Tokyo in 1983. As mangrove swamps constitute the extensive and characteristic coastal vegetation in this part of the world, subsequent post-IMC3 works focused primarily on the marine fungi in mangrove habitats. The first few papers were that of Gacutan and Uyenco (1983; Philippines), Kohlmeyer (1984, Thailand), Tan (1985, Singapore and Malaysia), Koch (1986, Thailand), and Jones and Tan (1987, Malaysia). Since then, intense interest and studies have yielded a wealth of information on the biodiversity of mangrove fungi in the region, with more than 100 new species and genera described. Apart from biodiversity, the proximity and accessibility of mangroves facilitated ecological studies undertaken to account for the observed occurrence, abundance and distribution of these fungal species. These included studies on fungal succession, vertical distribution, substrate specificity, and to a lesser extent, fungal interactions. Molecular taxonomy has also been studied in the last few years. Three IMCs later, how do we take stock of the progress in mangrove mycology ? There is certainly scope for further work on biodiversity and ecology. The question is: how can studies on mangrove fungi create a greater impact on life ?.

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55 - Diversity and phylogeny of *Halophytophthora* (Oomycetes)

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Halophytophthora species are straminopilous oomycetes inhabiting marine and brackish water environments as a first colonizer on the submerged fallen leaves. Fourteen species and two varieties have been described so far mainly from mangrove environments. They show wide variation in ecology and characters of asexual reproduction. For the purpose of investigating the phylogenetic relationship with other straminopiles and clarifying the species level taxonomy of this diverse group, 18S rDNA, D1D2 region of 28S rDNA and ITS1-5.8S rDNA-ITS2 were sequenced in 58 strains of *Halophytophthora* species and analyzed phylogenetically with other related oomycetes. The analyses revealed 1) the most species of *Halophytophthora* nested in the Peronosporomycetidae clade, but *H. spinosa* positioned apart from the main group but close to *Sapromyces* (Rhipidiomycetidae), which suggests at least removing *H. spinosa* to other (new) genus, 2) the *Phytophthora-Peronospora* clade nested within the main group of *Halophytophthora* as a sister group of *H. vesicula* complex, which includes *H. vesicula* and other morphologically similar species, 3) the species in the main group of *Halophytophthora* formed several clades, each of which corresponds to its specific character of asexual reproduction, 4) looking at the phylogenetic relationship between *Phytophthora* and *Halophytophthora*, the tree showed that the terrestrial or freshwater parasites might have evolved from the marine or brackish water saprobes.

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56 - Thai lignicolous aquatic fungi: From river to the sea

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Over the past 10 years our knowledge of Thai aquatic fungi has increased dramatically and with the description of many new taxa. The only previous records were those of Tubaki et al. (1983) on Ingoldian fungi. Although the major focus has been on their ecology, recent studies examines their molecular phylogeny, especially genera not assigned to an order or family for example *Bathyascus*, *Torpedospora* (Ascomycetes), *Dendryphiella* and *Sigmoidea* (anamorphic fungi). Currently some 600 higher aquatic fungi have been recorded for Thailand.

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57 - Marine fungi: A time to redefine?

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Many workers have tried to characterize physiologically and ecologically what is a marine fungi? Over 1,000 fungi have been recorded from marine habitats and they appear to be well adapted for life in this environment. However, some species have also been recovered from freshwater habitats and this raises the issue of how we define this unique group. Recently, molecular studies have demonstrated that members of the Halosphaeriales originated from a terrestrial ancestor, rather than evolution from ancestral marine taxa. Similarly, a number of marine bitunicate ascomycetes have been shown to group with the terrestrial Pleosporales. It is therefore time to reconsider our definition of what is a marine fungus and to look forward as to future studies in marine mycology.

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58 - A curmudgeon's view of priorities and economics

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The noun curmudgeon is defined and used here in two, not necessarily exclusive, ways: *n.* 1. *archaic:* a crusty, ill-tempered, churlish old man. 2. *modern:* (i) anyone who hates hypocrisy and pretense and has the temerity to say so; (ii) anyone with the habit of pointing out unpleasant facts in an engaging and humorous manner. The presenter's theses are that we have nearly all of our priorities backwards, that monetary and not scientific ends drive many of our research efforts, and that misjudgment frequently drives the rest. Topics to be addressed are (1) Where We Stand in Inoperculate Discomycete Systematics; (2) Asking the Wrong Questions; (3) Money, and the Failure of Universities: Grant Writing; (4) Milking the Grant Agencies; (5) Alpha Taxonomy Remains our Primary Necessity; (6) Training Students: How Did We Go So Wrong? (7) Journals, Proliferation and Blatant Errors in Judgement: Choosing Between Tree-Destruction and the www; (8) A View of the Future: the Presenter Exposed as a Crypto-Optimist.

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59 - Anamorphs - teleomorphs and the disparity of sampling

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Nannfeldt published his magnificent treatment of the Leotiales, one of the largest orders of Ascomycota, 70 years ago and today it still provides the clearest and most comprehensive view that we have of the classification of the inopcerculate discomycetes. Prior to his study the literature on these fungi was scattered and family delimitations were unclear. Nannfeldt 1932 system has been nearly universally followed with minor modifications, most notably the recognition of a few, generally, small segregate families such as the Hemiphacidiaceae, Vibrisseaceae, Loramycetaceae, Sclerotiniaceae and Rutstroemiaceae. These families were segregated based on ascomatal morphology, anamorph differences and, in the case of the Rutstroemiaceae, by a combination of morphological features and molecular phylogenetic data. The intent of this paper is to review current knowledge of helotialean anamorphs and the life history data. The notable situation of the families Dermateaceae and Leotiaceae/Helotiaceae, which remain problematic and largely unsampled in molecular phylogenetic data sets, will be discussed. The need for detailed biological studies of these fungi is of paramount importance in developing a reliable and predictive classification for this large and important group.

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60 - Neotropical discomycetes: Where do we stand and where do we want to go in systematics and biodiversity research?

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This talk will present an overview regarding the last 70 years of knowledge about neotropical Discomycetes. Areas to be discussed include the principal Discomycete collectors in the neotropics in this period, as well as numbers of publications dealing with neotropical Discomycetes in major journals and congress presentations. Knowledge of the total numbers of Discomycetes for some neotropical areas will be compared with other groups of fungi from the same geographical regions. A major contributor towards neotropical Discomycetes knowledge since 1949, date of his first expedition to Venezuela, is R. W. G. Dennis. In his famous and widely used 'Fungus Flora of Venezuela and Adjacent Countries' (Dennis, 1970), he treated 161 species of Discomycetes. Other mentors of this group of fungi, as well as their dedication and relevance to the area, will be presented. Advantages and disadvantages of conducting research on neotropical Discomycetes will be discussed in the sense of research funding possibilities and the consequences that these fund restrictions have had on Discomycetes knowledge. A correlation between the number of Discomycete collections and areas where these have been made, in comparison with publications resulting from these studies, will be analyzed. Based on achievements over the last 70 years, a general overview and recommendations of the paths to be followed in the future will be discussed.

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61 - Molecular diversity and phylogenetic affinities of mycorrhizal and root-endophytic fungi of the Helotiales

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Teleomorphic species of the ascomycete order Helotiales are abundant in nature, and range from hardly visible to some more prominent representatives. However, a number of asexual or sterile root symbiotic (endophyic, ericoid mycorrhizal and ectomycorrhizal) fungi have proven to belong to the Helotiales, yet their taxonomy, phylogeny and ecological roles are largely unresolved. We have studied the diversity and phylogenetic affinities of symbiotic root-associated ascomycetes of the Helotiales based on ITS1-5.8S-ITS2 (ITS) nrDNA sequences. Mycobionts were obtained from roots of ericoid plants and grasses and from *Piceirhiza bicolorata* ectomycorrhizas (*pb*ECM) on conifers and hardwoods, predominantly in burnt and metal-polluted habitats. The mycobionts were sequenced through the ITS and compared to sequences of known helotialean taxa. We recognised 132 fungal ITS-sequences with affinity to the Helotiales, of which 75% (54 different ITS-genotypes) grouped within the *Hymenoscyphus ericae* aggregate including *Phialophora finlandia*. This aggregate showed stronger affinity to members of the Hyaloscyphaceae and Dermateaceae than to *Hymenoscyphus fructigenus* (genus-type species; Helotiaceae). The *H. ericae* aggregate may be referable to a generic unit, and includes a diverse group of closely related ± darkly pigmented root-associated ascomycetes where the borders between intra- and interspecific ITS-sequence variation as well as different types of root-symbioses remain unclear.

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62 - How little we know about the inoperculates in China

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Three stages can be recognized in studies of inoperculate discomycetes in China. Saccardo's taxonomic treatments were applied by the early mycologists at the very beginning, the influence of Seaver's works constituted the second stage, and treatments by Nannfeldt and Korf were much involved after the 1950's. Revisionary work by Korf plus several other recent workers towards the natural classification system is accepted currently. Known species of the inoperculates in China increased from 67 in the 1930's and 1940's, 81 in 1963 by Prof. S. C. Teng and 140 in 1979 by Prof. F. L. Tai, and up to 424 in 103 genera at the end of 2001 in the third stage. Significant progress has been made through the efforts of mycologists from different institutions dealing with species diversity of the group in the past two decades and the known species increased dramatically. Examples revealing the species richness of the Hyaloscyphaceae and a number of genera in other families were provided. Gross morphology, detailed anatomic structures, and molecular data should be combined properly in order to discover the phylogenetic relationships among genera or families aiming at the establishment of correct species concepts and a reasonable taxonomic system for inoperculate discomycetes. We seem to be far from that goal.

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63 - Will ascus characters give meaning to future reclassifications of non-lichenized inoperculate discomycetes?

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Most families of the inoperculate discomycetes are still regarded as heterogeneous. Many genera are also still poorly delimited, although in recent years progress has been made by integrating molecular and phenotypic approaches in some monographic studies. With molecular tools we can rapidly trace the informative morphological characters. Although there is still an enormous amount of work to be done, we should also take action at suprageneric level, using a similar approach. It is clear that the inoperculate discomycetes are not monophyletic, and major reclassification is imperative, but this calls for robust multi-gene datasets, combined with congruent morphological character complexes that provide enough synapomorphies. Familiar characters such as excipular anatomy and gelatinous substances may fail at this point. Ultrastructural studies of selected taxa e.g., in the Helotiaceae and Hyaloscyphaceae, have shown that the ascus apical apparatus constitutes a promising set of microanatomical features and should be explored further. Although some fundamental differences in apical structures are only resolved by transmission electron microscopy, it is certainly feasible to screen larger groups of taxa using light microscopy. I will attempt to correlate morphological and molecular data currently available, however fragmentary they may be. Obviously, more data should be gathered before extensive conclusions can be drawn.

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64 - Progress and challenge: Helotiales in the new millenium

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After 70 years of progress, radical changes to the classification of inoperculates proposed by Nannfeldt have been few. Such changes are mainly recent, based on powerful tools such as DNA and ultrastructural analyses. They include demolition of the traditional Leotiaceae and Sclerotiniaceae, now regrouped into 5 families and, more extreme, the ousting of *Orbilia* as representative of a whole new class. In contrast, molecular study of Hyaloscyphaceae indicates surprising coherence of seemingly disparate taxa. But there remain conflicts in results and uncertainty about the placement of many taxa. Problems include an acute lack of data on the species involved. Two-thirds of these may remain undescribed, and only a handful have yet been sampled for modern analyses. Small subunit RNA sequences, for example, are known for less than 50 genera, only 7% of those described so far. Few species have received detailed morphological study, and many are scarcely represented at all in the world's herbaria. Identification remains fraught with problems and there is huge disparity in knowledge between different regions. Less than 200 species have yet been reported from the tropics, for example, fewer than from some sites in England. Yet rate of description of new species continues to fall. Clearly, there is still a pressing need for description and morphological study, to provide the basic taxa for application of modern techniques and to approach eventually a natural system for the Helotiales.

65 - Diversity in the genus *Penicillium*

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Penicillium is a tightly circumscribed but diverse genus, characterised by a distinctive asexual fruiting structure, the penicillus. Separation from other genera is usually unequivocal. Diversity is reflected in occurrence in a wide range of habitats. Some species, especially in subgenera *Aspergilloides* and *Furcatum*, are primarily associated with soil. Some species, especially in subgenus *Biverticillium*, are common in biodegradation, i.e. decaying plants and plant based materials. Others, especially in subgenus *Penicillium*, are found in seeds, which includes foods such as cereals, or dung. Few can be considered pathogens of animals or plants. A few are truly ubiquitous. *Penicillium* species are most common in cooler temperate climates, a reflection of relatively low temperature profiles for growth. Penicillium is associated with two sexual genera, *Eupenicillium* and *Talaromyces*, with mostly soilborne species. Some taxonomic questions remain. The possibility exists that *Penicillium* is not truly monophyletic, and that the species associated with the two sexual states are distinct in evolutionary terms, though not obviously so in ecological or morphological terms. The question of delimitation of subgenera, based on morphology, arises because molecular studies suggest that the subgenera *Aspergilloides* and *Furcatum* are not distinct from each other. And finally, the perennial question of what constitutes a species has been fuelled by molecular studies in particular.

66 - ISSR variation in *Penicillium miczynskii* and *Eupenicillium shearii*

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There is little evidence to indicate whether species of *Penicillium* are or are not genetically cohesive. To address this issue, I have been performing ISSR analyses on 190 strains of *P. miczynskii*. These strains were isolated primarily from four *Pinus* dominated sites in southeast Wyoming. Additional strains were isolated from a mixed hardwood forest in Denmark and a *Nothofagus* dominated forest in Chile. I also collected 130 strains of *E. shearii*, primarily from Costa Rica and India. Preliminary results indicate that *P. miczynskii* strains from SE Wyoming are very much alike, though some variation is present. Danish strains of *P. miczynskii* show ISSR patterns very different from those of the Wyoming strains, and also show considerable variation amongst themselves. Parsimony analysis using ITS sequences from several of these strains placed most of them in a clade including the type strain of *P. miczynskii*. In contrast, the strains of *E. shearii* clustered into two groups, irrespective of origin. These two groups may be interpreted as distinct species. This hypothesis is further supported by ITS sequences, which indicate that at least one of these groups is not closely related to the type strain of *E. shearii* (no representative of the second group has been sequenced yet). These results suggest that variation is structured quite differently in mitotic *Penicillia* than in meiotic *Eupenicillia*, and that lack of a meiotic cycle does not impose a constraint on intraspecific variation.

67 - Phenotypic diversity in *Penicillium* and its teleomorphs

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The genus *Penicillium* contains species of an impressive diversity and they are consistently found all over the world. In a detailed analysis of species occurrence in 16 soil microfungal surveys, our data indicate that: 1) Penicillia are abundant in certain soils, accounting for nearly 50% of all species obtained with 10 - 71% recently described or undescribed; 2) the soils with highest pendiversity appear to be soils from tropical and temperate rain forests (upper Amazon, Peru; ancient conifer forest, Oregon, USA) and coastal annual grasslands (California, USA); and 3) a great many soil species are reliable indicators of habitat type. *Eupenicillium* associated Penicillia are usually soil- or foodborne, while the very different *Talaromyces* associated Penicillia often are with plant polymer associated. Some species are associated to plants and one of them even produce myconodules in Elder trees. One species can grow in saturated cupper sulphate solution. Few species are xerophilic, most of them are still to be described. Some acidophilic species can only grow below pH 3.5 and are associated to wood, while others are alkalinophilic. Many species, mostly undescribed, are psychrophilic, while others are thermophilic. The chemical diversity of *Penicillium* species is equally impressive, isolates in each species being able to produce a large number of secondary metabolites. Most species produce 5-10 different families of secondary metabolites each with 3 to 20 members.

68 - Resolving the identification of *P. glabrum, P. purpurogenum, P. thomii* and *Eupenicillium lapidosum*

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The identification of *Penicillium glabrum* and other closely related species presents a challenge to mycologists because of the phenotypic similarity of these species. To address this problem, DNA sequences from ITS, beta-tubulin, calmodulin and translation elongation factor 1 alpha were determined from ex type and other isolates belonging to *P. glabrum*, *P. thomii*, *P. yezoense*, *P. grancanaraie*, *P. baiiolum*, *P. palmense*, *P. trzebinskii*, *P. terlikowskii*, *P. purpurescens*, *P. viridoalbus*, *P. oledskii* and *Eupenicillium lapidosum*. Prior studies showed that in the ITS region, total variation among these species is two base substitutions, although some of these species may be synonyms. *Ex-type* isolates of *E. lapidosum* and *P. thomii* had identical sequences in ITS, CF, EF1 and BT; *P. viridoalbus* and *P. purpurescens* were identical at 3 loci and differed by a single base at the other suggesting synonymies in each case. *Penicillium spinulosum* also resembles *P. glabrum* and is closely related. However, it is a genetically and phenotypically distinct species. The pattern of genetic variability at multiple loci suggests a complex of variable and closely related species centered on *P. glabrum*.

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69 - Species delimitation of Penicillia and Aspergilli; dilemma's between phenotypic and molecular characterization

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The taxonomy of *Penicillium* and *Aspergillus* and their teleomorphs has been subject to various methods of investigations. With the classical morphological approaches using characters of conidiophores, conidia and sexual propagules, delimitation of many taxa has been unclear and debatable. Introduction of physiological and culture criteria has only contributed little, particularly because standardisation of media for cultivating *Penicillium* and *Aspergillus* remains problematic. On the other hand chemotaxonomic profiles of secondary metabolites are promising showing fine distinction of taxa. With the introduction of various molecular tools delimitation of taxa have been solved, although one particular method does not always apply for all taxa of both genera resulting in different taxonomies. In some cases it is difficult to show similarities between the phenotypic and molecular characterization. This is particular true for important groups such as *Aspergillus* section *Nigri* and *Penicillium* subgenus *Penicillium*. Species of these groups are economically important and correct identification is crucial. In this paper examples with taxonomic constraints are discussed.

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70 - A polyphasic taxonomic study of *Fusarium avenaceum* and *F. tricinctum*

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A common set of 50 strains of *Fusarium avenaceum*, *F. tricinctum* and *F. stilboides* were examined by colony characters, image analysis of sporodochial conidia and conidia from the aerial mycelium, substrate utilization profiles (BIOLOG), rep-PCR (BOX, ERIC, REP primers), DNA sequencing (beta-tubulin, elongation factor 1-alpha) and metabolite profiling (HPLC, NMR). In general, DNA sequencing and rep-PCR results resulted in identical branching patterns in dendrograms. BIOLOG and rep-PCR detected more strain-level variation than DNA sequencing, but the BIOLOG phenogram differed in several respects from the molecular phylograms. Most strains identified as *F. avenaceum* (or its putative synonym *F. arthrosporioides*) clustered together in the molecular analyses, but two small groups of strains, one from Australia and the other from New Zealand, may represent distinct phylogenetic species. A population isolated from Turkish wheat formed a distinct cluster. *F. tricinctum* and *F. stilboides* formed distinct clusters except for a single identified strain of each species that appeared to be more distantly related. Representative strains of two subspecies of *F. avenaceum* subsp. *aywerte* and subsp. *nurragi*, were distantly related to *F. avenaceum* subsp. *avenaceum* and probably represent distinct species. Analyses of combined data sets and correlations between molecular, morphological and physiological data sets will be used to refine species concepts for these taxa.

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71 - Towards a polyphasic and predictive taxonomy for small-spored *Alternaria*

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Within recent years, several techniques have been used to study sub-generic classification in small-spored *Alternaria*. Examination of 3-dimensional sporulation patterns on standardized media has shown that there are more than seven stable patterns of sporulation. Molecular methods, such as RAPD-PCR, support this segregation, and metabolite profiling has shown that sporulation patterns are predictive of certain secondary metabolites. New methods to utilize phenotypic features unbiased have been developed and applied to *Alternaria*. Digital images of cultures, grown on standardized media at different temperatures, are captured and transformed into data matrices (VideometerLab). Similarly, total HPLC chromatograms are transformed into matrices (COW Tool). Each matrix is then subjected to statistical analysis. Adding other characters, such as pathogenicity or presence of specific genes, may strengthen existing species or species-groups based on morphology and reveal additional groups. By using multivariate statistics, it is also possible to combine several sets of different data derived from both current and new methods (e.g. RAPD and metabolites) in one analysis to get segregation of isolates into natural clusters of similar taxa. The purpose of this presentation is to introduce image analysis and multivariate statistics to analyse chemical, physiological and molecular data, separately and combined, in order to apply a polyphasic and predictive strategy to *Alternaria* taxonomy.

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72 - An assessment of research applications of Biolog MicroPlates^{TM}

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Biolog MicroPlates^{TM} are employed to characterize fungal strains based on differential assimilation of test substrates and redox reactions in a 96-well test plate. The Biolog method is potentially advantageous in being relatively simple, fast and economical; and data acquisition and identifications can be automated using a microplate reader and applicable software. Several research applications of the Biolog system are presented: i) the reliability of the Biolog system as a diagnostic tool is determined for identification of the 'green mould' disease of the commercial mushroom caused by *Trichoderma aggressivum*, ii) data from Biolog MicroPlates are compared with ITS sequence data, and their utility assessed in a survey of the biodiversity of *Trichoderma* strains from Asia, and iii) more than 900 *Trichoderma* strains are characterized using Biolog microplates, and the resulting database used to select strains that might be exploited for specific bioconversions. Strain level variation is more evident with Biolog data than with DNA sequencing or RAPD techniques, and Biolog data do not consistently reflect phylogenies constructed from molecular data. However, the Biolog system is an economical alternative method for surveying biological diversity, and provides data that complements molecular data in phylogenetic studies. Biolog data may provide a rational for the selection of strains for industrial applications.

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73 - *Penicillium* toxins in food and feed

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Penicillium species appear to dominate in storage damaged food and feed. Microbiological studies carried out at the National Veterinary Institute have shown that under certain conditions food may be severely infected by moulds, especially *Penicillium* species, and it is important to establish if mycotoxins are present. The *Penicillium* species identified belong to the most proficient known mycotoxin producing moulds. There are few analytical methods available for the analyses of *Penicillium* toxins in various matrices and therefore a screening method using liquid chromatography-mass spectrometry (LC-MS) to quantify the commercially available *Penicillium* toxins roquefortine C, griseofulvin, mycophenolic acid, verruculogen, chaetoglobosin B and penitrem A has been developed. *Penicillium crustosum* Thom., is very common in food spoilage worldwide. Several cases of intoxication caused by toxins produced by *P. crustosum* have been reported and the fungus is known to produce roqufortine C and the penitrems A-F. Investigations of isolates of *P. crustosum*, revealed the presence of penitrem A, but only small amounts of other penitrems (B-F) when the isolates were grown on rice. From the LC-MS traces of the rice extracts, two major components together with penitrem A could be seen. These two components have been isolated and identified as two new indole-alkalod isoprenoids, 18,19-dehydrosecopenitrem A and E.

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74 - Regulations in the EU for mycotoxins in foods

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The quality and safety of food may be threatened by a host of factors, including natural toxins. Of the large spectrum of natural toxins, mycotoxins are among the most widely studied and raise considerable concern, because of their ubiquity and potential deleterious effects on human and animal health. Mycotoxins are produced by certain fungi, which, under favorable temperature and humidity conditions, invade a wide range of agricultural crops in the field, and during post-harvest stages of production. In 1994 the EU started the harmonization process on standardization of certain mycotoxins in foods after its Scientific Committee on Food (SCF) expressed an opinion on -in their view at that moment-, the most important mycotoxins: aflatoxins, ochratoxin A and patulin. Aflatoxins and ochratoxin A have been more or less regulated now and recently certain emerging *Fusarium* toxins such as deoxynivalenol have been added to the list to be regulated soon. Estimation of the actual dietary intake

of mycotoxins is essential for risk assessment and will be used in determining whether there may be a relationship between observed adverse effects in humans and exposure to a particular mycotoxin. In order to apply the control on mycotoxins and to set standards for trading purposes, it is necessary to have collaborative tested and validated methods of anaylsis and sampling. The coherent approach of how EU-wide standards will be established will be addressed.

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75 - Ink cap sex and mushroom development

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Sexual development in *C. cinereus* occurs on the dikaryon under control of the mating type loci. A mating type proteins, homeodomain transcription factors, induce fruiting body initiation under appropriate light, temperature and nutritional conditions. Development starts with intense localized branching leading to a small loose structure (primary hyphal knot). Light and *A* genes are needed to transform it into a compact round aggregate (secondary hyphal knot) in which cap and stipe tissues differentiate. Once a primordium is fully established, light and the B mating type proteins (pheromone and their receptors) induce karyogamy and fruiting body maturation. Next to studying nutritional signalling, we identified some genes acting at the first steps in fruiting. One uncharacterised gene acts in primary hyphal knot formation, gene *cfs1* for a potential cyclopropane fatty acid synthase in the transition to secondary hyphal knots. Work in B. Lu's lab presented us two galectin genes, whose first expression correlates with formation of primary (*cgl2*) and secondary hyphal knots (*cgl1*). Structural analysis within the fruiting body suggests these protein to function in hyphal aggregation. Due to control by A mating type proteins, light, C and N sources, their promoters provide us with an ideal system to identify cis- and trans-acting elements in transcriptional regulation. Supported by the ETH Zurich, the Swiss National Science Foundation and the Deutsche Bundesstiftung Umwelt.

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76 - How mushrooms work

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With characteristic eccentricity, A. H. R. Buller (1931) determined the weight-lifting capacity of *Coprinus* fruiting bodies. From the mass raised by developing basidiocarps, he estimated that hyphae elongating within the stipe generated a pressure of 0.07 MPa (or two-thirds of an atmosphere). When all of the fruiting bodies emerging in a cluster exert pressure of this magnitude, their combined force is sufficient to crack compacted soil or decaying wood (and to dislodge paving slabs positioned over their mycelia in urban areas). For example, a pressure of 0.07 MPa applied over an area of 0.01 square meters (= 10 × 10 cm), produces a total force of 700 N. This is sufficient to lift the combined mass of the author of this presentation plus all seven volumes of Buller's *Researches on Fungi* (= 71 kg). Interest in these classical experiments has been revived by biomechanical research involving precise measurement of the forces exerted by single hyphae. The instrumentation used in these investigations is also effective for studying the mechanical behavior of multicellular fungal organs, and valuable information on the origin and control of force during fruiting-body emergence has been obtained during preliminary experimentation. Once the mushroom has surfaced, a variety of developmental adaptations optimize the discharge of spores from the gills and dispersal in air currents flowing beneath the cap. Recent work on basidiocarp form and function is also featured in this presentation.

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77 - Forceful invasion of corn leaves

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The corn pathogen *Colletotrichum graminicola* causes the anthracnose disease of several cereals and grasses. To infect the leaf, conidia germinate and differentiate a specialized infection cell called an appressorium (Deising et al. 2000). When an appressorium matures it accumulates osmotically active compounds to yield high concentrations. The resulting turgor pressure of more than 5 MPa is translated into forces of up to 17 µN by single appressoria (Bechinger et al. 1999; Bastmeyer et al. 2002). These figures indicate the importance of the rigidity of the fungal cell wall. We performed targeted inactivation of three chitin synthase genes of the corn anthracnose fungus and found that only CgCHSC is essential for vegetative growth and pathogenic development. To analyze the role of cell wall-degrading enzymes in the infection process we generated mutants defective in CgSNF1, a gene involved in activation of catabolite-repressed genes (Tonukari et al. 2000). Reduced growth rates on complex corn cell walls and reduced rates of infection suggest that cell wall-degrading enzymes assist forceful invasion by *C. graminicola*. Bastmeyer, M., Deising, H.B., Bechinger, C. 2002. Annu. Rev. Biophys. Biomol. Struc. 31:167-175 Bechinger, C., Giebel, K.-F., Schnell, M., Leiderer, P., Deising, H.B., Bastmeyer, M. 1999. Science 285:1896-1899 Deising, H.B., Werner, S., Wernitz, M. 2000. Microbes Infect. 2:1631-1641 Tonukari, N.J., Scott-Craig, J.S., Walton, J.D. 2000. Plant Cell 12:237-248.

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78 - Sexless superguns - the enigmatic life history and infection processes of the zoosporic nematode pathogen *Haptoglossa*

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The biflagellate zoosporic nematode pathogen *Haptoglossa* is placed in the Oomycete fungi, although its precise phylogenetic position has still to be resolved. All *Haptoglossa* species are characterized by having sausage-like thalli, which often completely fill the body cavity of their host. Most species produce discharge tubes which breech the nematode cuticle and through which the spores are released at maturity. *Haptoglossa* species fit into two major groups: zoosporic and aplanosporic. The latter group always seem to produce two or more different types of infection cell of varying karyology. No clear evidence of a sexual stage has been found, although internal nuclei-filled compartments have been observed within the thallus of one aplanosporic species, which is suggestive of a sexual stage. Synaptonemal complex-like structures have been seen in the nuclei of differentiated cysts of a zoosporic species. Therefore the exact nature of the life cycle remains enigmatic. The other characteristic structural feature of this genus is the production from cysts or aplanospores of complex infection gun cells. Barron likened them to ballistic missiles, although they actually function like hypodermic syringes with retracted needles. They are some of the most complex structures observed in any zoosporic fungi. Whilst the fundamental structural components have been conserved they show enormous phenotypic plasticity from one species to another.

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79 - Bug murder in the rainforest: A fatal attraction of sex versus celibacy with a potential for discovering new biocontrol agents

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In nature insect fungi are faithful in respect to those they kill. In agriculture: anything goes, promiscuity the order. In nature: promiscuity the exception. Work aimed at developing biocontrol agents is mostly with isolates from artificial ecosystems. And much work is with a few species of *Beauveria*, *Metarhizium*, *Nomuraea* and *Paecilomyces*. Sex rarely rears its ugly head in agro-ecosystems: I posit that asexual populations from agro-ecosystems become clonal and adopt a promiscuous attitude to their hosts murder using general-purpose pathogenicity genes. In nature, sexual forms are faithful to those they kill - pathogenicity genes more focused. The ancestral pathogen that moved from forest to field 15000 yrs ago had to adapt or die. A cicadellid pathogen from forest may have infected a leafhopper in a nearby rice field. That hopper may have died in a field where hoppers were rare but caterpillars common. Survival of the fittest favours a pathogenicity gene that could infect Homoptera and Lepidoptera. The cosy world of nature favours sex with fidelity where murder is the order. Agro-ecosystems favour asexual promiscuity when it comes to bug murder. 15000 years of agriculture has given the few biocontrol agents now being tested: the random consequence of sex and murder moving from forest to field. Isolations from insects in forest or from characterised soil isolates may provide a more targeted approach to discovering new biocontrol agents in the future.

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80 - Ectomycorrhizal development in wet alder carr forests: above- and below-ground diversity

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The aim of the study was to investigate ectomycorrhizal development in wet Alder carr forests in The Netherlands. In North-Western Europe, wet Alder carr forests are mostly found in peatlands and along streams forming an important component of wetland ecosystems. The soils are predominantly fed by groundwater and permanently waterlogged. The dominant tree species in wet Alder carr forests is *Alnus glutinosa* (Black alder). The present study showed that the majority of *A. glutinosa* roots in various wet Alder carr forests in The Netherlands were associated with ectomycorrhizal fungi, even in anoxic waterlogged soils. Ectomycorrhizal root tips and hyphae were observed up to two meters from the *A. glutinosa* trees in waterlogged soil. Ectomycorrhizal development under wet and oxygen limited conditions was related to radial oxygen loss from *A. glutinosa roots*. Field observations showed that the diversity of ectomycorrhizal sporocarps was higher in wet Alder carr forests in a peatland than along a stream. Moreover, PCR-based techniques showed that the abundance and diversity of ectomycorrhizal fungi below ground was higher in the peatland than along the stream. In the wet Alder carr forests in the peatland, a relatively high redox potential of the waterlogged soil was measured together with radial oxygen loss from roots of the dense understory vegetation indicating that ectomycorrhizal development in waterlogged soils is stimulated by oxygen input of the surrounding vegetation.

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81 - Mycorrhizal fungi in wetlands: the role of the extramatrical mycelium

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Wetland plants are generally considered non-mycorrhizal but recent surveys have confirmed that mycorrhizal fungi are common in wetland plants and that all of the usual types (VAM, orchid, ericoid, and ectomycorrhizas along with dark septate endophytes) can be found in appropriate host species. Among these fungi, we are interested primarily in the ascomycetous associates if peatland plants that have dematiaceous hyphae, a group that includes *Phialocephala fortinii* (DSE) and *Oidiodendron maius* (ericoid), among others. *Phialocephala fortinii* is a dematiaceous hyphomycete common in the roots of a variety of wetland plants. The ecological role of *P. fortinii* is poorly understood but it is an enzymatically diverse species, capable of degrading various organic nitrogen (N) and carbon (C) sources, including complex phenolic polymers, such as lignin, that are important constituents of peat. In many acidic bogs, *P. fortinii* is absent from the roots of ericaceous shrubs where *Oidiodendron maius* is the most frequent isolate. Our research demonstrates that *O. maius* is also an enzymatically diverse species, capable of utilizing organic C and N, and a proficient decomposer of *Sphagnum fuscum*, the primary component of bog peat. Our research suggests that the extramatrical mycelium of selected mycorrhizal fungi and other root endophytes may be important in nutrient cycling within wetland habitats.

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82 - Diversity of mycorrhizas in wet ecosystems, what we should consider: from a case study on alder EM to a new approach to screen AM

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The mycorrhizal status in wet ecosystems is complex, including non truly mycorrhizal associations with dark septate fungi to plants like poplars or alders forming both AM and EM. In the latter case, the scarce available results indicate that AM presence decreases and EM diversity increases from wet pioneer to dryer advanced succession phases. The aim of the paper is to present partial results and new technical developments on the basis of which exhaustive studies of the mycorrhizal status in wet ecosystems could be performed. A first point concerns a comparison between above and below ground diversity of EM at two sites of an alder forest near a lake in Northern Germany. By combining identification of fruiting bodies, morphotyping of the EM and analysis of the polymorphism in the ITS region of both kinds of samples, it was possible to demonstrate an increase of the EM diversity from the lake to the higher site of the stand. Direct investigations of AM diversity in the field are difficult for technical reasons. Identification on the basis of the spore morphology is possible, but spores only partially reflect the diversity of the AM themselves. AM anatomy is not convenient to determine the fungal partners at the 'species' level. Several methods based on molecular biology have been proposed, but the analyses are often hampered by contaminating fungi. We have developed an approach combining a Nested PCR with a restriction, which partially resolves the difficulty.

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83 - Contributions of arbuscular mycorrhizal fungi to plant performance in wet ecosystems: studies with *Lythrum salicaria*

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The impact of arbuscular mycorrhizal (AM) fungi on plant performance in wetland areas was investigated through studies on *Lythrum salicaria* (L.). Under field conditions plant performance and AM colonization levels varied with water availability and there was a negative correlation between plant performance and AM colonization levels. Partially submerged plants produce numerous, free-floating adventitious roots along submerged stem lengths. The adventitious roots, the primary root system, and AM fungal hyphae represent three possible pathways for resource acquisition. We tested the hypothesis that, to sustain shoot growth, a reduction in the ability of one or more pathways to acquire resources will require an increase in resource uptake in the remaining pathway(s). While greenhouse studies revealed that individually AM fungi did not have a significant effect on plant performance, the results did indicate a relationship among the three pathways supporting our hypothesis. Greenhouse studies were conducted to determine if, in inundated soils, AM fungi contribute to plant performance across a range of phosphorus availabilities. AM fungi did not enhance growth in inundated soils; in some cases performance was limited and it is unlikely that AM fungi enhance phosphorus nutrition in inundated soils. We suggest that because of the large differences between terrestrial and aquatic habitats, AM fungi may be performing different roles in each habitat.

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84 - Mycorrhization of endangered wetland plant species

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Arbuscular mycorrhizal (AM) fungal diversity in soil seems to influence plant biodiversity. As information concerning the mycorrhization of endangered plants is lacking, we determined the mycorrhizal status of *Serratula tinctoria* (Asteraceae), *Betonica officinalis* (Lamiaceae), *Drosera intermedia* (Droseraceae) and *Lycopodiella inundata* (Lycopodiaceae), occurring at two different sites (bog and fen meadow) in the county of Salzburg, Austria. In addition to AM fungi, infection by dark septate endophytes (DSE) was quantified. The infection intensity of AM fungi and DSE appeared to be higher in the fen meadow than in the peat bog. The roots of *S. tinctoria* and *B. officinalis* were heavily infected by AM fungi and both, vesicles as well as arbuscules were observed over the vegetation period. *L. inundata* showed AM fungal infection, too; in spring vesicles were observed frequently, in autumn they were less numerous. In *D. intermedia* roots AM fungal infection intensity was lower than in the species mentioned before; however, fungal structures typical for AM fungal infection were observed, especially in spring. *S. tinctoria* and *B. officinalis* were heavily infected by DSE, while *L. inundata* was just slightly infected and *D. intermedia* did not seem to be infected by DSE at all.

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85 - Molecular genetics of gibberellin biosynthesis in *Gibberella fujikuroi*

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As well as being phytohormones, gibberellins (GAs) are present in some fungi and bacteria. GAs were first discovered in the fungus *Gibberella fujikuroi*, from which gibberellic acid (GA3) and other GAs are produced commercially. Although higher plants and the fungus produce identical GAs, important differences in the pathways and enzymes used have become apparent with the identification of the genes for GA-biosynthesis in *Arabidopsis thaliana* and *G. fujikuroi*. These profound differences indicate that higher plants and fungi have evolved the complex biosynthetic pathways to GAs separately and not by horizontal gene transfer. In *G. fujikuroi*, the 7 genes of the gibberellin (GA)-biosynthetic path-way including four cytochrome P450 monooxy-genases, are located in a gene cluster. To study the function of several genes of this cluster, we used a gene replacement approach followed by GC-MS and HPLC analysis. Most of the genes encode multifunctional enzymes. The availability of the genes allows the study of molecular mechanisms of gene regulation. Because the GA biosynthesis is regulated by nitrogen metabolite repression, we cloned several genes which are involved in nitrogen regulation, such as the general nitrogen regulators, areA and nmr. Gene replacement of areA led to a significant reduction of gibberellin formation by repressing the expression of the GA-pathway genes. In contrast, NMR does not play the role as a general counterpart of AREA as expected.

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86 - Involvement of ethylene in a plant - *Botrytis cinerea* interaction

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The role of ethylene was studied in the interaction of *B. cinerea* with the host plants tomato, French bean and *Arabidopsis thaliana*. Infected resistant *Arabidopsis* produce less ethylene as compared with infected sensitive plants. Ethylene promotes the disease development whereas inhibition of plant ethylene production resulted in disease suppression. Increased plant tissue reactive oxygen species (ROS) levels resulted in elevated ethylene and severe disease. The actual contact with pathogen cells (e.g. dead conidia) induced ethylene and ROS in plants. This effect was also obtained by leaf injury, H_2O_2, pH reduction, oxalic acid and botrydial; the compounds and effects are produced by the pathogen. Antioxidants reduced ethylene production and disease severity. Interestingly, ethylene promoted conidia germination and subsequent penetration to the host. Ethylene signaling mutants of *Arabidopsis* showed extremely high susceptibility to *B. cinerea*; it is possible that the pathway that leads to susceptibility is independent of other ethylene signal transduction pathway. *B. cinerea* has the potential to produce ethylene, thus it is possible that the ethylene originates not only from the plant. In conclusion, in *B. cinerea* - plants interaction ethylene induces auto-catalytic production of the same hormone in exposed tissues thus promoting the deterioration of the infected tissue and the ROS development and vice versa.

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87 - Abscisic acid biosynthesis genes in *Botrytis cinerea*

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Botrytis cinerea causes the grey mould disease in more than 200 plant species. Like several other phytopathogenic fungi, *B. cinerea* has been shown to produce different kinds of phytohormones in axenic culture. Although the impact of this biosynthetic capacity on host-parasite interaction is unclear, it has been suggested that production of the phytohormone abscisic acid (ABA) is supporting the infection process. A molecular approach has been initiated to study the role of phytohormones in the pathogenicity of *B. cinerea*. Our aim is to clone genes encoding enzymes of the biosynthetic pathways, especially the ABA pathway, to study their expression and to analyse the pathogenicity of deletion mutants. Therefore, a cDNA library of an ABA overproducing strain was established and differentially screened. Several cDNA clones derived from genes expressed during ABA production were characterised.

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88 - Ethylene production and ethylene-induced genes in *Botrytis cinerea*

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We defined the biosynthetic pathway of ethylene in the pathogenic fungus *Botrytis cinerea*, and characterized the conditions that affect ethylene production in vitro. During the first 48 h of culture the fungus uses methionine to produce a-keto g-methylthiobutyric acid (KMBA). In darkness, KMBA accumulates in the medium and no or very low ethylene levels are produced. In light KMBA is photo-oxidized and ethylene is released. Ethylene production rates in the light were the highest after 24 h (7 ml/g/h) and declined thereafter, whereas in the dark ethylene was detected only after 48 to 96 h of culture. A cDNA library was constructed from fungal mycelium that was grown for 24 h in the light in methionine-enriched medium. The library was differentially screened with mRNA probes from light (ethylene +) and dark (ethylene -) grown cultures. Over 20 differential clones were identified. These clones represent transcription factors, glycolysis enzymes, ribosomal proteins and unknown ESTs. Northern hybridization was conducted to confirm the differential expression pattern of the clones and to further define the expression pattern of clones that were highly induced by ethylene. This work provides the first molecular evidence for ethylene response in fungi.

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89 - Alteration of plant-pathogen interaction by phytohormones

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The effect of plant growth regulators on *in vitro* growth of the pathogenic fungus *Sclerotinia sclerotiorum* and on white mold disease that is caused by the pathogen was investigated. Naphthalene acetic acid at concentrations of 200-400 µg/ml inhibited the fungal growth in culture and reduced white mold severity on bean and cucumber plants. Gibberellic acid at concentrations of 50-250 µg/ml promoted both mycelium growth and white mold disease severity on plants. Methyl jasmonate at concentrations of 75-250 µg/ml inhibited mycelium growth in culture and suppressed bean and cucumber white mold. Absiscic acid at concentrations of 100-300 µg/ml decreased mycelium growth but promoted disease development on bean and cucumber plants. Ethylene released from ethephon (200-600 µg/ml) increased bean and cucumber white mold severity. *S. sclerotiorum* has the capacity to produce ethylene in culture. Ethylene production by *S. sclerotiorum* reached peak (400 µl/g/h) after 6 days of incubation followed by a decline to 155 µl/g/h after 10 days. The ethylene biosynthesis inhibitor aminoethoxvinyleglycine (AVG) suppressed white mold severity on bean and cucumber plants at concentrations up to 300 µg/ml. The results demonstrate a variable effect of plant hormones on the development of white mold. The changes in disease development may be due to both the effect of the plant hormones on the susceptibility of the plant to infection as well as due to a direct effect on the fungus.

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90 - Differential effect of auxin-compounds produced by two *Pythium* species with different pathogenicity

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Pythium ability to produce phytohormones-like compounds has been frequently assumed to be involved in plant *Pythium* relationship. This study shows that within *Pythium* genus, two fungi: *P. oligandrum* and *Pythium* group F, exert different effects on plant growth; both of them produce auxin compounds through the tryptamine pathway under the same cultural conditions. Indeed, the ability of *Pythium* group F to produce indole-3-acetic acid (IAA) in the immediate vicinity of roots had no positive effect on plant development. On the contrary, *P. oligandrum* production of tryptamine (TNH_2) was associated with increased plant growth. Although analysis revealed marked differences in auxin-compounds produced by both *Pythium* in the plant nutrient solution, their differential influence on tomato growth likely results from different fungal-plant relationships. *Pythium* group F is a minor pathogen: it causes symptomless infections, and then yield losses in tomato soilless cultures; its hydrolytic enzymes, have a destructive, though limited, impact on the root cell-walls of the outer cortical area. So, one may hypothesise that the effect of auxins on already damaged tissues dramatically disturbs the host physiology. As a consequence, abnormal root swellings and irregular plant development was observed. On the contrary, *P. oligandrum*, increased root development by inducing neither damages nor disturbance of TNH_2 absorption by roots, tryptamine has a positive impact on plant development.

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91 - Use of molecular biological techniques to directly monitor fungi with laccase activities in different soil compartments

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Fungal oxidative exo-enzymes without substrate specificity play a central role in the cycling of organic matter in soils. Due to their broad ecological impact and their favorable gene structure, i.e. four highly conserved regions encoding copper binding sites, laccases appeared to be the best appropriate enzymes to develop a technique to monitor fungi with an oxidative potential in soils without taking them into culture. Several degenerated primers, specific for basidiomycetes, were designed to perform single and nested PCR and assessed on DNA extracts from pure cultures. A single PCR allowing amplification of laccase gene fragments of about 200 bp gave highest success rates and was chosen for an application on two forest and one agricultural soils. Diverse fragments of around 140 and 200 bp were amplified and the polymorphism revealed by analyzing their sequence indicated that the method allows to monitor laccase genes from a broad spectrum of basidiomycetes. The analyses also showed a certain specificity of the distribution of fungal laccase genes in the soils and within the horizons, which probably reflects a specific distribution of corresponding fungal species. For the forest soil horizons, the higher diversity of laccase genes appeared in the litter which contained mainly 140 bp fragments and decreased with the depth. This work illustrates the potential of molecular biological tools to screen fungal genes with a precise function in complex environment compartments.

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92 - Phosphate uptake by communities of arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal fungi (AMF) constitute an important component of ecosystems by their ability to influence nutrient capture and structure of plant communities. The crucial role of the root-external mycelium in the soil-plant P transport by AMF has been unambiguously demonstrated by means of growth systems encompassing one or more mesh-enclosed soil compartments. The mesh excludes root growth, but allows for in-growth of hyphae and thereby for the direct measurement of hyphal uptake of P isotopes added to the root free soil. Examples will be given on the successful use of such experimental system to demonstrate the P uptake by communities of AMF in various field studies. There is increasing evidence for marked inter- and intraspecific differences between AMF in their rate and pattern of mycelial growth and in their effectiveness of P acquisition, but the relationship between the P uptake and the diversity of an AMF community is only poorly understood. Future research challenges within this area will be discussed, including the identification of the dominating components of AMF communities in roots and the subsequent test of the hypothesis that it is these dominating AMF, which are responsible for the major proportion of P uptake by the communities.

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93 - Determining mycorrhizal versus saprotrophic status of fungi by isotopic methods

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Mycorrhizal status of fungi is often difficult to determine because of difficulties in culturing functional symbioses. In addition, the possibility of facultative mycotrophy means that mycorrhizal status may vary in different field situations. Recent studies indicate that isotopic patterns of nitrogen (N-15), carbon (C-13), and C-14 (radiocarbon) often distinguish between mycorrhizal and saprotrophic fungi, and may therefore be a potent new tool to examine fungal functioning in situ. N-15 patterns in mycorrhizal fungi are correlated with proteolytic capabilities and N dynamics between fungi and plant hosts. C-13 patterns in mycorrhizal fungi appear to indicate source C and have therefore been used to determine plant hosts in mycorrhizal fungi of broad host specificity. Saprotrophic fungi are consistently enriched in C-13 relative to source C in litter and wood, suggesting that saprotrophic fungi primarily incorporate carbohydrate-derived C (C-13 enriched), with little incorporation of lignin-derived C (C-13 depleted). Radiocarbon indicates the age of fungally-assimilated C with a precision of about 1 year, and can therefore readily distinguish between mycorrhizal fungi (relying almost exclusively on current photosynthate) and saprotrophic fungi (relying on litter and wood of a range of ages). Continued improvement in our ability to interpret isotopic patterns in fungi will require culture studies to better understand C and N isotope fractionation during metabolism.

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94 - Weathering, a new role of ectomycorrhizal fungi in ecosystems

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Ectomycorrhizal fungi play a major role in the nutrient acquisition of trees. Mycelial uptake and transport of dissolved nutrients as well as fungal access to organic N and P sources have received much attention. Recent research suggests a third role of ectomycorrhizal fungi: mobilisation of P and other essential plant nutrients directly from minerals through excretion of organic acids. The mobilised cations are subsequently translocated to the ectomycorrhizal trees and thus by-pass the soil exchange complex. The primary source of all essential plant nutrients except nitrogen is the weathering of minerals. Low molecular weight organic acids excreted by plant roots and microorganisms are considered to be the most important biological weathering agents in soils. Commonly released into their environment by different fungal species are oxalic and citric acid, both strong chelators of trivalent metals such as Al3+. As the concentration of Al3+ in the soil solution diminishes through chelation, weathering rates of Al silicates such as feldspars increase. The awareness that ectomycorrhizal fungi contribute to this process has increased since the discovery of hypha-shaped tunnels inside mineral grains. The contribution of ectomycorrhizas to mineral weathering may change traditional ideas on nutrient cycling and soil formation in forest systems. Latest results on measurements of fungal weathering activities will be presented and ecological implications will be discussed.

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95 - Decomposition of humic substances, lignin and organopollutants by soil-litter decomposing basidiomycetes

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Humic substances (HS) are ubiquitous nonliving organic materials in all terrestrial and aquatic environments, and represent the major fraction of organic matter in soils and sediments. Consequently, microbial degradation of HS is essential for maintaining the carbon cycle. Despite this fact, only less is known about the particular microorganisms which decompose humic matter. Our main objective has been to examine the potential of litter-decomposing basidiomycetes colonizing forest soils to disintegrate HS and other recalcitrant compounds (lignin, organopollutants). Within the scope of several screening test, we have selected *Nematoloma frowardii*, *Collybia dryophila*, *Stropharia coronilla* and *Agrocybe praecox* as particularly active species degrading different HS, lignin and/or several organopollutants in liquid as well as in sterile and nonsterile solid-state cultures. These agaric fungi produce manganese peroxidase (MnP) as the predominant oxidative enzyme which is secreted into their microenvironment in response to high Mn^{2+} titers. In combination with certain low-molecular mass effectors, isolated MnP was found to attack recalcitrant aromatic substances unspecifically via one-electron abstractions giving rise to instable radicals, which tend to disintegrate spontaneously into polar fragments and CO_2.

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96 - Arbuscular mycorrhizal communites in rare and common *Pulsatilla* spp.: towards restoration of endangered plant species

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In the context of plant conservation it can be hypothesized that the presence of compatible arbuscular mycorrhizal fungi (AMF) directly contribute to the growth and survival of endangered plants. Here, we investigated a congeneric rare vs. common species pair, *Pulsatilla patens* and *P. pratensis*, in Estonian soils. Seeds were sown in soil inocula from four localities and germination, seedling establishment and root colonizing AMF species communities were assessed. A lack of soil treatment-related differences in *P. pratensis* germination and seedling establishment rates strongly contrasted with the significantly higher establishment rates of the rare *P. patens* in soil inoculum from a Scots pine forest where both target species co-exist in the understorey. Distinct communities of AMF species, identified following rDNA (SSU) sequence and phylogenetic analyses, were detected in roots of established plants and test seedlings grown in soil inocula from an agricultural landscape and a Scots pine forest. In seedling roots of both species exposed to forest soil inoculum, SSU sequences exhibited high homology to woodland *Glomus* sequences, earlier detected on *Hyacinthoides non-scripta* in a deciduous UK woodland site. The data provides support for the involvement of specific AMF in early seedling establishment of *Pulsatilla* species. Greater host species-linked dependency towards particular AMF may explain the loss of the rare *P. patens* in mycobiont limited disturbed or managed landscapes.

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97 - Net transfer of water between plants connected by common mycorrhizal networks

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Common mycorrhizal networks (CMNs) may play a major role in mediating water parasitism associated with hydraulic lift. Neighboring plants that share a CMN with the lifting individual may be able to derive the greatest benefit from this process. The ability of CMN to directly transfer significant amounts of water between plants was evaluated in the obligate mycorrhizal species coast live oak (*Quercus agrifolia*). Mycorrhizal (ecto- + arbuscular) oak seedlings were grown in microcosms comprising two adjacent compartments separated by air gaps and mesh barriers that could be crossed by hyphae but not by roots. Oak seedlings in one of the compartments had access to a taproot chamber with unlimited supply of water and conducted hydraulic lift. Following an 11 day drought period, during which watering was withdrawn from the upper two compartments, we detected a net transfer of water from the lifting oaks to neighboring plants through CMNs. *Quercus*, *Salvia* and *Eriogonum* seedlings in the adjacent compartment showed up to 20% higher leaf water content compared with their experimental counterparts with severed hyphal connections at the onset of the drought period. Isotopic data obtained after injecting deuterium-depleted tracers into the taproot chamber also indicated that neighboring droughted seedlings received up to 19% of their water from the lifter oaks through CMNs.

98 - Fungal biodiversity: What do we know? What can we predict?

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This years IMC comes roughly 10 years after published reassessments that greatly increased the estimated numbers of fungal species. We feel it timely to review these estimates based on a decade of data gathering and thinking on the topic. Each talk in the symposium will present the current status of known diversity for a major group of fungi with a geographical focus. Contributions will cover macro-, micro-, lichenized, aquatic, soil-inhabiting, and arthropod-associated fungi, plus a summary that integrates each of these presentations. Each talk will address the following questions for its fungal group: How many species are known worldwide? How accurate is this number? How is this group geographically distributed? Which geographic areas are well versus poorly known? What are the species overlap between different geographic regions? And what are the estimated centers of diversity? Thus, each presentation will function both as a meta-analysis of current data and a gap assessment to indicate where future research efforts should be concentrated. In addition to providing a summary of the state of our knowledge on fungal diversity, a goal of this symposium is to help inspire future work on fungal diversity and biogeography so that significant progress toward our understanding of these issues will be made by the next IMC.

99 - Global diversity and distribution of macrofungi

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We have compiled data on macrofungal diversity and distribution patterns for major geographical regions of the world. For this presentation, we have defined macrofungi to include ascomycetes and basidiomycetes with large, easily observed sporocarps that fruit epi- or hypogeously. Co-authors provided data on a particular taxonomic group or geographic area. We then employed a meta-data analysis to investigate species overlaps between areas, levels of endemism, centers of diversity, and estimated percent of species known for each geographic area for each taxonomic group and for the combined macrofungal data set. Thus, we provide both a meta-analysis of current data and a gap assessment to help identify research needs.

100 - Diversity of saprobic microfungi

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Recent studies in Australasia, Hong Kong, South Africa and Thailand have provided new data towards quantifying the magnitude of fungal diversity. High global fungal species numbers rely heavily on there being a relatively high ratio of unique fungi to host plant species. Data on host specificity for saprobic fungi is sparse, but a study of four New Zealand native plant genera; *Agathis* (1 species), *Metrosideros* (x taxa), *Nothofagus* (5 taxa) and *Rhopalostylis* (2 species) has found that 7 out of 10 saprobic microfungi described on *Agathis* are unique to *Agathis* in New Zealand, 0 of 2 are unique to *Metrosideros*, 16 of 20 are unique to *Nothofagus*, and 6 of 9 are unique to *Rhopalostylis*. Figures for fungi on Proteaceae and Restoniaceae in Cape Fynbos, S. Africa are also presented. Is this enough diversity to account for the estimated numbers of fungal species in the world? This paper will explore techniques available to assess microfungal numbers in a habitat or region and present results from some of the studies. It will concentrate on efforts to access whether saprobic microfungi are host or tissue specific and comment on reasons for these relationships. Databases can be used to establish the numbers of fungi on different plant hosts. As databases become more comprehensive it will be possible to examine the true extent of specificity amongst saprobic microfungi.

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101 - Biodiversity of soil-inhabiting fungi

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We count filamentous fungi living in soil layers but exclude those from the often more diverse litter. The Centraalbureau voor Schimmelcultures (CBS) preserves a great diversity of these fungi. The database contains 5768 isolates from soil or roots, distributed over appr. 2430 spp., including unpublished names and a few double counts for anamorph and teleomorph names. 2430 - (131 zoosporic + 89 macromycete basidiomycetes) = 2210 spp. of soil fungi (? 70% of those known). Basidiomycetes often account for the majority of fungal activity in a soil but are better assessed in mycorrhizal and wood/litter-decomposing inventories. Recently, the general increment in the numbers of soil fungi is only slightly accelerated, after CBS has broadened its accession policy and since the advent of molecular taxonomic tools. Particularly some poorly defined genera and species yield new taxa. Among thousands of isolates from any soil novelties will be found. Soil fungi spread easily and are regarded as cosmopolitans. Even in tropical forests many taxa are similar to those found in temperate latitudes. Molecular studies tell us that continental barriers determine a phylogeographical differentiation for slimy-spored species of *Fusarium* (now 138 in CBS) and *Trichoderma* (now 49 in CBS). These are predicted to increase in number of known taxa by not more than 100%. In the more ubiquitous genera *Penicillium* and *Aspergillus* (now ca. 300 and 200 spp.) an increment of about 100% is anticipated.

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102 - Global diversity and distribution of fungi in aquatic habitats

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We have compiled data on the diversity and distribution patterns of fungi and fungal-like straminipiles that occur in aquatic habitats ranging from freshwater to marine. Major groups included in this assessment are: Chytridiomycetes, Ascomycetes, Basidiomycetes, Hyphochytriomycetes and Oomycetes. Co-authors provided data on a particular taxonomic and/or ecological group. We then analyzed data to determine the total number of species, the number of species per habitat and/or substrate type, species overlap among habitats and substrates, and latitudinal and continental distribution patterns in diversity. We also estimated levels of endemism and centers of diversity for the various taxonomic/ecological groups.

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103 - Biodiversity of lichens

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Estimates for lichen diversity on the planet range between 13500 to 19000 species. A more precise estimate is hardly possible, for several reasons: First, the species concept of several lichen groups is still unsettled. Second, there are numerous unresolved taxonomical problems, especially in crustose lichens, and third, the lichen flora in several areas of the world - especially in the tropics - is still under-explored. However, there is already an enormous amount of data from all over the world, which needs to be compiled and validated to reach a better estimate of species diversity. Lichen information is already accessible on-line from several, heterogeneous sources (see e.g. e.g. ITALIC, http://dbiodbs.univ.trieste.it/, http://biobase.kfunigraz.ac.at/lichen/medlich2.html). To interconnect the different sources and to provide corresponding software tools is part of the ongoing project 'Global biodiversity of lichens' by T. Feuerer and G. Rambold (http://www.checklist.de/). Until the end of 2003, data from all 193 states and 300 adjacent geographical units will be available (For an experimental version see http://141.84.65.132/ChecklistsDe/Lichens/index.html). In support of this project, the International Association for Lichenology has established a Commitee, which coordinates and gathers lichen diversity information for the global checklist project.

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104 - Biodiversity of arthropod-associated fungi: What do we know? What can we predict?

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One of the greatest uncertainties in attempting to estimate global species richness of fungi is being able to draw inferences from the known number of taxa associated with hyperdiverse groups such as arthropods. Knowledge of the diversity and biogeography of these interactions is improving, but for many groups remains an imprecise foundation for extrapolation. Arthropods and fungi are closely associated in nearly all types of terrestrial, freshwater, and marine environments. Here we discuss only those fungi that use arthropods as a resource. Associations along the mutualist-pathogen continuum vary, and these as well as life history traits are discussed for each major group of fungi in relation to their known and potential diversity. We review the current state of knowledge regarding diversity, host relations, and biogeography of Laboulbeniales, Trichomycetes, Entomophthorales, *Septobasidium*, *Cordyceps*, and endosymbiotic yeasts. For the most diverse and one of the best biogeographically-studied groups, Laboulbeniales, patterns of diversity and host utilization at different geographical scales are analyzed. On the bases of these datasets, species discovery patterns and host associates, and the latest estimates of global insect species richness, we extrapolate as to the potential magnitude of the biodiversity of insect associated fungi and discuss ways in which the estimates may be refined. We also address the major lacunae that require coordinated effort from systematists.

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105 - Fungal diversity: Estimates, predictions and future challenges

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This presentation summarizes the data from the other presentations in this symposium. Based on that information, we present an estimate of the total number of fungal species currently known to science. We also make a conservative estimate of the number of fungi yet to be discovered. This is done by using data from geographic regions which are relatively well explored for fungi to estimate the number of new taxa yet to be found in poorly known areas. We also identify where there are gaps in our knowledge, both geographically and taxonomically.

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106 - Comparative methods for managing phylogenetic uncertainty

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Comparative analyses across species must be based upon good phylogenetic information to distinguish similarity that arises from common ancestry from that which arises from parallel or convergent evolution. This means that phylogenies are necessary for studying ancestral states, for calculating rates of evolution, and for investigating correlations among two or more traits. I will briefly describe these sorts of analyses, giving examples, then go on to describe a difficulty that arises in comparative studies. The difficulty is that phylogenies are seldom known with certainty and different phylogenies can give different answers to the comparative question. A relatively new technique in comparative studies make it possible to take phylogenetic uncertainty into account. Bayesian methods for inferring phylogenies, based upon Markov Chain Monte Carlo methods, allow the investigator to draw a random sample of phylogenetic trees, from the universe of possible trees. The comparative hypothesis can then be tested in each tree, in effect, removing phylogenetic uncertainty from the hypothesis. These methods are becoming increasingly easy and practical to implement on desktop computers. I give examples from several areas of research.

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107 - Major fungal lineages are derived from lichen symbiotic ancestors

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About one-fifth of all known extant fungal species form lichen symbioses. Lichens are widely believed to have arisen independently on multiple occasions, accounting for the high diversity and mixed occurrence of lichenized and non-lichenized (42% and 58%, respectively) fungal species within the Ascomycota. Depending on the taxonomic classification chosen, 15-18 orders of the Ascomycota include lichen-forming taxa, and 8-11 of these orders (representing about 60% of the Ascomycota species) contain both lichenized and non-lichenized species. Here we report a phylogenetic-comparative analysis of *RPB*2, and the small and large subunit of the nuclear rRNA genes from about 60 species representing about 75% of the species diversity of the Ascomycota, a phylum that includes > 98% of known lichenized fungal species. Using a Bayesian phylogenetic tree sampling methodology, combined with a statistical model of trait evolution, we take into account uncertainty about the phylogenetic tree and ancestral state reconstructions. Our results, based upon a sample of 19,900 phylogenetic trees, show that lichens evolved earlier than believed, and that gains of lichenization have been infrequent during Ascomycota evolution, but have been followed by multiple independent losses of the lichen symbiosis. As a consequence, major Ascomycota lineages (Ascosphaerales, Eurotiales, Onygenales and Chaetothyriales) of exclusively non-lichen-forming species are derived from lichen-forming ancestors.

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108 - Evolution of interkingdom host-jumping of *Cordyceps* and the Clavicipitaceae

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The genus *Cordyceps* includes over 300 described species of fungi that are pathogens of arthropods and fungi. It is a member of the Clavicipitaceae (Ascomycota, Hypocreales) which also includes symbionts of plants, mainly of the Poaceae. Current subfamilial classification of the Clavicipitaceae comprises the Clavicipitoideae, which includes all of the grass symbionts, and the Cordycipitoideae, which includes all of the pathogens of arthropods and fungi. This represents an evolutionary hypothesis that implicitly assumes inter-Kingdom host jumps are rare and irreversible. To test this hypothesis we initiated a molecular phylogenetic study of the Clavicipitaceae with a particular emphasis on *Cordyceps*. We have assembled a four-gene region dataset that includes data from the nuclear SSU and LSU rDNA, beta-tubulin, and elongation factor 1-alpha for 75 taxa. Phylogenetic analyses reject the monophyly of *Cordyceps* and the Cordycipitoideae and do not support host jumps as being rare and irreversible. Three clades of clavicipitaceous fungi, which all contained pathogens of animals and fungi, were resolved in these analyses. Pathogen of animals is resolved as the primitive symbiosis for the Clavicipitaceae with a single jump to the Poaceae that was most likely followed by a reversal to animals. Multiple jumps have occurred from animals to fungi with at least one reversal to animals. Character coding of host affiliation and testing character state reconstructions will be discussed.

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109 - Molecular phylogenetics and breeding systems in the ascomycete fungi

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By a combination of phylogenetic approaches, we are studying the evolution of breeding systems among fungi in the Ascomycota. Within the family Pleosporaceae, combined data from the glyceraldehyde-3-phosphate dehydrogenase gene and ribosomal ITS regions provide good resolution. Our phylogenetic analysis shows that the 'asexual' species and the selfing species are nested among outcrossing sexual species, indicating that outcrossing sexuality was the ancestral condition for the family. In sexual filamentous ascomycetes, opposite mating type information at the MAT1 locus regulates mating and the opposite mating type genes each have a clonal, non-recombining phylogenetic history. Many species in the Pleosporaceae have no known sexual states, but where tested, these supposedly asexual fungi have intact, functional mating type genes. We used PCR to amplify and sequence fragments of the opposite mating type genes from supposedly asexual species in the genus *Alternaria*, in the Pleosporaceae. Each haploid fungal isolate had just one mating type, but both mating types were present in each species. We sequenced the ribosomal ITS regions for isolates of opposite mating types, for three asexual species and four known related sexual species. Analysis of the probability of substitution patterns indicated that, if sexuality had been lost, it was lost after the most recent ITS substitutions had evolved in each species.

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110 - Trends in morphological evolution in homobasidiomycetes

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Homobasidiomycetes display an incredible diversity of fruiting body forms, ranging from simple corticioid forms, which lie flat on their substrates, to elaborate, developmentally integrated forms, such as stinkhorns. More than half of all described homobasidiomycetes have pileate-stipitate fruiting bodies, with gills or other configurations of the hymenophore. A functional (if not phylogenetic) distinction can be made between gasteromycetes, which lack ballistospory and produce spores internally, and hymenomycetes, which retain ballistospory and produce spores externally. Much of the research in higher-level homobasidiomycete systematics in the last century has focused on tracing patterns of evolution in fruiting body forms, and has revealed extensive convergence. Our recent analyses concern trends in the evolution of fruiting body morphology. Examples of the

questions that concern us are: 1) Are simple resupinate forms evolutionarily more labile than complex forms? 2) Is there a general trend toward the evolution of pileate-stipitate forms? 3) Can we reject the hypothesis that the loss of ballistospory is irreversible? To address these questions we are employing binary and multistate maximum likelihood methods to develop and test models of fruiting body evolution, using a large (ca. 500 species) phylogenetic tree derived from rDNA sequences.

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111 - Concepts and approaches during 50 years of biological control of fungal plant pathogens

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There are now more than 80 products near to or on the market that have biological control activity against plant pathogens in soil, root, aerial and post-harvest environments. Most of these been developed relatively recently in response to environmental concerns which have forced reductions of fungicide and fumigant use and availability. Bacterial products are dominated by *Pseudomonas*, *Burholderia* and *Bacillus* species and fungal products by *Gliocladium* and *Trichoderma* species. With the exception of products based on *Agrobacterium radiobacter* for control of crown gall, *Phlebiopsis gigantea* for control of stem and root rot of pine and some *Trichoderma*-based products, few have been on the market for 10-20 years or more. Indeed, several have come and gone. Key features for achieving a successful biocontrol product must be cost-effective and reproducible disease control. In the last 15 years, the importance of understanding the ecological interactions between a biocontrol agent, its target pathogen and host plant before this can be achieved has gradually become appreciated. Against this background, several successful biocontrol agents have been selected, screened for activity, characterised and then undergone registration and marketing. Reassessment of the concepts and processes involved in natural disease suppression has also provided novel biological approaches to disease control.

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112 - Biological control of fungal plant pathogens-recent advances and future perspectives

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Two different approaches have been described to control plant pathogens: either introduce a selected biological control agent or enhance naturally occurring biological control. The first approach has been the most commonly followed during the 30 last years. Most emphasis was devoted to the study of the modes of actions of selected antagonists and less to the study of the conditions required for successful application. More recently, the development of molecular tools led to the creation of transformed biological agents possessing several modes of action, or having other beneficial traits such as a greater ability to survive or colonize the target. These 'improved strains' are supposed to possess an enhanced biological control activity, but demonstrations in nature of these advantages are still missing. Biological control of fungal pathogens remains very limited due to the narrow specificity of the biological control agents and the inconsistency of their efficacy. Today there is a renewed interest for studying the effects of organic matter and compost amendment on the soil inoculum potential, with the objective of enhancing the natural potential of suppressiveness that exist in any soil. Whatever the method used, biological control should be part of an integrated pest management strategy. Research in this field requires an holistic approach taking into account not only the microbial interactions and the plant defense reactions but also the whole agronomical system.

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113 - Premier bacteria and fungal plant disease suppression

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Take-all, caused by *Gaeumannomyces graminis* var. *tritici*, is an important root disease of wheat worldwide. Take-all decline (TAD) is the spontaneous decrease in the incidence and severity of take-all that occurs with monoculture of wheat or other susceptible host crops after one or more severe outbreaks of the disease. In Washington State, USA, TAD develops in wheat fields because of the build up of strains of *Pseudomonas fluorescens*, which produce the antifungal metabolite 2,4-diacetylphloroglucinol (DAPG). DAPG producers are abundant in other suppressive soils. Whole-cell repetitive sequence-based (rep)-PCR with the BOXA1R primer distinguished 17 different genotypes (A through N) within worldwide collections of DAPG producers. Genotype D, which is primarily responsible for TAD in Washington soils, aggressively colonizes wheat and barley and suppresses take-all when applied at very low doses (as few as 10^2 per seed or gram of soil). Field studies have verified that introduced populations of genotype D strains are sustained throughout the growing season, survive between crops, and reestablish on the roots in successive years above the threshold required for suppression of take-all.

114 - Risk assessment of fungal biocontrol agents - How can RAFBCA help?

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Fungal biocontrol agents (BCAs) secrete a wide range of metabolites. Many have been shown to play an important role in pathogenesis-antagonism and the successful control of the target organism. Current registration procedures for products that purport biocontrol of plant pests and diseases require risk characterization and evaluation of known, and potential exposures to human health and nontarget organisms in the environment. Evaluation of all the metabolites produced by BCAs would be expensive and time-consuming. One of the objectives of the EU-funded RAFBCA project is to develop the methods and tools to assess the risks of fungal BCA metabolites. This paper will describe: (1) some of the methods and tools being developed for high throughput analysis such as biosensors, and microtitre plate assays and (2) studies that show that the quantities of secondary metabolites produced by fungi in-vivo are usually much less than those secreted in nutrient rich liquid media. Additionally, information on intra-species and inter-species variability in the production of selected metabolites will be provided, as well as data on the spatial-temporal distribution of fungal metabolites. Furthermore, strategies will be presented which could standardise the risk assessment of fungal BCAs.

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115 - Molecular approaches to studying fungal interactions in relation to biocontrol

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Trichoderma-based biofungicides are a reality in commercial agriculture, although they represent only a niche market in comparison to that of chemicals. However, more than a dozen formulations are available today as registered products in the USA and Europe, and the application of these biocontrol agents, both as biopesticides or biofertilizers, is steadily increasing also in organic farming. The modes of action of these beneficial fungi are many and very complex. Therefore several research strategies, also at a molecular and gene level, have been applied to identify the main genes and compounds involved in this complex, three-way interaction among the antagonist, plant and pathogen. We have used different reporter systems and genetic manipulation to identify molecules that are involved in the cross-talk between *Trichoderma* and its host, and have found opportunities for developing new disease control methods. In addition, we have been able to monitor the interaction in vivo and in situ between *Trichoderma* and its host and correlate the occurrence of mycoparasitism to the biocontrol effect.

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116 - Weed biocontrol by fungal plant pathogens - research approaches and practical application

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Weeds are among the major pest constraints for many human activities, and their management represents the major cost of agriculture. Pathogens that infect weeds have been considered to partially replace traditional control methods, using two main approaches, the classic and the inundative method, but their success has been quite limited. The best results are mainly against alien weeds of pasture or forest, but also some mycoherbicides are available as commercial products. In despite of the partial success, in the last years some argumentations have further risen the interest in weed biocontrol, e.g.: increased consumption of organic products, ban of dangerous herbicides, lack of registered herbicides for niche crops, forbidden use of chemicals in anthropical environments (parks, archeological sites), resistance. To not disappoint these expectations, more efficacious agents are needed. Different strategies could be used to enhance effectiveness of fungal pathogens, including formulations, distribution systems (precision agriculture), selection of better characters and genetic enhancement, or integrated approaches, as mixing fungal pathogens, combining bacteria or bioactive metabolites with fungal pathogens, or managing traditional and biological methods. The presentation will focus mainly on the different strategies of weed biocontrol using fungal plant pathogens and will discuss the possibilities to improve the herbicidal properties of weed pathogens.

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117 - Xerophilic fungi, a physiological wonder

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Xerophilic fungi are defined as those that can grow below a water activity of 0.85, which means the ability to grow in 60% glucose, 45% glycerol or 20% NaCl - all (w/w). Few organisms on earth, other than halophilic bacteria, are able to grow under these conditions. To grow at reduced water activities, microorganisms must balance the external

environment by an equivalent water activity inside, as growth requires turgor. Bacteria concentrate K+ ions and certain amino acids to achieve this, while fungi have evolved the ability to use polyols - glucose, mannitol and in the limit glycerol - as the internal solute. At its limit, *Xeromyces bisporus*, the most xerophilic known organism, is able to grow at a water activity of 0.65, equivalent to a solution containing 65% (w/w) glycerol. As *X. bisporus* is known to accumulate glycerol to balance the outside environment, that means that life processes, including all enzyme functions, must be carried out in a thick glycerol syrup. *X. bisporus* and the yeast *Zygosaccharomyces rouxii* are capable of sexual reproduction at 0.7 water activity, i.e. in 60% (w/w) glycerol. A few of these fungi are able to grow in very concentrated NaCl solutions, but use the same internal solute system, so are correctly classified as halophilic xerophiles. These remarkable fungi, a number of genera and species, are all Ascomycetes, and may only have evolved once. Some further details of these fascinating fungi will be provided in this paper.

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118 - Molecular ecology of anaerobic fungi (Anaerobic fungi-do we know them all?)

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Anaerobic fungi are important components of the rumen ecosystem but their physiology and enzymology have attracted more interest than their ecology. As such, information relating to the mechanisms and dynamics of spread between hosts and niche differentiation between species remain unanswered. It is not clear how many species may coexist in a single host animal, nor whether the various species differ in their patterns of substrate colonisation. We have conducted detailed analysis of fungal populations from rumen digesta and faeces of silage-fed cows, and have investigated the effect of environmental conditions on survival of these fungi in faeces. Using the MPN technique with various substrates as an enrichment source, quite different fungal taxa were isolated. For instance, *Caecomyces* spp. were more abundant on cellobiose. In the course of this work we also isolated and characterised a new genus of anaerobic fungus *Cyllamyces* (type species *C. aberensis*). Difficulties in reliable morphological identification led us to use PCR-RFLP of the ITS region of the rRNA locus as a tool for identifying fungi isolated from MPN tubes. Sequence analysis of these regions was used to validate restriction enzyme patterns. Ambiguous RFLP patterns were found to be attributable to between-repeat polymorphisms within the ITS region of single isolates. ITS2 sequences from a single isolate diverged by 5% or more and there was more variability between repeats than between quite unrelated isolates.

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119 - Psychrophilic fungi - do they really exist?

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Many food-borne Penicillia and some soil-borne species grow well at low temperatures (0-5 °C) but most of these species also grow quite well at 30 °C and could thus be regarded as psychrotrophic. Isolates from Svalbard and Antarctis have also been known species that were psychrotrophic and well known. The only new species of *Penicillium* described from Antarctis, *P. antarcticum*, is psychrotrophic to mesophilic. Some psychrophilic lichens and anamorphic ascomycetes like *Humicola marvinii* with optimum temperatures at 15 °C have been reported. A rather large number of psychrotrophic Penicillia have been isolated from Alpine or polar regions and some of them can be regarded as real psychrofiles. These fungi hardly grows (or do not grow at all) at 25 °C, and have an optimum temperature for growth near 15 °C. In few samples from Zachenberg in Greenland all Penicillia appeared to be new species, except one known taxon *P. nordicum*, known from refrigerated meat in Europe. Taxonomically the new species belonged to subgenus *Furcatum* and have some resemblance to common soil-borne species in series *Canescentia*. All species produced a surprisingly large number of bioactive secondary metabolites, including different combinations of kojic acid, penicillic acid, griseofulvin, chaetoglobosins and tryptoquivalins. Nearly all species produced some closely related cyclic peptides, and we hypothesise that these peptides are involved in adaptation to cold temperatures.

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120 - Halophilic fungi - are they widespread in the fungal kingdom?

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Recently the novel observation was made that fungi, representing the only kingdom not so far known to sustain extremely saline natural conditions, populate manmade eutrophic and oligotrophic salterns, saturated or nearly saturated with NaCl. The majority of isolates from all sampled eutrophic salterns were black meristematic yeast like fungi: *Hortaea werneckii*, *Phaeotheca triangularis*, *Aureobasidium pullulans* and *Trimmatostroma salinum*. They were lacking in the oligotrophic ones, where saprophytic members of the genus *Cladosporium* prevail.

These species are all closely related, belonging to the single order Dothideales. A large number of g. *Cladosporium* strains, along with reference strains of frequently isolated species were sequenced and their morphology and physiology are currently studied. The resulting clusters contain ubiquitous isolates originating from different extreme environments and at considerable phylogenetic distance away a separate branch, consisting of saltern isolates. From both types of hypersaline environments, xerophilic nonmelanized filamentous fungi, known primarily as food contaminants were also frequently isolated: *Wallemia sebi*, *Penicillium* and *Aspergillus* with teleomorphic stage *Eurotium*. Further analyses of these isolates show in some cases distinct differences in xerophily, contrary to the general belief, that with fungi it is determined primarily by the water activities of the culture media and not by the chemical nature of the solute.

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121 - Resolving differences between thermophilic and mesophilic *Paecilomyces*

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Paecilomyces Bainier was erected for the thermotolerant *Paecilomyces varioti*. Brown & Smith (1957) broadened the concept of the genus to include mesophilic species off insects. Samson (1974) provided a modern morphology-based treatment of the genus. With few, plastic morphological features available for study confident identification of many *Paecilomyces* is problematic. The availability of molecular phylogenetics provides the opportunity to combine morphology and sequences to more fully resolve differences at the genus/species level. Isolates from the Samson Monograph were sequenced (18S, ITS and â-tubulin gene) to compare mesophilic and thermophilic *Paecilomyces*. Thermophilic section *Paecilomyces* form a separate clade to the mesophilic section *Isarioidea*. This work accepts the placement of section *Paecilomyces* in the Eurotiales and the placement of section *Isarioidea* in the Hypocreales. *Paecilomyces varioti* forms four clades and is apparently a cryptic species. Two of these clades have a *Byssochlamys* teleomorph. Evidence suggests that the thermophilic *Talaromyces*/*Penicillium* may be derived from a thermophilic *Byssochlamys*/*Paecilomyces* ancestor.

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122 - Enzyme discovery from extremophilic fungi

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Discovery of enzymes from fungi living in extreme environments has primarily focused on thermophiles. Among the thermophilic fungi very rich enzyme producers have been found and also industrially exploited, e.g. from *Humicola* and *Scytalidium*. However, recent discovery efforts in our group have shown that much more is still to be found! The presentation will include data on new enzymes found from thermophiles (e.g. from *Trichophaea saccata*) but also new data of enzyme discoveries from other extreme environments as the gut flora of the termite larvae and the Iberian snail, from psychrophilic fungi, from xerophiles (as *Talaromyces*) and from alakali tolerant *Stilbella*. The presentation will further cover suggestions to new molecular methods of discovery from fungi from extreme environments, building on the rapidly rising amount of fungal sequence information available.

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123 - Lichen 'oases' in the Namib Desert

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Lichenized filamentous fungi dominate large areas of the coastal Namib Desert, the most arid region in Southern Africa. The existence of these lichen 'fields' is due to coastal fog depositing sufficient water for lichen growth. The most well-developed lichen communities are dominated by the fruticose lichen *Teloschistes capensis* which in this region is at the northernmost part of its range. While fog events occur along much of the Namibian coast, *Teloschistes* fields are very localized. The most extensive occurrence of *T. capensis* is inland from Cape Cross, the location of one the world's largest seal (Cape fur seal) colonies. We show that NH_3 emitted from the seal colony can be detected over the desert from 32 $\mu g\ m^{-3}$ at 250m from the colony to 0.2 and 0.02 $\mu g\ m^{-3}$ at 3.6 and 26 km, respectively. The NH_3 is enriched in ^{15}N, and δ^{15} N in *T. capensis* declines from +3.6‰ at 2.1 km from the colony to -5.5 and -7.2‰ at 11 and 26 km, respectively. Uptake of NH_4^+ from simulated fogwater can be demonstrated under laboratory conditions and this is greatly enhanced by the addition of PO_4^{3-}. Chemical analysis of fogwater revealed that the seal colony is also a source of PO_4^{3-} aerosol in addition to NH_3. Anecdotal evidence suggests that many members of the Teloschistales have an ecological preference for eutrophicated habitats and we hypothesise that NH_3 and/or PO_4^{3-} output from the seal colony has promoted the development of *Teloschistes* fields in this area.

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124 - Fungi and lichen: microbial ecology in Antarctic rock desert

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Fungi and prolichens account for a substantial proportion of the endolithic biomass of rock. To explore the development and survival of epilithic and endolithic fungi, visualisation technologies have to be stretched to their full potential. These techniques have enabled the observation of biologically transformed minerals in rocks from Antarctica, including diagenetic iron hydroxide nanocrystals and clays around chasmoendolithic fungi in sandstone, and calcium oxalate and silica deposits close to fungal cells in granite. As for any ecosystem, the study of the rock microhabitat, requires previous knowledge of the components and the processes that take place within it. The structure and function of each component of the lithic microecosystem needs to be established by quantifying and identifying the fungi present in each lithobiontic niche and defining the mineralogical features of these hidden microhabitats. Once we have selected the techniques to observe these components *in situ* and locate the presence of water, the questions that need to be addressed are: how are the fungi organised in the fissures and cavities, which fungi are present and how many are there, what water relationships are there and what effects do fungi have on the substrate's minerals? This last question is crucial, since mechanical and chemical changes in minerals and mineralisation of fungal cells can lead to physical and/or chemical traces (biomarkers), even after the death of the microorganism.

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125 - Exploring the transcriptome of the ectomycorrhizal symbiosis

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Studies of the ectomycorrhizal symbiosis have highlighted the role of transcriptional regulation in controlling the morphological stages of symbiosis differentiation. To examine gene-activity changes associated with the development of the ectomycorrhizal symbiosis, we have performed expression profiling using poplar, eucalypt and Pisolithus cDNA arrays. A marked change in the gene expression in the mycobiont and the host-plants was observed at multiple levels: (a) a general activation of the protein synthesis machinery probably supporting an intense cell division/proliferation, (b) an increased accumulation of transcripts coding for cell wall proteins in hyphae and roots probably involved in the symbiotic interface formation, and (c) the upregulation of energy metabolism in colonised roots. This data suggests a highly dynamic environment in which symbionts are sending and receiving signals, are exposed to high levels of stress conditions and are remodeling their tissues. With multiple EST/cDNA array programmes dealing with ectomycorrhizal associations, we will have in a near future an unparalleled opportunity to ask which genetic features are responsible for common/divergent traits involved in this symbiosis. A few of the many possible breakthroughts will be in characterisation of common transcriptional and transduction networks and new insights into unique metabolic routes critical for mycorrhiza functioning.

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126 - Responses of mycorrhizal fungi to heavy metals: a cellular and molecular investigation

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Within the fungal kingdom mycorrhizal fungi represent direct links between plants and soil and are often needed to ensure plant survival in heavy metals polluted areas. A sterile mycelium PSIV, an ascomycete establishing ericoid mycorrhiza, was used to understand how zinc ions affect the cellular mechanisms of fungal growth. Chitin, the most characteristic shape-determinant of fungal wall, was quantified and located by specific probes. Conspicuous changes in hyphal morphology were observed in zinc-treated mycelium, where hyphal walls were thicker and chitin labelling was more intense. Chitin synthase genes belonging to two classes were differentially expressed. Morever, molecular mechanisms leading to protection against heavy metals were investigated in an arbuscular mycorrhizal fungus (*Gigaspora margarita* BEG34) and led to evidence of a structurally novel metallothionein. The gene, designated *GmarMT1*, encodes a functional polypeptide capable of conferring increased tolerance against cadmium and copper as revealed by complementation assays in yeast. The *GmarMT1* RNA is expressed in both presymbiotic spores and symbiotic mycelia even in the absence of metal exposure, but it is significantly less abundant in the latter stage. An opposite pattern was observed upon copper exposure, which upregulated *GmarMT1* expression in symbiotic mycelia, but not in germinated spores. Part of this work is supported by the European Project GENOMYCA (QLK5-CT-2000-01319).

127 - Dissecting carbon metabolism in AM fungi: Implications for symbiosis and biotrophy

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The obligate biotrophic nature of arbuscular mycorrhizal fungi (AMF) has long been known but never overcome, making all efforts of culturing these organisms continuously under axenic conditions unsuccessful. This has considerably limited progress in basic research and biotechnological strategies for the large scale production of AM fungal inoculum. All cytochemical, biochemical, metabolic and genome investigations carried out the last 25 years indicated that AMF resemble saprophytes (e.g. genome size, metabolic capabilities, capacities for DNA, RNA and protein synthesis, etc.), however we are still unable to make these fungi complete their life cycle in the absence of a suitable host plant. One of the reasons proposed for this failure is the existence of a metabolic lack in their C metabolism. However, neither assays of involved enzymatic activities revealed the origin of such a metabolic blockage, nor did any of the numerous C sources assayed in synthetic media induce the fungus to fulfil its life cycle axenically. In recent years the application of a range of experimental techniques including AM monoxenic cultures, molecular biological methods, NMR spectroscopy and in vivo microscopy has greatly contributed to our understanding of C metabolism in AMF, both under asymbiotic and symbiotic conditions. The results have shed some light in the difficult, but fascinating question of the obligate biotrophic nature of AM fungi.

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128 - Carbohydrate and nitrogen dependent gene-regulation in the ectomycorrhizal fungus *Amanita muscaria*

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Carbohydrate and nitrogen support has a profound impact on gene regulation and fungal physiology in saprophytic ascomycetes. As saprophytic fungi, ectomycorrhizal fungi live in environments with a different carbohydrate and nitrogen support (e.g. extramatrical hyphae versus mycorrhizal hyphae). To understand ectomycorrhizal fungal physiology in soil and the symbiotic structure, the effect of carbohydrate and nitrogen nutrition on fungal gene expression is of special interest. Northern blot analysis was carried out for selected genes of the ectomycorrhizal fungus *A. muscaria*. The fungus was grown in liquid culture at different carbohydrate and nitrogen conditions as well as in ectomycorrhizas. Genes investigated so far encode a monosaccharide transporter, a phenylalanine ammonium lyase, an excreted protease and an amino acid transporter. These studies revealed an interconnection between carbohydrate and nitrogen dependent regulation of gene expression in *A. muscaria* sharing homologies but also some differences to ascomycetes. Ectomycorrhizal hyphae are not uniform but consists of two 'fungal networks', the Hartig net that is attached to root cortical cells (forming together the plant/fungus interface), and hyphae that are ensheating the infected root. Since both structures have different functions with regard to carbohydrate and nitrogen uptake, storage and partitioning, we also compared the expression of fungal genes in both hyphal networks.

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129 - Functional genomics of fungal-plant communication in the arbuscular mycorrhizal symbiosis

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Fossil data indicate that arbuscular mycorrhizal (AM) fungi already colonized early land plants so that AM formation probably belongs to one of the earliest developmental programs plants evolved. At the same time, rRNA sequence analysis has shown that AM fungi form a monophyletic group (Glomeromycota) of which little is known about the biology of the possible progenitor. Analyses of the symbiotic programme in AM interactions have targeted fungal genes involved in cell wall synthesis, nutrient metabolism or membrane transport, and plant genes associated mainly with interactions with pathogenic organisms or symbiotic rhizobia. Several non-targeted approaches have also been used to further identify genes involved in AM formation and/or function but very limited information has been gained so far about fungal gene expression. This has prompted us to adopt alternative strategies of transcriptome analysis in a more extensive search for genes involved in different stages of AM development, based on suppressive subtractive hybridisation and large scale cDNA sequencing. Expression profiling has identified ESTs which correspond to fungal genes that are induced by host root exudates in pre-symbiotic phases or in mycelium during symbiotic root interactions. Such global approaches of functional genomics of AM will contribute to a better molecular understanding of fungal-plant communication in these widespread symbiotic associations and of how they promote plant vigour.

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130 - Mycorrhiza-bacteria interactions and functioning in boreal forest soils

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Boreal forest trees are highly dependent on root symbiotic ectomycorrhizal (ECM) fungi for growth in low pH, lignin-rich podzolic soils. In Scots pine seedling microcosm studies, extensive mycorrhizal fungal colonization of the constituent upper humus (O) and underlying mineral (E and B) horizons was visualised and horizon-specific ECM identified following ITS-RFLP/sequence phylogenetics. Soil N and P mobilisation and uptake was confirmed in analyses of mycorrhizosphere compartment-specific gene and/or enzyme expression and linked to organic acid production. Bacterial biofilms in O horizon mycorrhizospheres were shown to be mainly comprised of forest soil specific gram -ve bacteria, *Bacillus* spp. and Crenarchaea based on carbon profiling and 16S sequence phylogenetics. Preferential organic acid utilisation by bacteria in the external mycorrhizosphere highlights mycorrhizal fungal involvement in weathering of E and B horizon minerals. Mycorrhizospheres developed in the O horizon did not host *Pseudomonas fluorescens* which was further confirmed in the rapid loss of marker gene tagged *P. fluorescens* introduced into mycorrhizal silver birch root systems. However, Scots pine mycorrhizospheres developed on petroleum contaminated soils do support catabolic plasmid harbouring fluorescent pseudomonads and increased hydrocarbon oxidation activity. Based on these data it is hypothesised that, in functional terms, the mycorrhizosphere behaves as an external rumen/gut of the host tree.

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131 - The *Paxillus involutus* / *Betula pendula* symbiosis: Gene expression in ectomycorrhizal root tissue

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Ectomycorrhizas (ECM) are symbiotic associations formed between plants and soil fungi. To identify genes and metabolic pathways specifically expressed in the mycorrhizal root tissue, 3,555 Expressed Sequence Tags (ESTs) were analyzed in a cDNA library constructed from ECM formed between the basidiomycete *Paxillus involutus* and *Betula pendula* (birch). In parallel, cDNA libraries from saprophytically growing fungus (3,964 ESTs) and from axenic plants (2,532 ESTs) were analyzed. By assembly of all ESTs (10,051), 2,284 contigs were identified, each representing a unique transcripts of either fungal or plant origin. Of those, 650 contigs (28%) were uniquely expressed in the mycorrhizal tissue. Based on homology to sequence information in the GenBank (nr) protein database a majority of contigs could be assigned putative functional and metabolic roles. By comparing redundancies between libraries, transcripts related to protein synthesis were found down-regulated, whereas transcripts related to cell rescue, defense, cell death and ageing were up-regulated in the mycorrhizal root tissues as compared to the free-living fungus. Furthermore, the mycorrhizal root tissue displayed an up-regulation in transcripts related to nucleotide metabolism and carbon utilization as compared to the saprophytically growing fungus, whereas transcripts related to amino-acid metabolism and lipid, fatty-acid and isoprenoid metabolism were down-regulated.

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132 - Nutrient-regulated expression of functionally diverse surface proteins in truffles

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Inorganic nitrogen is often the most limiting nutrient in the rhizosphere and N-availability is one of the environmental cues that influence ectomycorrhizae formation. This presentation will focus on four distinct, N-status-regulated surface protein genes from *Tuber borchii*. Two of them code for transmembrane proteins involved in inorganic-N internalization that resemble related transporters from other (symbiotic and non-symbiotic) fungi. They specifically respond to N-shortage, but do so at a surprisingly slow rate. Much more intense responses (to both N and C starvation) were measured for the genes encoding two other Surface Proteins, which harbor secretion signal peptides at their N-termini, are both loosely associated to the cell wall, and bear only a very restricted (TbSP1) or no (TbSP2) resemblance to polypeptide sequences found in databases. The TbSP2 gene, which contains starvation stress response elements in its promoter, codes for a cysteine-rich, 11 kDa structural protein of as yet unknown function. The product of the TbSP1 gene, instead, is a calcium-activated phospholipase A2 that is both secreted and cell wall-associated in pre-symbiotic mycelia, but also accumulates in symbiosis-engaged hyphae as well as in fruibodies. Generalized surface remodeling and lipid-mediated events thus appear to predominate in the *Tuber* response to nutrient shortage. The results of ongoing experiments aimed to understand the physiological significance of such events will be discussed.

133 - Rarity of mushroom species, a numerical approach

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Abundances and yearly frequencies of mushroom species are tightly correlated in the data sets on a forest plot in Switzerland and on several forest and grasland plots in Austria. The geographic range of species seems correlated with abundance and yearly frequency. Species that score low on the three parameters can be called rare. None of the plots studied for 21 and 7 years, respectively, seems to have shown its full species richness. Many (?) rare species may thus have gone unnoticed. Rare species run the risk of extinction. Therefore rare species need to be considered for a 'red list' (according to IUCN criteria). The red lists of the Netherlands and Germany were evaluated for the presence of rare species. The fungal biota of both countries are well studied. The evaluation was done by comparing the red lists with distribution data. Distribution data of the Netherlands have been published (Arnolds, Dam & Dam-Elings, 1995). German distribution data were estimated on a basic estimate that the whole country holds 6000 species and on the basis of distribution data of higher plants, mosses, birds, mammals, beetles and butterflies. The impression is that very rare mushroom species are underrepresented on the red lists of both countries.

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134 - Diversity of soil fungi in different Namibian biomes

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Within the framework of the BIOTA project (Biodiversity Monitoring Transect Analysis in Africa) the biodiversity of soil fungi is the aim of this survey. The scientists of the frame project (Southern Africa) study the influence of climatic changes and management systems on different organisms along the rainfall gradient spanning from the Cape of Good Hope to the Angolan border. This presentation concentrates on results of Namibia, where sampling started in 2001. Soil samples were taken in different biomes of Namibia: dry forest, thorn bush savannah, Nama Karoo and the Namib. The soil fungi were isolated by the soil washing method. Most cultures belong to the Fungi Imperfecti (Hypho- and Coelomycetes), not so many are Asco- or Zygomycetes. Only a few Oomycetes and one Basidiomycete have been observed up to now. Members of the genus *Aspergillus* are frequent. In nearly half of the soil samples of the dry forest of northern Namibia a member of the *A. niger*-group (*A. niger* respectively *A. japonicus*) was isolated, but nowhere else. Fewer cultures were gained from the soil of the Nama Karoo. In the first sampling the dominating isolate was a Coelomycete, most likely a member of the genus *Phoma*. However, in this area the samples of 2001 were taken before the onset of the raining season, while the raining season in the other areas was well under way. In 2002 the sampling was after the annual rain was fallen.

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135 - Arbuscular mycorrhizae in Namibian grasses: a comparison between North and South

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Arbuscular mycorrhizae (AM) are studied within the framework of the BIOTA Southern Africa project. One aim is an inventory of species along the rainfall gradient from the Cape of Good Hope to the Angolan border. In addition to this, different land management systems are studied with respect to their influence on biodiversity. This survey is focussed on Namibia, comparing the South (with an annual rainfall of approx. 150 mm) to the North (with 450 mm rain/year). Grasses were chosen as host plants of AM as they occur along the whole transect and are associated with AM fungi under normal conditions. Morphological studies revealed different rates of mycorrhization in the North and South. Molecular studies (sequencing of the ITS region of nuclear ribosomal genes) of grass roots infected with arbuscular mycorrhizal fungi showed that several AM species (eg *Glomus intraradices*) occur along the whole transect while others occur only in the North or the South. These results are backed by morphological identification of spores isolated from surrounding soils. Comparing different management systems in the South, differences in rate of mycorrhization can be detected; although no differences were noted in species composition of AM fungi.

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136 - Biodiversity of endophytic fungi from *Pinus tabulaeformis* in China

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In a survey of biodiversity of endophytic fungi from *Pinus tabulaeformis* in China, A total of 25 taxa and 6 morphotypes of mycelia sterilia were found. In the 6 morphotypes, 18 isolates were arbitrarily selected from white morphotype and identified into various taxonomic levels based on rDNA sequence analysis. The 5.8S gene and ITS regions of rDNA from the 18 white morphotypes

were amplified and sequenced. Phylogenetic analysis based on the 5.8S gene sequences showed that these 18 white morphotypes belonged to the Ascomycota. Further identification of the white morphotypes to lower taxonomic levels was conducted by means of sequence similarity comparison and phylogenetic analysis of both the 5.8S gene and ITS regions. The origins of WMS9 and WMS10 were inferred to be the genus *Lophodermium* of the Rhytismataceae, and the origins of WMS11, WMS13, and WMS18 were the Rhytismataceae. WMS2, WMS3, WMS4, WMS5, and WMS6 were identified to the genus *Rosellinia*, WMS1 to the genus *Entoleuca*, and WMS14 to the genus *Nemania* of the Xylariaceae, and the origins of WMS7, WMS8, WMS12, WMS15, WMS16, and WMS17 were the Xylariaceae. The value of using DNA sequence analysis in the identification of endophytic fungi is discussed.

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137 - Assessing diversity of leaf-inhabiting pathogenic coelomycetes in south-east Europe

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Leaf-inhabiting pathogenic coelomycetes are difficult to monitor in nature because of their microscopic size, large number of inadequately characterized and classified morphs. About 1100 species of these anamorphic fungi have been recorded from south-east Europe. Fungal diversity assessment in these temperate and submeridional regions allow one to predict an eventual total of around 15300 microfungi on the 5100 known vascular plants. Some names need critical revision at species rank, other anamorphic names need reassessment in the light of teleomorph names and the application of one name for the holomorph. An eventual 1500-2000 leaf-inhabiting pathogenic coelomycetes can thus be expected for south-east Europe. Main centres of biodiversity are the Carpathian mountains and Crimean peninsula. About 500 leaf-inhabiting coelomycete pathogens were observed in mountain forests, subalpine meadows, forest-steppes and steppes of Crimea. The eastern Carpathian virgin broadleaf and conifer forests, and alpine plant communities are characterized by over 260 of these pathogens, but this number is not final. The species richness of leaf-inhabiting coelomycetes is based on a combination of abiotic factors and host-plants diversity. Decline of the total population of these fungi, at 43% in the Carpathians, and accumulation of species with agressive pathogenic characters has been observed in unstable ecosystems. Study of these fungi can provide a way to monitor stability of natural ecosystems.

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138 - Functional biodiversity of grassland saprotrophic fungi

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The aim of this project is to characterise the functional biodiversity of saprotrophic decomposer fungi in a grassland community. The presence of all saprotrophic fungal species in a grassland community may not be necessary to maintain the function of the ecosystem, i.e. some species may be functionally redundant in the process of decomposition because several species may break down the same carbon and nitrogen substrates. Fungal isolates obtained from an upland grassland soil in Scotland, were selected to cover not only the most abundant species (common isolations), but also a cross section of the fungal community (occasional isolations) from different treatment plots (control, lime, nitrogen and nitrogen plus lime) at the field site. Isolates were tested for their ability to grow on specific substrates, in defined and semi-defined media. Primarily the activity of cellulolytic enzymes, ligninolytic enzymes, pectinase, amylase and chitinase were tested on solid media. Twelve isolates were chosen for further screening for the utilisation of smaller molecular weight carbon and nitrogen sources using the BIOLOG system. Further work is in development to test the paired isolates ability to utilise and compete for natural substrates available in the grassland.

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139 - Guatemalan Macrofungi: diversity and uses

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Many fungal species from North to South America and vice versa found place in Guatemala due to its geographical position and orographic relief. Many of them are mycorrhizal with pines, oaks, alders and fir. In addition, there is also a large diversity of saprophitic species, especially in the tropical areas. Mayan people in Guatemala have used mushrooms as part of their rituals and as a source of food since many centuries ago. Currently mushrooms are used as food and as medicine but exceptional uses as insecticide or decorative elements are reported. The main researches on macrofungi in Guatemala have been directed for 10 years ago by the University of San Carlos. Currently its Mycological Herbarium contains a large collection of saprophitic and mycorrhizal dried samples from almost all the regions of the country. The most important collections of mycorrhizal mushrooms come from the highlands because of their diversity and knowledge of the Mayan people. Species described only for North America as *Boletus edulis*, *Catathelasma ventricosa*, *Gomphus floccosus*, *Lactarius salmonicolor*, *Rhizopogon evadens* and others were found. New and possible endemic species are present in the genera *Amanita*, *Boletus*, *Cortinarius*, *Lactarius*, *Russula*, *Tylopilus*, but more studies are necessary. Interesting findings on distribution of species in the Caribbean area and south east of the country have also been reported.

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140 - Effect of host tree species on fungal community composition on a tropical rain forest in Panama

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Wood-inhabiting ascomycetes and basidiomycetes represent a diverse group of taxa which play an important role in decomposition and nutrient cycling. Little is known about how communities of these organisms are organized or how tree host influences community structure. To determine whether host tree species influences the composition of fungal communities, the ascomycetes and basidiomycetes present on three host species: *Prioria copaifera*, *Quararibea asterolepis*, and *Trichilia tuberculata* were sampled on a plot on Barro Colorado Island, Panama. Fungal diversity was high. Sampling of 181 trees and branches found dead over three years yielded 75 ascomycetes and 112 basidiomycetes species. The highest diversity of both fungal groups was found on *Prioria*, but the asymptote of species accumulation curves was not reached for any host tree. Ordination of fungal communities revealed distinct differences between hosts, but strong similarity within hosts across years. The most abundant fungal species of both ascomycetes and basidiomycetes were generalists found on all three hosts. However, *Quararibea* harbored more species unique on this host. Randomization tests revealed that there were significantly fewer host generalist fungi than expected for ascomycetes but not for basidiomycetes. These results indicate that host composition plays a role in structuring both ascomycete and basidiomycete communities, but that the most successful species are capable of colonizing multiple host species.

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141 - Conservation of biodiversity: Effects of varying levels of green-tree retention on ectomycorrhizal fungus diversity

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The Demonstration of Ecosystem Management Options (DEMO) experiment examines the effects of green-tree retention on mycorrhizal fungi, vegetation, wildlife, insects, soils, hydrology, and social perceptions of forested landscapes. Ectomycorrhizal (EM) fungi were sampled from three replicated treatment blocks. Sporocarp production and ectomycorrhizae were assessed before and after trees were cut. 197 EM species were identified from sporocarps (57 hypogeous, 140 epigeous) and 188 EM morphotypes were described from soil cores. Hypogeous sporocarp production of the genera *Gautieria* and *Rhizopogon* was greatly reduced by heavy thinning as was mushroom production in the genera *Cortinarius*, *Inocybe*, and *Russula*. Moderate thinning retained higher levels of sporocarp production. The cumulative number of EM types was estimated for each treatment via the Abundance-based Coverage Estimator (ACE). The ACE attempts to account for species that are present in a population but not observed in the sample. Fewer EM types per soil core in the most heavily cut areas translated into a severe reduction in the rate at which species accumulated. The rarer species were disproportionately affected in the heavily cut areas and EM diversity was greatly lowered, especially as compared to the moderately thinned areas. Both the sporocarp data and the EM data suggested that diversity indices responded in a non-linear manner to varying levels of green-tree retention.

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142 - *Hygrocybe* and *Cuphophyllus* as ecological indicators

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A total of 46 species and 7 varieties of *Hygrocybe* sensu lato are known from Norway. Most species are been found during investigations of seminatural grasslands. *Hygrocybe* species are also found in rich forests, fens, and in alpine areas. Data on *Hygrocybe* species from 650 grassland localities have been collected in the years 1992-2001, with a total of 3650 records excl. duplicates. Some species show a southern distribution pattern, e. g. *H. intermedia*. Studies of vertical distribution show that there are lowland species, e. g. *H. irrigata*, indifferent species, e.g. *H. conica*, *H. pratensis*, and 'summer farm species' with the highest frequency in north boreal (subalpine) region: e.g. *H. nitrata*, *H. turunda*. There are species occuring mainly in calcareous areas, e.g. *H. colemanniana*. *H. vitellina* seems to have an oceanic distribution pattern, some other species may be suboceanic. The frequencies of the grassland species are presented. Most rare grassland species seem to be good indicators of species rich localities with a long grazing continuity. The fragmentation of *Hygrocybe* localities in the cultural landscape is a continuing process. The county of Møre og Romsdal has a much higher density of species rich localities than Denmark and the Netherlands. It is a challenge to save the *Hygrocybe* localities for future generations.

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143 - Autecology of *Hygrocybe* spp. in temperate grasslands

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Members of the genus *Hygrocybe* are ubiquitous and colourful components of many undisturbed and nutrient-poor grasslands in Northern Europe. Through surveys of the distribution of *Hygrocybe* spp. and of other macrofungal genera showing similar patterns of occurrence, a picture is gradually emerging of the more important waxcap grassland sites, and of those species in greatest need of protection. As part of a UK-based Soil Biodiversity Programme (http://mwnta.nmw.ac.uk/soilbio/sourhope.htm), we have monitored the effect of various management regimes on fruiting of *Hygrocybe* spp. Fine-scale mapping combined with genetic analysis (AFLP/ISSR) is being used to measure the extent of individual genets, with species-specific PCR probes being used to establish the vertical location of mycelia. Analysis of the natural abundance of the stable isotopes in fruitbodies showed that grassland *Hygrocybe* spp. show significant depletion for ^{13}C(-28 to -30‰) and enrichment for ^{15}N(+12 to +18‰), a pattern that sets them apart from most macrofungi previously examined. Furthermore, the other macrofungi associated with these undisturbed grasslands (eg Clavariaceae, Geoglossaceae) have very similar isotope signatures despite being taxonomically unrelated. Experiments using plant litter enriched in ^{15}N are currently underway and will further clarify our understanding of the role of these fungi in nutrient cycling and explain why they are so adversely affected by many agricultural practices.

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144 - What is *Agaricus cossus*? - Phylogeny of the white *Hygrophorus* species in Northern Europe

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The genus *Hygrophorus* Fr. comprises about 40 species in the Scandinavian countries. They are characterized by medium to large, fleshy basidiomes that are whitish or colored in gray, brown, yellowish orange to red. All species grow in woodlands and are obligate mycorrhiza formers. There are at least ten white to whitish species in the Nordic countries. In traditional classification color or color- changes, viscidity, odor etc. of the basidiome have been the most important characters. However, these characters are often vague and also dependent on environmental conditions. For these reasons great taxonomical and nomenclatural problems among the white *Hygrophorus* species exists. The internal transcribed spacer regions 1 and 2 and the first 900 basepairs of the 5' region of the nuclear large subunit ribosomal DNA was sequenced for 35 ingroup and outgroup taxa. Phylogenetic analysis shows that *Hygrophorus* is a well-defined genus but the white species do not form a monophyletic group. Host specificity seems to be high among the analyzed taxa.

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145 - Tropical Hygrophoraceae: convergent evolution in *Hygrocybe*, and support for retention of *Hygroaster* based on molecular evidence

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The family Hygrophoraceae is highly diverse in tropical regions where all the genera are represented (*Camarophyllopsis*, *Cuphophyllus*, *Hygroaster*, *Hygrocybe*, *Hygrophorus* and *Humiditis*). *Hygroaster* and *Hygrocybe* Section *Firmae* are restricted to the tropics and subtropics. Using ribosomal DNA sequences, we have obtained a preliminary phylogeny. Despite having ornamented rather than smooth spores, the genus *Hygroaster*, is a good member of the Hygrophoraceae. With the exception of Sections *Firmae* and *Glutinosae*, the molecular phylogeny is concordant with the current division of *Hygrocybe* into two subgenera: *Hygrocybe* and *Pseudohygrocybe*. Section *Glutinosae* forms a separate clade from the other genera, supporting recognition of the genus *Gliophorus*, but more taxon sampling is needed. Within *Hygrocybe* ss., Section *Firmae* is included in the subgenus *Hygrocybe* clade and is basal to sections *Hygrocybe* and *Chlorophanae*. The most basal members of section *Firmae* (*H. hypohaemacta*) and *Chlorophanae* (*H. glutinipes*) that we have sampled have similar macro- and micromorphology, except for the presence of dimorphic basidia and spores in Section *Firmae*. Section *Firmae* should be reclassified in subgenus *Hygrocybe*. Although section *Firmae* may be derived from section *Coccineae* in subgenus *Pseudohygrocybe* as suggested by Heinemann, our results suggest that convergent evolution is responsible for the similarity of micromorphology in terminal species in the Sections *Firmae* and *Coccineae*.

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146 - Recent advances in molecular phylogeny of temperate Hygrophoraceae and concordance with morphology and ecology

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Phylogenetic relationships within Hygrophoraceae (*Basidiomycota*) were studied using parsimony analysis of nuclear LSU, ITS1-2 and SSU rDNA. The analysis included 114 sequences (30 LSU; 23 SSU; and 61 ITS of 41 species) from Russia, the USA and Europe. For comparisons, a phylogenetic reconstruction was also made using morphological and ecological data (30 characters in total). Results from the molecular analyses are largely concordant with each other as well as with the analysis of morphological end ecological data. However, the ITS sequences varied too much between representatives of different genera, so this region is most appropriate for phylogenetic studies within genera of the Hygrophoraceae. The results suggested that the Hygrophoraceae appears to be monophyletic group. The family contains 9 separate clades that support the following genera: *Cuphophyllus*, *Neohygrocybe*, *Camarophyllopsis*, *Humidicutis*, *Gliophorus*, *Pseudohygrocybe*, *Hygrocybe*, *Neohygrophorus* and *Hygrophorus*. The taxonomic position of some species should be reevaluated. Including several representatives of the Tricholomataceae s.l. (i.e. *Omphalina*) in the analysis demonstrated that some of them may be related to the Hygrophoraceae and probably should be transferred to this family. (This study was partly supported by grants from Hesler Endowment Fund and Russian Foundation for Basic Research).

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147 - Biogeographical implications of Trichomycete distributions

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Known distributions of Trichomycetes worldwide fall into several patterns depending upon their host types, habitats, and host specificity. It is hypothesized that in all cases autonomous dissemination of the gut fungi does not occur except within boundaries of their immediate host populations, and that dispersals over greater distances are the result of particular active or passive mechanisms. Examples of vicariant distributions of the symbionts in both the Northern and Southern Hemispheres are presented. The hypothesis that this fungus-arthropod association is very ancient is supported by historical biogeography.

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148 - Progress toward a rDNA based phylogeny of the Harpellales

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The Harpellales (Trichomycetes), gut fungi, are unique endosymbionts associated with larval aquatic insects worldwide. Their evolutionary relationships have been difficult to infer because of the paucity of morphological characters and unculturability of most of the 35 known genera. Previous DNA studies have included at most four genera of culturable Harpellales. This study used thalli taken from guts (mixed genomic template) to generate 18S and 28S rDNA sequences to infer the phylogeny of the Harpellales using cladistic analyses. Sixteen genera of Harpellales were included, with 64 of 72 sequences from unculturable samples. The genus *Orphella* fell outside an otherwise monophyletic group of Harpellales, more closely allied to the Kickxellales. The two largest genera, *Smittium* and *Stachylina*, are polyphyletic, perhaps masked by convergent and limited morphological characters. The cladograms did not reveal any pattern that corresponds with the two most important morphological criteria, trichospore shape and zygospore type, as well as appendage number for both. The current two family system of classification is not supported, suggesting that the Legeriomycetaceae may need to be dropped as a rank. However, the need to add more sequence data, pursuit of a protein gene, and continued collections for subsequent cladistic analyses are discussed.

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149 - Systematics of non-culturable orders of Trichomycetes based on molecular markers

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The Eccrinales and Asellariales are morphologically diverse orders of the class Trichomycetes (Zygomycota). These gut fungi inhabit a wide range of hosts: Crustacea, Insecta and Diplopoda in varied habitats (marine, freshwater and terrestrial). The order Eccrinales is characterized by unbranched, nonseptate, multinucleate thalli and sporangiospores that are formed basipetally from the thallus apex; they live attached to the cuticle lining of the digestive tract of the host by a secreted, basal holdfast. The order Asellariales, on the contrary, has branched,

septate thalli that produce arthrospores in their fertile branches. They attach through holfast cells that have unique morphology commonly used for species identification. Sexual reproduction is not known for either group. No species of either order has been axenically cultured so far. Most of their taxonomy, if not all, is based on a few micromorphological characters. Molecular markers have been developed to study the relationship of these orders to other groups of fungi. Ribosomal gene (18S and 28S) sequence analyses do not support a close association of these orders to the Harpellales, their putative sister taxon.

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150 - Recent advances in trichomycete ecology and physiology

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Although the systematics of trichomycetes represents a fairly mature level of knowledge, studies of trichomycete ecology and physiology have lagged behind. To address this imbalance, we have investigated the ecology of trichomycetes and aspects of their physiology. We asked whether the distributions of trichomycetes in time and space were predictable. A two-year study revealed that the prevalence of trichomycetes inhabiting black flies is related to season. In some streams, *Harpella melusinae* was most prevalent in spring and fall. Efforts to relate prevalence to environmental characteristics showed that the prevalence of *H. melusinae* is greatest in mountain streams. Prevalence of this species in the black fly *Simulium tuberosum* is related to stream pH and conductivity. We also investigated physiological aspects of establishment of trichomycetes in hosts. Zygospores of *H. melusinae* and *Stachylina* spp. are rare, but formation can be stimulated by elevated levels of potassium and pH (as KOH). *Smittium megazygosporum* has a disjunct distribution and large zygospores (150 µm long). Large zygospores might enable trichomycetes to persist in the environment. The role of zygospores in the life history of trichomycetes remains unclear. *Smittium culisetae* inhabits specific regions of the host hindgut, which might be related to the timing of spore germination as spores transit the gut. Nutritive characters of the hindgut regions might determine which areas are habitable.

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151 - Dispersal mechanisms in species of Harpellales

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Fungi of the Harpellales (Trichomycetes) live attached to the mid-gut (Harpellaceae) and hind-gut (Legeriomycetaceae) of their dipteran hosts. The hosts include species of both simuliid and culiciid vectors for river blindness and malaria respectively. Transmission of these fungi within host populations is by means of asexually produced sporangia termed trichospores. These spores are produced by the thallus within the host gut. Light microscopic images of these spores from representative genera will be shown and an explanation made of the mode of transmission and infection. Some members of the Harpellales have a chlamydosporic stage which is associated with the eggs of host Diptera. These spores are thought to provide a means of dispersal to new or ephemeral habitats. Data relating to the occurrence of these spores at various collection sites will be presented. Light and scanning electron micrographs showing the structure of this stage will also be provided for three species of the Harpellales: *Genistellospora homothallica*, *Harpella melusinae*, *Smittium* sp. Results of infection trials in order to establish positively a process for reinfestation within the host by these spores will be given. The mechanism(s) by which species of the Harpellales invade the tissue of the adult fly is unknown. Transmission electron micrographs of pupae and gravid adults will be provided to show a possible pathway of infection.

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152 - Selected habitats of *Xylaria* species of Mexico

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Due to its diverse floristic composition, México is, perhaps, the area of the New World with more species of *Xylaria*. In this contribution, 50 species of *Xylaria* are reported growing on different habitats, i.e., on wood and fruits of *Quercus* spp., on *Guazuma ulmifolia*, *Liquidambar styraciflua* and *Magnolia* spp. fruits, on pods and pod-like structures, on fallen leaves, on wood of several dicots and monocots, on different fruit remains and soil and dung. Morphologic and host relationships are presented for the following species of *Xylaria*: *X. adscendens*, *X. alata*, *X. albisquamula*, *X. amphithele*, *X. apiculata*, *X. aristata*, *X. castilloi*, *X. coccophora*, *X. delicatula*, *X. dichotoma*, *X. diminuta*, *X. duranii*, *X. enteroleuca*, *X. equina*, *X. eugeniae*, *X. feejeensis*, *X.* cf. *filiformis*, *X. gracillima*, *X. guazumae*, *X. inaequalis*, *X. ianthino-velutina*, *X. ianthino-velutina* large-spored variety, *X. jaliscoensis*, *X. juniperus* var. *asperula*, *X. lancea*, *X. liquidambar*, *X. longiana*, *X. longipes*, *X. magniannulata*, *X. magnoliae*, *X.* cf. *magnoliae*, *X. mexicana*, *X. michoacana*, *X. microceras*, *X. multiplex*, *X. oxyacanthae*, *X. palmicola* small-spored variety, *X. perezsilvae*, *X. phyllocharis*, *X. poitei*, *X. rhizomorpha*, *X. quercinophila*, *X.* sp. aff. *hypoxylon*, *X.* sp. aff. *longiana*, *X.* sp. *cubensis* complex, *X. scabriclavula*, *X.* cf. *scruposa*, *X. striata*, *X. subcoccophora*, and *X. tumulosa*.

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153 - The Xylariaceae of Ecuador - an update

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Besides the early effort by Lagerheim and Patouillard (1890s) not much was known about Ecuadorean Xylariaceae until the current effort initiated in 1987. Even today many areas of high potential remain unexplored but at least a rough picture of the xylariaceous biodiversity can now be presented. Ca. 17 genera have been recorded, and *Xylaria* rules with more than 98 species, many of which lack formal names. *Camillea* has the highest country record known with 23 spp. *Hypoxylon* is also represented with ca. 23 spp., but a high proportion are only known from single records, so this number should increase. The recent survey has documented the presence of *Entonaema pallida* in the country. *Anthostomella* and *Rosellinia* remain poorly known, but *R. evansii* has been found as new to South America. It is clear that the Amazon basin has the highest diversity with more than 110 species, but many of the novelties discovered occur at higher altitudes. Also the Choco region of the northwest and the southern provinces have produced promising results. *Alnus acuminata* was investigated in order to establish whether it shares xylariaceous fungi with its temperate relatives, and both *Hypoxylon multiforme* and *H. fuscum* were recorded together with the more subtropical/tropical *Daldinia childiae*. Mycogeographically it is interesting to note that members of the genera *Phylacia*, *Thamnomyces* and *Leprieuria* so far only have been recorded within the Amazon basin.

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154 - Where are the Xylariaceae? The Southeast Asia connection

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The family Xylariaceae is truly cosmopolitan but the greatest concentration of species occurs in the tropics. In the past it has always been acknowledged that tropical South and Central America house the greatest diversity. However, recent surveys in Thailand, Malaysia and Papua New Guinea suggest that the 'species gap' between the old and the new tropics is not as exaggerated as previously suggested. The lack of collectors, and indeed specialists in Southeast Asia, has contributed significantly to under-recording in the region. For example *Camillea* has always been considered to be a South American and Central American genus but two new species have recently been described from Malaysia and *C. tinctor* has been found to be regularly collected in Malaysia, Thailand and Papua New Guinea. A total of 12 genera of Xylariaceae are reported from Malaysia and at least 15 taxa are considered to be previously undescribed. The Xylariaceae is now known to be well represented in Thailand with 17 genera recognised. Studies in Papua New Guinea also reveal a strong presence there. It is of some significance that in each of the countries surveyed a considerable number of the collections of *Hypoxylon* and *Xylaria* are probably undescribed whilst others are infrequently reported. The range of species reported from the region will be discussed, together with variation associated with habitat and host, and the findings compared with currently available records from Africa and tropical America.

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155 - Can lifestyle be used to find the rare or the new among the Xylariaceae?

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Although the Xylariaceae are seen primarily as wood inhabitants genera such as *Hypocopra*, *Poronia*, *Podosordaria*, and *Wawelia* are specifically dung inhabitants. *Wawelia* is also xerophilic. Other members of the family are litter, fruit or seed inhabitants and others are associated with soil or insects. The majority are saprotrophic but an increasing number of taxa are being recognized as phytopathogens through latent invasion in stressed hosts. The Xylariaceae also exhibit an outstanding presence as endophytes, especially in the tropics. Many taxa also have a strong host preference and some seem to be host specific. Within the wood inhabiting species not only is host important but also the nature of the host. Some species grow only on wood which retains its bark others only on decorticated wood and some only on well-rotted and water soaked wood. Thus the nature of the substratum and the habitat strongly influence the species which will be found. Studies on endophytic Xylariaceae show that some of the species isolated appear not to have been recorded from that vicinity and in some cases they appear to be previously undescribed. There must be teleomorphs somewhere? A number of xylariaceous taxa have been found to be associated with mangrove ecosystems and apparently even marine situations. It is proposed that host, habitat and climate all interact to dictate which taxa are present and that a knowledge of these conditions should prove useful in detecting rare and new species.

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156 - *Rosellinia* morphology: the first step towards molecular studies

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The genus *Rosellinia* is cosmopolitan in its distribution and is reported from hardwoods, dicotyledonous plants, coniferous litter and occasionally from monocotyledonous hosts. The stromata are usually uniperitheciate and embedded in a persistent or evanescent subiculum. Species of *Rosellinia* can be characterised by ascospore shape, combined with stromatal, subicular and anamorphic characters. About 70 taxa are validly described, belonging roughly to six spore shape groups. (1) *R. necatrix*-type spores are 30-120 µm long, slender, with short germ slits and tapering ends surrounded by a slimy sheath. (2) Five taxa possess ellipsoidal spores. Taxa belonging to groups 1-2 are associated with a *Dematophora* anamorph. (3) The third group is characterized by 60-120 µm long, broadly rounded ascospores with a long germ slit, slimy sheath or caps. (4) In the fourth group ascospores range from 25-50 µm, are ellipsoidal, with a long germ slit and one or two cellular, semiglobose appendages and slimy sheath. (5) About five taxa possess ascospores with two large, conical, cellular appendages. (6) The largest and most heterogeneous group includes approximately 40 taxa and has 10-60 µm long, ellipsoidal ascospores with long, short or without germ slit, with slimy caps or sheath, occasionally with a cellular appendage. The subiculum is evanescent. Groups 3-6 are characterised by a *Geniculosporium* anamorph.

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157 - Interesting xylariaceous fungi from Taiwan

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Results from cultural and anamorphic studies on some recently collected xylariaceous fungi from Taiwan are highly interesting and are valuable in settling taxonomic problems related to these particular taxa. *Penzigia cranioides* is the type species of the genus *Penzigia*. The *Xylocoremium* anamorph produced in culture indicates that *P. cranioides* is in fact a *Xylaria* species and *Penzigia* is considered in synonymy with *Xylaria*. *Poronia pileiformis* had been reported to have an anamorph typical of *Xylaria* and could be accommodated in the genus *Podosordaria*. Our Taiwan collection, however, produced a *Lindquistia anamorph*; we thus feel justified in accepting the species in *Poronia*. A new *Theissenia* species produced elongated conidia which were sympodially borne on conidiogenous cells. This fungus has bipartite stromata and evanescent asci lacking an apical ring. It may represent an interface between Xylariaceae and Diatrypaceae. We accept two sections in the genus *Hypoxylon*: sections *Hypoxylon* and *Annulata*. *Hypoxylon ravidoroseum* belongs to section *Hypoxylon* of the genus *Hypoxylon*. One collection is peculiar in having a thickening on the ascospore perispore and in having both papillate and umbilicate ostiolar openings. A closer examination revealed that the 'papillae' of the papillate ostiolar openings are indeed homologous to the overlying stromatal tissue of ostiolar discs in *Hypoxylon* section *Annulata* which are pushed away during perithecial development.

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158 - New evidence on the taxonomy and the secondary metabolism of *Daldinia* and *Hypoxylon*

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During the past years, we evaluated the taxonomy of *Daldinia* Ces & De Not. was by a polyphasic taxonomic approach, using classical morphological studies of teleomorphic and anamorphic characters in combination with scanning electron microscopy, HPLC-based secondary metabolite profiling and different methods of PCR fingerprinting. Several new species were discovered, and some new chemotaxonomically relevant secondary metabolites were identified. Once the evaluation of most European *Daldinia* species had been accomplished, our studies were meanwhile extended to tropical *Daldinia* species and to those members of the allied genus *Hypoxylon* Bull. that occur in temperate climates. Besides a short review of previous work, recent results on the taxonomy of *Daldinia* and novel metabolites from *Hypoxylon* will be presented. References: [1] Stadler, M. et al. 2001. Mycotaxon 77, 379-429. [2] Stadler, M. et al. 2001. Phytochemistry 56, 787-793. [3] Wollweber, H. & Stadler, M. 2001. Z. Mykol. 67, 3-53. [4] Stadler, M., et al. 2001. Mycotaxon 80, 167-177. [5] Stadler, M., Baumgartner, M. & Wollweber, H. 2001. Mycotaxon 80, 179-196. [6] Stadler, M. et al. 2001. Mycological Research, 105, 1191-1205. [7] Stadler, M. et al. 2002. Mycological Progress 1, 32-41. [8] Mühlbauer, A. et al. 2002. Submitted to Mycological Progress.

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159 - Transposable elements in *F. oxysporum*: impact on gene expression, genome organization and evolution

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The *F. oxysporum* genome harbors diverse representatives of most known families of transposable elements (TEs). Different active transposons have been identified determining a broad spectrum of mutations. The TE-mediated changes range from changes in the size and the arrangement of the whole genome to changes in single nucleotides. In addition, some TE-induced mutations, such as read-out transcription, or local rearrangements due to aberrant transposition process may be of evolutionary significance to their host by bringing new regulatory elements in proximity to exonic sequences. Analysis of the distribution of some elements in natural populations revealed that their dynamics within and between species appeared to be the result of different evolutionary processes: vertical transmission, stochastic loss, inactivation via a RIP-like mechanism, burst of amplification, and horizontal transmission. These investigations showed the capacity of TEs to restructure the *F. oxysporum* genome in interesting ways and their potential as valuable tools for tracing populations.

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160 - *Fusarium* species big and small, and the interface between systematics and population genetics

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Multilocus phylogenetic approaches have revolutionized our view of species in fungi, particularly in the genus *Fusarium*. Using these approaches, phylogenetic units can be identified that correspond to coherently defined species, within which varying degrees of phylogenetic structure are observed. Deciding where to draw the line between species and subspecific lineages requires consideration of a variety of phenotypic, biogeographic and ecological factors. In this talk, I will compare and contrast several fusaria that have been well-characterized phylogenetically, discussing how species are usefully delimited and what we know about genetic structure within those species. Case studies will include *Fusarium avenaceum* (little phylogenetic structure), *F. hostae* (some phylogenetic structure), *F. proliferatum* (much phylogenetic structure) and species lineages of the *F. solani* complex (varying levels of phylogenetic structure). As has been demonstrated in other groups of fungi, phylogenetic partitions within species may be useful as landmarks for defining the boundaries within which population genetic processes occur in Fusarium. The DNA sequence databases of Fusarium species that have emerged from these studies are also extremely useful as tools for identification.

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161 - Phylogenetic analysis of *Fusarium* based on partial TEF-1α gene sequences

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In an attempt to develop rapid molecular detection methods for economically important mycotoxin producing *Fusarium* species, we have sequenced a part of the translation elongation factor 1α (TEF-1α) gene of more than 13 species of the sections *Sporotrichiella*, *Roseum*/*Arthrosporiella*, *Gibbosum* and *Discolor*. This gene was chosen because it was expected to provide a high degree of phylogenetic signal and because the gene phylogeny and the taxon phylogeny in related taxa has been reported to be highly concordant. Our sequence data were combined with additional data retrieved from sequence databanks, aligned and subjected to phylogenetic analyses (PAUP*). The resulting phylogeny largely supports the subdivision of the taxa into the four sections, but a few exceptions were observed indicating that the sections *Sporotrichiella* and *Discolor* are polyphyletic. The taxa representing the section *Roseum*/*Arthrosporiella* were found to form a monophyletic clade, but our results suggest that it may be more appropriate to include some of the taxa currently classified in the sections *Sporotrichiella* and *Discolor* in section *Roseum*/*Arthrosporiella*. We found a high degree of concordance between the clades and taxa known to produce DON/nivalenol and T-2/HT-2 mycotoxins. The delimitation of taxa is discussed in light of the observed phylogeny.

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162 - Evolution of the *Fusarium graminearum* species complex

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Fusarium head blight or scab of cereals is one of the most devastating plant diseases worldwide. These pathogens cause significant reduction in seed quality and yields and often contaminate seeds with trichothecene and estrogenic mycotoxins. Genealogical concordance phylogenetic species recognition was used to investigate species limits within this complex in order to understand the host range, geographic distribution and mycotoxin potential of these fusaria. Because trichothecene chemotypes are not well correlated with the species phylogeny, we investigated the evolutionary history of the trichothecene mycotoxin gene cluster. Results of these phylogenetic analyses demonstrated that polymorphism within these virulence-associated genes is trans-specific and appears to have been maintained by balancing selection acting on chemotype differences that originated in the ancestor of this important group of plant pathogens.

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163 - Medicinal mushrooms: Past, present and future

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Medicinal mushrooms have been known in Oriental medicine for hundreds of years as beneficial for health. In 2001, the value of world mushroom production and medicinal mushroom products was estimated to be worth approximately 18 billion US dollars. Mushrooms make up a vast and yet largely untapped source of powerful new pharmaceutical products. Many if not all Basidiomycetes mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium, and culture broth. The data about mushroom polysaccharides are summarized for 651. These polysaccharides are of different chemical composition; the main ones comprise the group of β-glucans. β-(1->3) linkages in the main chain of the glucan and further β-(1->6) branch points are needed for their antitumor action. Most of the antitumor clinical evidence is from commercial polysaccharides lentinan, PSK (krestin), and schizophyllan. Mushroom polysaccharides prevent oncogenesis, show direct antitumor activity against various allogeneic and syngeneic tumors, and prevent tumor metastasis. Cultivation and development of edible and medicinal mushrooms can positively generate equitable economic growth that had already an impact at national and regional levels. This impact is expected to continue increasing and expanding in the 21st century Therefore, sustainable research and development of mushroom production and mushroom product can become a "nongreen revolution" (Chang, 1999).

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164 - Beneficial effects of edible mushrooms in Japan

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The aqueous extracts of edible mushrooms showed so high growth-inhibitory activity aganist solid sarcoma 180. Among many antitumor polysaccharides, a glucan from Lentinus edodes was used clinically as an injection of a narrow adaptation in Japan. However, substances having the antitumor activity by p.o. were a low molecular weight protein- bound polysaccharide from one of the most popular edible mushrooms, *Flammulina velutipes*. An epidemiological study in Nagano Prefecture, Japan indicated that the cancer death-rate of farmers producing *F. velutipes* as a main occupation was remarkably lower than that of common people in the Prefecture. Another edible mushroom, *Hypsizygus marmoreus* had also high antitumor activity and preventive effect for tumor metastasis. Cancer preventive effect of the mushroom was proved by comparison of carcinogenesis between mice bred with an feed containing the dried fruitbodies and mice bred with an ordinary feed. The mechanism of cancer inhibitory and preventive activities of edible mushrooms was due to immunopotentiation and antioxidant activity. Thus, the intake of mushrooms proved to be effective in cancer prevention and growth-inhibition. Based on these results, EEM (Extracts of Edible Mushrooms), a suppliment was proved to have a clinical effectiveness. It has been said in traditional Oriental medicine from the ancient times that Medicine and Food have the same origin. This is identical with our conclusion from mushroom-studies.

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165 - Identification of species and strains of medicinal mushrooms using repetitive elements

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A comparison of a previously isolated retrotransposon of the gypsy-family from the white button mushroom *Agaricus bisporus* with published retrotransposons of the matsutake mushroom (*Tricholoma*) showed a high similarity in translated sequence of the reverse transcriptase gene. Based on conserved sequences degenerate primers were designed and used to amplify similar sequences in a number of gilled mushrooms that are known for their medicinal potentials. From strains of species examined (*Pleurotus ostreatus*, *Agaricus subrufescens*, *A. blazei*, *Stropharia rugoso-anulata*, *Ganoderma lucidum*), fragments were amplified with the expected length. Sequence analyses showed that all fragments indeed represented the relevant part of the reverse transcriptase gene of retrotransposons. Southern analyses showed that most clones hybridized only to the DNA of the species from which the clone was isolated, thus provide species-specific probes. Transposons were subsequently used to study relationships between strains within a species. Using transposon-tagging to map individual transposon copies, phenograms were constructed of a collection of wild strains of *A. bisporus*. The relationships found within this collection agree with previously constructed phenograms using mitochondrial DNA and RFLP markers. This study shows that retrotransposons are, in principle, useful to identify mushroom species and individual strains within species.

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166 - Standard methods for the determination of immunomodulatory properties of medicinal mushrooms

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A variety of edible fungi have been used in the past hundreds of years to prevent or cure a variety of diseases, e.g. various malignancies, infectious diseases, cardiovasculair diseases, hypertension and even stomach ache after serious drinking. Many serious studies were carried out mostly in China and Japan into the effects of mushroom derived polysaccharides, i.e. beta-glucans. on solid tumor growth. *Ganoderma lucidum*, *Lentinus edodes*, *Pleurotus* spp. and many others have been described as potentially anti-tumorigenic based on changes of in vivo or in vitro transplantability of laboratory tumor cell lines in mice. In recent years studies have been carried out into immunomodulation as the basic mechanism behind those anti-tumor effects. However, comparison of the outcome of the different experiments is almost impossible 1. due to inadequate identification procedures of the fungal species and strains used and 2. due to different methods of measuring immune activity. The present contribution aims to internationally standardize methods employed 1. in isolation and characterization of fungal beta-glucans, 2. measuring modulation of both innate and adaptive mouse resp. human immunity in vitro and ex vivo. Reliable methods will be proposed.

167 - Genoprotective activities of mushrooms

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Selected mushroom species have been examined as a source of natural antioxidants for offsetting the adverse effects of 'reactive oxygen species' linked to numerous pathological conditions including coronary heart disease, various types of cancer and neurodegenerative conditions. Aqueous extracts of the sporophores of *Agaricus bisporus*, *Auricularia auricula*, *Flammulina velutipes*, *Hypsizygus marmoreus*, *Ganoderma lucidum*, *Lentinula edodes*, *Pleurotus sajor-caju* and *Volvariella volvacea* were assessed for ability to prevent H_2O_2-induced oxidative damage to cellular DNA using the Comet assay. Genoprotective effects were observed with cold (20 °C) and hot (100 °C) water extracts of *A. bisporus* and *G. lucidum* fruit bodies but not with the other mushroom species tested. The genoprotective effect of *A. bisporus* is associated with a heat-labile protein present in the mushroom fruit body. This protein has been purified and identified as tyrosinase on the basis of its catalytic and electrophoretic properties, and inhibition studies. Genoprotection is dependent upon the enzymic hydroxylation of tyrosine to L-DOPA and subsequent conversion of this metabolite to dopaquinone and other oxidation products that enhance the oxidation status of the cell. These findings indicate that some edible mushrooms represent a valuable source of biologically active compounds with potential for protecting cellular DNA from oxidative damage.

168 - Lentinan as a possible "oral vaccine" against tumor development

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Lentinan, a purified b-glucan isolated from *Lentinus edodes* has remarkable anti-tumor and anti-viral activities in animal models when given through intravenous or intra-peritoneal injection. We investigated the efficacy of oral administration of lentinan using the AKR mice model. Results obtained showed that pre-feeding the mice for 7 days at an optimum dose of 3mg/mouse was most effective against tumor induction. A tumor inhibition rate of 94.44% was observed. The mice remained tumor-free several months after this treatment. In addition to feeding, passive transfer of lentinan-primed lymphocytes from AKR mice was able to restrict tumor development in immuno-deficient mice. Lymphocytes removed from the spleens of either lentinan-fed or buffer-fed AKR mice were inoculated simultaneous with human colon carcinoma cells into both the nude mice (athymic mice) and SCID (severe combined immuno-deficient) mice. Large tumors developed in mice that received lymphocytes from the buffer-fed AKR mice whereas very small or no tumors were found in mice receiving lymphocytes from the lentinan-fed AKR mice. Electron microscopy studies and TUNEL assays indicated that the number of tumor cells was reduced by the host immune system through apoptosis. DNA fragmentation, increment in proteases' activities and cytokine production were also observed. Tumor cells isolated from lentinan-fed mice displayed higher levels of caspase 3 and caspase 8 as compared to that of the buffered-fed control mice.

169 - Application of inhibition ELISA using antibodies to antitumor polysaccharides in screening of mushroom possessing immunomodulating activities

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Mushrooms have recently become attractive as health beneficent foods, and as a source material for the development of drugs. Many investigators have isolated and identified antitumor polysaccharides from edible mushrooms. We have attempted to quantify exactly and easily the antitumor polysaccharide in mushrooms by enzyme linked immunosorbent assay (ELISA). And, the inhibition assay of ELISA using antibodies to certain polysaccharides has been established to determine the contents of antitumor polysaccharides. It has been recognized that lentinan from *Lentinula edodes* and β-glucan from *Grifola frondosa* (GGF) were the antitumor polysaccharides possessing strong immunomodulating activities. In this study, we applied the inhibition assay of ELISA using antibodies against lentinan and GGF in order to screen the mushrooms containing a high level of these antitumor polysaccharides. And, the relationship between their contents and immunomodulating effect of each mushroom on cytokine production, such as tumor necrosis factor-α (TNF-α), and nitric oxide (NO), was investigated. It was ascertained that *Lentinula edodes*, *Flammulina velutipes*, *Grifola frondosa*, and *Meripilus giganteus* containing the positive polysaccharides to anti-lentinan and anti-GGF antibodies at high level augmented the production of TNF-α and NO from mouse peritoneal macrophages. These results showed that an inhibition assay of ELISA was a powerful approach to determine of antitumor polysaccharides in mushrooms.

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170 - TNF-α and NO production from macrophages is enhanced through up-regulation of NF-κB by polysaccharides purified from *Agaricus blazei* Murrill

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The Basidiomycete fungus *Agaricus blazei* Murrill has medically been used as a health food for the prevention of cancer. We chromatographically purified polysaccharide from alkaline-soluble fraction of *A. blazei*, which showed the highest activity of TNF-α and NO production from macrophage cell line, RAW264.7. The result of time course experiments on TNF-α and NO production from RAW264.7 stimulated with the purified polysaccharide was indicated that TNF-α was produced 5 h earlier than NO. To investigate the effect of the purified polysaccharide on mRNA expression of TNF-α and iNOS, the level of mRNA was evaluated by RT-PCR. The levels of TNF-α mRNA was induced at 0.5 h after stimulation, peaked at 3 h. On the other hand, those of iNOS mRNA was induced at 3 h and thereafter maintained the same levels. Furthermore, DNA binding activity of NF-κB was rapidly increased even at 0.5h after treatment, and peaked at 2h. When RAW264.7 was stimulated with the purified polysaccharide simultaneously in the presence of anti-TNF-α antibody, TNF-α mRNA expression did not affect and that of iNOS was down-regulated. Taking consideration into these results, it was ascertained that TNF-α production from RAW264.7 stimulated with the purified polysaccharide was enhanced through up-regulating NF-κB, and then the newly produced TNF-α enhanced NO production with autocrine pathway. Thus, the purified polysaccharide possessed the immunomodulating activity and might have anti-tumor activity.

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171 - Synergistic molecular interactions in the *Trichoderma* biocontrol system

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Recent studies have shown that the antagonist mechanism of *Trichoderma* spp. is much more complex than expected because of the numerous factors involved and the variety of mechanisms activated during the interaction with the host and the plant. Most of the research at a molecular level has been focused on the role of fungal cell wall degrading enzymes and antibiotics in the mycoparasitic relationship with phytopathogenic fungi. Pathogenicity factors have been identified among chitinases and glucanases, and from this, several opportunities have arisen for developing new biotechnologies for disease control. We have investigated the interaction between enzymes and antibiotics and found that *Trichoderma* applies these factors in synergistic combinations during attack. Synergism has also been found among different types of enzymes, and when the encoding genes are transgenically expressed in plants or other biocontrol microbes. Also synergisms appears to be the mode that some chitinases and glucanases interact in releasing mycoparasitism inducers from the host cell wall during the early recognition phase. Finally, a high level of synergism has also been found when *Trichoderma* or its antifungal compounds (enzymes or antibiotics) are applied in combination with *Pseudomonas* spp. and other biocontrol agents. We show that functional synergism between differently acting compounds is critical in biocontrol and is a key to understand mycoparasitic mechanisms.

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172 - Chitinases and plant induced systemic resistance: new insights in *T. asperellum* (*T. harzianum* 203)

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Two main modes of action can be discerned in the biocontrol mechanism of some *Trichoderma* species: mycoparasitism and plant systemic induced resistance. In order to better understand the role of chitinases in mycoparasitism of *T. asperellum*, we have studied regulation of the expression of the endochitinase chit36 and the N-acetylglucosaminidases coding for Chit73 and Chit102 using Northern blots and a promoter-GFP reporter system. Despite their close sequence similarity, chit73 and chit102 are differentially regulated under stress conditions and by chitin degradation products. chit36 expression is up-regulated *in vivo* during pathogen confrontation. Induced plant resistance by *Trichoderma* was studied using a spatially separated challenge system and real-time PCR. We could show that the ethylene and jasmonic acid signaling pathway are triggered in cucumber roots inoculated with *T. asperellum*. As the result of this different PR proteins and antibacterial phenolic compounds are systemically induced in the leaves of the plant. These preliminary data provide initial insights at the molecular level on the *Trichoderma*-plant interaction.

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173 - Role of β-1,3-glucanase from *Trichoderma harzianum* cect 2413 in antagonism against phytopathogenic fungi

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T. harzianum CECT 2413 grown under conditions of simulated mycoparasitism secreted several β-1,3-glucanases; the most abundant, BGN13.1, was purified and the corresponding gene, *bgn13.1* was isolated. *bgn13.1* is strongly induced by chitin, autoclaved mycelia or plant extracts and is only expressed at very narrow pH ranges (around 5.5). There is a weak expression of the gene under carbon starvation, and under some stress conditions. The promoter sequence has putative boxes which respond to stress, carbon (but not nitrogen) starvation, regulation by pH and regulation under mycoparasitism (MYC). The presence of MYC boxes in the promoter and the gene induction by chitin strongly suggests that BGN13.1 is involved in antagonism. BGN13.1 was overexpressed in *T. harzianum* and tobacco plants. *T. harzianum* transformants were more aggressive than the wild type in *in vitro* assays against *Botrytis*, *Phytophthora* and *Rhizoctonia* but maximal growth inhibition did not correlate with maximal levels of β-1,3-glucanase activity. Combination of BGN13.1 transformants with transformants which overexpress other enzymes showed synergistic effects against fungal strains, but the efficiency of both single transformants or their mixture vary according to the antagonized strain. Plant protection experiments with different *Rhizoctonia* strains, *T. harzianum* and tobacco plants, both wild types and BGN13.1 transformants are currently in progress.

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174 - Signaling cascades involved in the mycoparasitic process by *Trichoderma*

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In the presence of a fungal host *T. atroviride* produces hydrolytic enzymes and coils around the host hyphae. In a biomimetic assay, different lectins induce coiling around nylon fibers; coiling in the absence of lectins can be induced by applying cAMP or the heterotrimeric G protein activator mastoparan. We isolated a *T. atroviride* G alpha subunit gene (tga1). Transgenic lines generated that overexpress the Ga protein show very delayed sporulation and coil at higher frequency. Furthermore, transgenic lines that express an activated mutant protein with no GTPase activity do not sporulate and coil at a higher frequency. Lines that express an antisense version of the gene are hypersporulating and coil at much lower frequency in the biomimetic assay. Loss of Tga1 in these mutants correlates with the loss of GTPase activity stimulated by the peptide toxin mas-7. In contrast, cAMP levels and cAMP-dependent protein kinase activity drop when diffusible host signals are encountered and the mycoparasitism related genes ech42 and prb1 are highly expressed. Herewith we show that the prb1 is subjected to Nitrogen Catabolic Repression. Accordingly, induction of prb1 transcription by *Rhizoctonia solani* cell walls requires the release from a repressed condition, which is determined by nitrogen availability. prb1 transcription in response to nitrogen limitation, may be mediated by a MAPK signaling cascade.

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175 - Components involved in regulation and signaling of the mycoparasitic response of *Trichoderma atroviride*

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T. atroviride (former *T. harzianum*) strain P1 acts as a mycoparasite and is applied as biological control agent against a number of plant pathogenic fungi. The mycoparasitic interaction is host-specific and not merely a contact response. It is thus likely that signals from the host are recognised and provoke antifungal activities which are accompanied by morphological changes and the secretion of hydrolytic enzymes. To understand the signalling mechanisms involved in this process, we have begun to investigate the cis- and trans-acting factors controlling expression of the chitinases, using nag1 as a model because its disruption leads to a complete loss of expression of all chitinases. Three motifs were detected and characterized in vivo and in vitro. A gene (seb1) encoding a protein binding to one of these motifs (a CCCCT-box) was cloned from *T. atroviride* and its biochemical and functional characterization will be reported. Further, towards an elucidation of the link between chitinase gene expression and the mycoparasitic response, we have cloned key components of the cAMP and MAP kinase signaling pathways in *T. atroviride* and started their functional characterization by gene disruption. First results from this will be presented.

176 - Taxonomic perspectives on some ascomycetous endophytes of mycorrhizas

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Roots are habitat for a wide variety of fungi including a large number of ascomycetes. Several groups of ascomycetous anamorphs consistently appear during isolation protocols using traditional culturing methods or where diversity estimates are made using DNA analyses. Among the taxa recovered, *Phialocephala fortinii*, *Leptodontidium orchidicola*, *Scytalidium vaccinii* and similar species (teleomorphs apothecial or suspected as such), *Oidiodendron* spp., and *Geomyces pannorus* (teleomorphs cleistothecial) are frequent Leotiomycetes. Other taxa include *Heteroconium chaetospira* and species of *Phialophora*, *Cladosporium*, *Phoma*, and large numbers of unidentified or partially characterized sterile taxa. In most cases, the nature of the association between these fungi and the host plant roots is unknown but their well developed saprobic abilities suggest their role as mutualists with plants is probably not mycorrhizal in the usual sense. Continuing isolation work along with morphological, enzymatic and molecular characterization, coupled with resynthesis studies with host plants, will lead to a clearer understanding of the niches occupied by these fungi in roots and mycorrhizas.

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177 - Non-mycorrhizal root endophytes - aspects on their ecology

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Non-mycorrhizal root endophytes are a heterogeneous group of fungi with possible beneficial associations with their host plants. Recent data from a primary successional sub-alpine ecosystem and a secondary successional temperate grassland ecosystem suggest greater abundance of non-mycorrhizal endophytes than mycorrhizal fungi. The abundance of mycorrhizal and non-mycorrhizal, root-inhabiting fungi may fluctuate according to physiology or phenology of the host. Two competing hypotheses are presented: 1) these fungi are parasitic and tap into the host photosynthate translocation during seasonal changes in host physiological activity, 2) these fungi are actively involved in controlling host photosynthate reallocation. To identify the dominant fungi in rhizosphere, a shotgun cloning experiment was conducted using environmental DNA from soil and rhizosphere samples. Fungi in the shotgun cloned rDNA libraries represented ascomycetes, basidiomycetes, chytridiomycetes and zygomycetes. Detected fungal communities were concluded to be very similar in soil and rhizosphere. This is interpreted to indicate that root-inhabiting fungi frequently possess extramatrical hyphae extending to the soil. Alternatively, large proportion of the root and rhizosphere inhabiting fungi are common soil fungi and facultatively colonize host tissues. The potential of soil-inhabiting fungi having biotrophic and/or endophytic phases will be discussed.

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178 - Ectendomycorrhizas - specialized associations between members of the Pinaceae and ascomycete fungi

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Ectendomycorrhizas (ECM) are formed in two genera, *Pinus* and *Larix*, when roots are colonized by a few ascomycetous fungal species, primarily *Wilcoxina mikolae* and *Wilcoxina rehmii*. Two dematiaceous species and *Sphaerosporella brunnea* have also been reported to form ECM with these genera. Some of these fungi are capable of forming typical ectomycorrhizas with other conifer genera and with angiosperm species. Reports of other genera of conifers and angiosperms possessing ECM are based on field collections of roots of unknown age and thus may represent senescing ectomycorrhizas. ECM are common in *Pinus* and *Larix* seedlings in nurseries, regenerated seedlings on degraded sites, and in some natural forest sites. The identifying structural characteristics of ECM are a limited mantle, an Hartig net, and intracellular hyphae forming hyphal complexes. The latter form soon after Hartig net development. Intracellular hyphal complexes occur in epidermal and cortical cells and are surrounded by host-derived plasma membrane and an interfacial matrix of unknown composition. Although ultrastructural features of the fungus-root cell interface suggest that these may be sites of nutrient exchange, there is no evidence for this. It is known that some of the fungi involved are able to breakdown complex carbohydrates but carbon transfer to young seedlings has not been demonstrated. Suggestions have been made that ECM may be important in the establishment of seedlings in harsh environments.

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179 - Community structure and phylogeny of ericoid and epacrid mycorrhizal fungi

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To understand the community structure of ericoid mycorrhizal fungi in the roots of ericaceous and epacridaceous plants, researchers have cultured hundreds of fungal isolates from thousands of mycorrhizal root

pieces. Because many isolates do not sporulate and therefore cannot be identified, researchers have turned to molecular tools: RFLP and sequence analysis. We have mapped the fine-scale distribution of the fungi cultured from roots and found that in most cases the same fungus did not grow out of adjacent 2mm root segments. However, not all ericoid or epacrid root endophytic fungi are culturable. We have found two different communities of fungi when isolating and cultivating versus cloning PCR-amplified DNA from the same roots. We have also accessed the 200+ sequences in GenBank of ericoid and epacrid mycorrhizal fungi and root endophytes from a number of research groups and carried out phylogenetic analyses. These analyses indicate there are only 7 ericoid mycorrhizal fungi or fungal groups in the parts of 3 continents that have been studied thus far. These same 7 fungi can also be found in the roots of a single plant on a site on northern Vancouver Island, British Columbia.

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180 - Ascomycetous mycorrhizae from prescribed burn in ponderosa pine forest from Northwest, USA

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Post-fire Pezizales include members that fruit as early as two weeks and others as late as two years after a fire. Despite frequent occurrence, little is known about their ecological function. Ponderosa pine forests have a natural fire regime that includes low intensity fires at a 15 to 30 year return interval. Our objective is to determine if members of the post-fire Pezizales associated with ponderosa pine forests are mycorrhizal, which may aid in re-generation of these forests. Because fungi are patchily distributed over the landscape, and the location of associated mycorrhizal root tips difficult to determine, we collected soil cores directly beneath Pezizalean sporocarps from three sites that had been burned to mimic the natural fire regime. Root tips from the soil cores were sorted into morphological groups. Restriction fragment length polymorphism (RFLP) patterns were obtained for each morphological group to compare to RFLP patterns of collected sporocarps. Twenty three patterns were found but did not match any of the five genera and ten species of Pezizalean sporocarps collected. Sequence analysis using the small ITS region of the nrDNA was done for comparison with Ascomycetous sequences, representing both mycorrhizal and reputed non mycorrhizal species. Matches were found with *Geopora* spp. and *Wilcoxina* spp.

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181 - Mycorrhiza-associated sterile fungi in a Mediterranean environment: systematic heterogeneity versus ecological convergence?

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Mediterranean ecosystems, featuring high plant diversity and co-dominance, offer an interesting scenario for the study of root-endophytic dark sterile mycelia (DSM) in different hosts. Investigation of ectomycorrhizal *Pinus halepensis* and endomycorrhizal *Rosmarinus officinalis* plants in Italy indicated that neighboring healthy individuals of these two hosts harbor several DSM morphotypes. Sequence analyses of nuclear ITS and 18S rDNA regions of the dominant morphotypes allowed their identification as a unique spectrum of DSM, being quite diverse and distinct from those so far described in other host plants investigated. Recognition of systematic affinities was possible with varying degrees of resolution for the different morphotypes, ranging from species-level identification to placement within Ascomycete subclasses. Association of these fungi with both hosts spanned at least eleven years; they thus appear to be true root colonizers coexisting with the accredited mycorrhizal symbionts. In spite of their taxonomic diversity, such DSM displayed some morphophysiological convergence justifying their recognition as an ecological group. One morphotype was identified as *Rhizopycnis vagum* D.F. Farr, a recently described fungus so far known as a root pathogen of crop plants. Studies of population genetics of this fungus and pathogenicity assays are unraveling the actual range of its ecological plasticity.

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182 - Mutualistic colonization of roots by endophytic fungi

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Fungal colonization of plant roots is often mutualistic, a well known example being that of mycorrhiza, but also interactions of dark septate endophytes (DSE) and the mycorrhizal-like basidiomycete *Piriformaspora indica* with roots of their hosts may counteract the negative effects of stress or improve growth of the host. We compared endophytic colonizations of the roots with those of above-ground plant organs. Endophytic infections of *Fusarium* spp. in the shoots of bean and barley were asymptomatic,

intercellular, remained localized and had no significant effects on yield, carbohydrate metabolism, stress tolerance or induced resistance of the host. In contrast, endophytic colonization of the roots of bean with *Fusarium* sp. and larch with *Cryptosporiopsis* sp. and *Phialophora* sp. (a DSE), was extended and both inter- and intracellular and improved growth of the host. A culture extract of *Phialophora* sp. not only improved growth of the host, but also led to increased branching of the roots of the treated seedlings. The mutualistic effects may in part be due to the capability of the endophytes to synthesize IAA. We hypothesize that fungal colonization of the roots is more likely to be mutualistic than that of the above-ground organs: a) endophytic root colonization in contrast to that of the shoots is frequently systemic, b) the shoot synthesizes its own metabolites and c) microorganisms in roots are in close contact with an environment harboring many degradative products.

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183 - Is Asia the centre of origin for the megagenus *Cordyceps*?

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Cordyceps is panglobal with many anamorphs: most poorly known. Of its anamorphs, *Beauveria*, *Hirsutella*, *Metarhizium*, *Nomuraea*, *Paecilomyces* and *Verticillium* have been assessed as potential biocontrol agents. *Beauveria*, *Metarhizium* and *Paecilomyces* have species that seem to have lost the *Cordyceps* form, becoming panglobal and with wide host ranges. Significantly, all 3 genera are linked to *Cordyceps* in Asia (China, Hong Kong, Japan, Korea and Thailand). *Cordyceps* of *Beauveria* and *Metarhizium* are from Coleoptera while that of *Paecilomyces* is from Lepidoptera. These insect orders have the most *Cordyceps*: 88 spp. from Thailand with 43 off Coleoptera (15) and Lepidoptera (28). This is ca. 2x the number for N. America. Although major surveys of *Cordyceps* are known from several countries (Brazil, Congo, Ecuador, Ghana, Japan, Korea, N. America, Sri Lanka, Taiwan) only 13 of 88 species from Thailand are reliably named to species. Significantly, comparative surveys in Thailand and Japan indicate that both countries have a rich but disparate biodiversity. A survey of Thai *Cordyceps* has no slowing in discovery of new records/species: few countries enjoy such scrutiny. However, I posit that significant surveys from other countries suggest that if *Cordyceps* producing *Beauveria*, *Metarhizium* or *Paecilomyces* were present there should, by now, be records. That there are not implies an important role for East Asia as a possible Centre of Origin for *Cordyceps* and its anamorphs.

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184 - Changes in nutritional mode within lineages of insect-associated fungi

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Diverse groups of insects support the growth of fungal parasites. They range from deadly pathogens to obligate biotrophs that derive nutrition without apparent harm to their insect hosts. Some of the biotrophs have reduced thallus morphology resulting in a loss of characters that would be useful in phylogenetics. The absence of morphological characters puzzled eminent mycologists such as Roland Thaxter, who studied the fungi. More recent phylogenetic studies of the fungal parasites of insects, including termites and beetles, support several independent origins of fungal associates of insects. Furthermore, the studies suggest that one group of endosymbionts arose from within a lineage of lethal pathogens. Other examples provide evidence that certain fungal biotrophs probably arose from within lineages that included mycoparasites with complex life histories. The reliance on insects to disperse mycoparasitic fungi to fungal hosts may have lead to the fungal host being bypassed with a host shift to the insect as the sole host. Although some hypotheses (Cope's Law), propose that obligate parasites are evolutionary dead ends, this may not always be so.

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185 - Field studies employing a recombinant mycoinsecticide (*Metarhizium anisopliae*) reveal that it is rhizosphere competent

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In the summer of 2000, we released genetically altered insect pathogenic fungi onto a plot of cabbages infested with *Trichoplusia ni* (cabbage looper caterpillars) at a field site in Maryland. The transformed derivatives of *Metarhizium anisopliae* ARSEF 1080, designated GPMa and GMa, carried the *Aequorea victoria* gfp gene alone (GMa) or with additional Pr1 protease genes (GPMa). The study: a) confirmed the utility of gfp for monitoring *M. anisopliae* in field populations over time; b) demonstrated little dissemination of transgenic strains, and produced no evidence of transmission by non-target insects; c) found that recombinant fungi were genetically stable over one year under field conditions, and d) determined that deployment of the transgenic strains did not depress the culturable indigenous fungi. Monitoring the fate (survivorship) of transformants under field conditions revealed that they decreased over several months, except in the inner rhizosphere demonstrating that rhizospheric soils are a potential reservoir for *M. anisopliae*. Overall, our results place a sharp focus on the biology of the soil/root

interphase as a site where plants, insects and pathogens will interact to determine fungal efficacy, cycling and survival. However, the rhizospheric effect was less marked for GPMa, and overall it showed reduced persistence in soils compared to GMa.

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186 - Entomophthorales host-pathogen relationships: ecology, pathobiology and molecular characterization

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Fungi from the order Entomophthorales are pathogenic to arthropods, especially insects. They are highly specialized and provide us with valuable insight in the development of host-pathogen relationships and are also potential biological control agents. Attention will be given to three host-pathogen systems, which recently were studied with respect to ecology, pathobiology and molecular characterization. 1) *Entomophthora muscae* complex and hosts from Diptera. Morphological and pathobiological characters allow us to discriminate between the limited number of recognized species, but the inclusion of molecular data strongly suggest that each host-pathogen system is independent. Each fly species thus harbors its own selection of genotypes 2) *Strongwellsea* spp. and hosts from Diptera. This genus disperses conidia from abdominal holes in the still living hosts. We have found infections in many dipteran families, including in the arctic. Morphological and pathobiological characters indicate a high diversity and specialization. 3) *Pandora neoaphidis*, *Neozygites fresenii* and *Entomophthora planchoniana* and hosts from Aphididae. These fungi are adapted to the holocyclic life-cycle of many host aphids. Further, a recent study has revealed that two clonal populations of an anholocyclic aphid host each harbored only one of these pathogens.

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187 - The occurrence and stability of the entomogenous hyphomycete fungi *Metarhizium anisopliae* and *Beauveria bassiana*

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There is considerable interest in the development of entomogenous, hyphomycete fungi, such as *Metarhizium anisopliae* and *Beauveria bassiana*, for the control of arthropod pests. The successful deployment of these biocontrol agents will depend on a thorough understanding of their ecology and population dynamics. Biochemical and molecular markers have been developed to study: (1) the occurrence of indigenous strains, (2) the efficacy and fate of released (exotic) strains, (3) impact of fungi on non-target organisms, and (4) fungal stability. The latter has received considerable attention because it influences sporulation, virulence, host specificity and ecological fitness which, in turn, influences production costs and efficacy. *In vitro* studies show that some strains of entomogenous fungi are more stable than others. Some of the changes observed may be due to parasexual recombination, gene silencing or gene deletion. Recombinants were recovered from insects co-infected with two strains of either *M. anisopliae* or *B. bassiana*.

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188 - Biodiversity of thermophilic fungi

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Critical knowledge on the biodiversity of thermophilic fungi has been hindered by the proposal of several unwarranted nomenclatural and taxonomic decisions which have led to a misunderstanding of the taxonomic concepts of some thermophiles. The situation has been further aggravated by the current chaotic state in binomial citations of common members of the group. The systematics of thermophilic fungi has been recently re-appraised in a series of contributions which have appeared almost 30 years after the last major monograph on their taxonomy, biology and economic importance (Cooney and Emerson, 1964). To date, about 50 new names have been introduced at both species and varietal levels; a total almost equal to earlier names reported for the group. However, some of these introductions proved not to be justified. Indeed, some name changes do not appear to be supported by sound taxonomic arguments. Thermophilic fungi now acceptable as valid from a nomenclatural point of view comprise about 40 taxa. Thus some Mucorales, several Ascomycetes and hyphomycetes and a single agonomycete could appear to be true thermophiles. However, from a taxonomic point of view, the status of some species still requires further study. Once these basic studies have been completed, they will guarantee that a sound database exists for thermophilic fungi. Such taxonomic stability will also ensure that any new discoveries of interest have the potential for eventual industrial application.

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189 - Heat and the physiology of thermophilic fungi

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The thermophilic fungi compose a small ecological group defined solely on temperature requirement for growth, ranging from 20 °C to at or above 50 °C. There are only about 40 known species of thermophilic fungi and for only

a small number of these fungi the parameters for growth have been investigated. Thermophilic fungi are found in the environment eg compost pilesand bird nests where they will be exposed to different and shifting stress factors like high temperatures and nutritional limitations. In higher organisms, thermal adaption requires a concerted action at different levels whereas non-motile microorganisms exclusively have to cope with this type of changes by cellular mechanisms. The permissive temperature for a thermophilic fungus is above the heat-shock temperature for mesophilic fungi. This means that even the housekeeping proteins of the thermophiles may need some kind of heat-shock adaption at its optimal growth termperature. This implies a general heat-adaptive mechanism already constitutively at work in thermophiles compared to mesophiles. Different aspects of growth at elevated temperatures will be evaluated and a verification of maximum growth upto 62 °C of a thermophilic fungal strain will be presented. The stress response of different thermophilic fungal species/isolates will be compared in order to determine, whether the thermophilic fungi can be characterized by other criteria than the temperature range for growth.

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190 - Functional diversity of thermophilic fungi in mushroom compost

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The species richness of thermophilic fungi in mushroom compost (and its ingredients) was 22 species. Mixed ingredients, starting to self heat, were species rich as was the alternatively surveyed substrate of pigpen litter (sawdust based). We assume that more species were present and ways of finding them will be discussed. The final product, mushroom compost, is an artificial, strongly manipulated, ecosystem. *Scytalidium thermophilum* dominates and *Talaromyces thermophilus* and *Thermomyces lanuginosus* can only be isolated after the selective killing of *Scytalidium*. *Scytalidium*'s high growth rate and its cellulolytic ability are probably important for its success. *Talaromyces* and *Thermomyces* tolerate higher temperatures but grow relatively slow. Under mesophilic conditions, after inoculation with mushroom mycelium, *Scytalidium* stimulates the extension rate of growth of the mushroom mycelium. Also other organisms, some pure cultured thermophilic fungi, like *Myriococcum thermophilum*, and enrichment cultures of prokaryotes in compost, are stimulatory to mushroom mycelium. The interactions of these species with the substrate and with the other species present will be discussed. We will try to identify the key characteristics of species related to these interactions. Our research contributes to the general understanding of composting (turnover, emissions, stabilization) and the rational use of agricultural wastes for new products.

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191 - An industrialists view on thermophilic fungi as sources for industrial applications

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Many industries need enzymes that are active at, or can resist, high temperatures in compliance with the nature of the industrial processes and substrates. If the enzyme can be added in an existing industrial step without justification of temperature or pH, savings of costs and efforts can be done. Many bacteria and Archaea from thermal areas have been explored for the industry, and have provided it with enzymes which are thermoactive and stable at above boiling temperatures. Fungi can not live in as high temperatures, and generally do not display as thermoactive enzymes as some prokaryotes do. On the other hand, fungal enzymes are generally more acid stable than bacterial enzymes, which is another important factor for many industries. Also, fungi can produce enzymes with different functions than some bacterial enzymes, and are, despite their less thermophilic nature, of large interest for the industry. However, can we expect that thermophilic fungi, with optimum growth temperatures up to only ca 20-30 °C above that of non-thermophilic fungi, make enzymes that are more thermostable than the enzymes from these fungi? The general question 'Do fungal growth profiles correlate with enzyme characteristics' is both of academic and industrial interest and can be enlighted with thermorelated studies, as this physiological niche probably is more explored than any other physiological growth niche.

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192 - Settling differences: Evolution of recognition genes

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Mating compatibility genes, vegetative incompatibility genes and in plants, genes conferring resistance to pathogens have evolved under similar evolutionary pressures and consequently often display common genealogical patterns of diversification. Fungi sense their biotic environment as a series of encounters with other organisms. The recognition genes they carry help to classify such biotic interactions as same or different - same or genotype, species, or a compatible mate. Similarly, in host plant-pathogen interactions, plant fitness can be dependent upon recognition and response to fungal invaders. Because the biotic environment directly affects organismal fitness, genetic systems allowing recognition and response to other genotypes within a species and to other species have evolved. We will discuss our research results in mating genes as well as plant resistance genes in order to illustrate of common and different evolutionary themes.

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193 - Pheromone receptors of the basidiomycete *Schizophyllum commune* distinguish single amino acid differences among active mating pheromone ligands

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The linked, multigenic loci B-alpha and B-beta of the homobasidiomycete *Schizophyllum commune* contribute to the specification of more than 20,000 mating types. Nine versions each of B-alpha and B-beta have been described by genetic methods; only portions of a few of these versions have been described by molecular techniques. These versions carry different but related sets of genes encoding lipopeptide pheromones and 7-transmembrane-domain receptors. We sequenced the linked pair B-alpha3-B-beta2 and we can account for all known mating activities of a B-alpha-B-beta complex for the first time. There are eleven pheromone genes and two receptor genes within the B-alpha3-B-beta2 complex. The amino acid sequences of predicted pheromones and receptors from B-alpha3-B-beta2 were compared with other *S. commune* mating pheromones and receptors to decipher how specificity is achieved in their interactions. These pheromones and receptors can be functionally expressed in *Saccharomyces cerevisiae* and show the same specificity as seen in *S. commune*. Using a combination of heterologous and homologous expression systems, we can show that single amino acids in some pheromones control which receptors (i.e. mates) are activated for pheromone response. The receptors likewise can be manipulated to have altered pheromone recognition or to be active in the absence of pheromone. Thus, a finely tuned set of molecules maintains outbreeding while prohibiting self-activation of the mating process.

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194 - Mating recognition in ascomycetes

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In heterothallic Euascomycetes, mating is controled by the mating-type alleles (idiomorphs) which allow compatible strains to recognize each other and to proceed to fertilization. Fertilization is not immediately followed by karyogamy. Instead, the parental nuclei divide mitotically several times inside the female organ and are intermingled in plurinucleate cells. Then nuclei from male and female origin, *i. e.* nuclei with opposite idiomorphs, recognize each other and form dikaryotic hyphae. This step involves at least two key events: internuclear recognition (IR) and cellularization. The dikaryotic hyphae divide mitotically, maintaining a strict ratio of 1:1 of each parental nucleus. Eventually pairs of nuclei fuse and meiosis and ascospore formation ensue immediately. A major challenge is to understand the relations between the idiomorphs and the developmental events of the sexual cycle. *Podospora anserina* is used as a model system for investigating the functions of its *mat*+ and *mat*- idiomorphs and the molecular mechanism of IR. The *mat*+ sequence contains one gene, *FPR1* and the *mat*- sequence contains three genes: *FMR1*, *SMR1* and *SMR2*. *FPR1*, *FMR1* and *SMR2* encode transcriptional factors which control fertilization and IR. A model, based on genetics, has been proposed for the control of IR by the *mat* genes and will be presented. IR is followed by a developmental arrest which is overcome by *SMR1*. The current hypotheses about the molecular mechanism of IR will be discussed.

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195 - Gene deletion and unidirectional mating type switching in *Ceratocystis*

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Four of the five major clades within *Ceratocystis* have homothallic species. Within the *C. coerulescens* clade there are two closely related species with contrasting mating systems: *C. eucalypti* is heterothallic, while MAT-2 strains of *C. virescens* are self-fertile due to unidirectional mating type switching. Most strains of *C. virescens* recovered from nature are self-fertile and behave as MAT-2 in crosses, progeny from selfings or from crosses segregate in a 1:1 ratio for mating type, and MAT-1 progeny are self-sterile. Mating type idiomorphs and flanking regions were sequenced for both mating types of these two species. In *C. eucalypti*, the idiomorph has either the *MAT1-1-2* and the *MAT1-1-1* genes or only the *MAT1-2-1* gene, depending on mating type, typical of a heterothallic ascomycete. All strains of *C. virescens* have the *MAT1-1-2* and *MAT1-1-1* genes (as in MAT-1 strains of *C. eucalypti*), but self-fertile strains also have the *MAT1-2-1* gene and another copy of the *MAT1-1-1* gene. Half of the progeny recovered from selfings retain this arrangement of four mating type genes in the idiomorph and are self-fertile, but the other half (MAT-1, self-sterile) are missing a 4.7 kb fragment that includes the *MAT1-2-1* gene and one copy of the *MAT1-1-1* gene. The inherited deletion irreversibly changes the mating type to MAT-1 and apparently allows mating with MAT-2 nuclei. This is the first sequencing of the DNA fragment deleted in unidirectional mating type switching.

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196 - Examining the relationship between nonself recognition, heterokaryon incompatibility and hyphal fusion in *Neurospora crassa*

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Filamentous fungi grow by tip extension, branching and hyphal fusion to form a hyphal network. Filamentous fungi can also undergo hyphal fusion between individuals to make vegetative heterokaryons. Recognition of nonself in such heterokaryons is mediated by genetic differences at *het* loci. Heterokaryons or transformants that contain alternative *het* alleles show severe growth inhibition, conidiation suppression and death. We use *Neurospora crassa* to study the molecular mechanism of hyphal fusion and the consequences of fusion between *het* incompatible individuals. Live cell imaging of hyphal anastomoses showed a self-signaling phenomenon which initiates the multi-stage process of fusion. We identified four mutants that fail to undergo hyphal fusion. Two mutations are in genes encoding signal transduction components; these mutants also suppress heterokaryon incompatibility mediated by the *mat* locus. Nonself recognition during heterokaryon incompatibility has been shown to be mediated by the formation of a HET heterocomplex. Suppressor analyses identified two additional loci that are required for mediating heterokaryon incompatibility. One of these mutants suppresses heterokaryon incompatibility at both the *mat* and *het-c* locus. By these analyses, we hope to shed light on nonself recognition mechanisms in filamentous fungi that underlie heterokaryon incompatibility and explore its relationship to the hyphal fusion process.

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197 - Somatic incompatibility in basidiomycetes. Genetic analysis in *Heterobasidion*

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In basidiomycetes, intraspecific recognition of self and non-self is mediated through the somatic incompatibility (SI) system (similar to the vegetative compatibility system in ascomycetes). It is expressed in the vegetative phase of growth both in homokaryons and in heterokaryons when dissimilar genotypes meet. In the homokaryotic stage, the system can be overridden by mating. SI involves post-fusion cell death, and depending on the species involved, activation of phenol oxidases, melanin production, formation of clear zones of collapsed aerial hyphae, hyphal knot cells or dense so called barrages. The genetic control of SI has been studied in less than ten species. In members of the genus *Phellinus*, SI was under control of one major gene, while 3-4 genetic loci have been implied in *Heterobasidion*, *Collybia* and *Pleurotus*. In this presentation we describe a QTL approach to characterise the genetic and molecular background to SI in *Heterobasidion annosum*. A genetic map was constructed using AFLP markers for a cross between a S and a P homokaryon of *H. annosum*. Siblings were mated with the parent mycelia and resulting heterokaryons, differing in one sib-related nucleus, were paired in all combinations. Putative loci for SI were mapped based on the incompatibility reactions. Implications for future work will be discussed.

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198 - Overview of fungal lignin degradation

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Lignocellulose, a major component of woody biomass, is the most abundant renewable organic material in the biosphere and has important applications as a resource for producing pulp and paper, animal feeds, and industrial chemicals. Lignin, which limits access of microbial enzymes to cellulose and hemicellulose in woody materials, is a key limiting factor not only in the efficient conversion of the latter to useful materials but also in global carbon cycling. White-rot basidiomycetes are efficient lignin degraders in natures and produce one or more of three major classes of lignin-modifying enzymes (LMEs) designated: laccases, lignin peroxidases (LiPs), and manganese peroxidases (MnPs). LMEs are extracellular, non-specific, metalloenzymes and catalyze radical-mediated oxidative degradation reactions. Cumulative evidence indicates that a large number of isoenzymes of LiPs, MnPs, and laccases are produced by various ligninolytic fungi and that different isoenzymes appear to be encoded by individual genes which are distributed on multiple chromosomes. Regulation of production of LMEs appears to be subject to complex interplay of nutritional, environmental, temporal, and genetic factors. An overview of the studies on the physiology and molecular biology of LMEs as well as applications of ligninolytic fungi for bioremediation of priority environmental pollutants will be presented.

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199 - Biochemical and molecular aspects of lignin degradation by *Pleurotus* species

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Pleurotus species are investigated due to their ability to degrade lignin selectively. Their ligninolytic system is different from that of the model white-rot basidiomycete *Phanerochaete chrysosporium*. Extracellular oxidoreductases have been characterized from *Pleurotus eryngii* including versatile peroxidase (VP), aryl-alcohol oxidase (AAO) and laccases. These enzymes are of biotechnical interest for degradation of lignin, aromatic compounds and dyes. *P. eryngii* laccases oxidize lignin *via* natural redox mediators and contribute to oxygen activation by redox cycling of lignin-derived quinones. AAO and VP, which have been recently cloned, crystallized and expressed in *Escherichia coli* and *Emericella nidulans*, are characteristic of the ligninolytic system of *Pleurotus* (and *Bjerkandera*) species. AAO provides hydrogen peroxide for peroxidase activity and generation of active oxygen species. VP represents a third type of ligninolytic peroxidase combining the catalytic properties of lignin peroxidase and manganese peroxidase (first described in *P. chrysosporium*) due to an hybrid molecular architecture including sites for oxidation of both Mn(II) and aromatic substrates. AAO and VP molecular models were obtained by homology modeling (using crystal structures as templates), and the VP crystal structure has been recently solved. Future studies include confirmation of active sites by site-directed mutagenesis, and modulation of catalytic properties by protein engineering techniques.

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200 - Role of reactive oxygen species in wood decay by fungi

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Extracellular reactive oxygen species (ROS) have long been thought to have a biodegradative function in wood decay by fungi, but the nature of these species and the mechanisms for their production have not been elucidated. We monitored the hydroxylation of a synthetic aromatic polymer, phenethyl polyacrylate, to estimate the magnitude of extracellular ROS production by two brown rot basidiomycetes, *Gloeophyllum trabeum* and *Postia placenta*, on cellulose. The results showed that ROS production was sufficient to account for much of the cellulose depolymerization in the cultures. Both fungi produced extracellular 2,5-dimethoxyhydroquinone, a metabolite that rapidly reduces ferric iron and dioxygen, thus yielding hydroxyl radicals via the Fenton reaction. We purified and characterized a *G. trabeum* flavoprotein NADH:quinone reductase that probably drives this chemistry by regenerating the hydroquinone. The gene that encodes the reductase shares substantial similarity with quinone reductase genes of other fungi, including some nonlignicolous species.

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201 - Biotechnological applications of wood decay fungi

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The ligninolytic systems of white-rot fungi have great potential for applications in wood-processing such as pulping, pulp bleaching and wood composite manufacture. For example, the use of white-rot fungi to treat wood chips prior to mechanical pulping (biopulping) is already approaching industrial scale. Furthermore, the low specificity of these systems allows for the conversion of various aromatic pollutants and industrial wastes such as contaminated soil (bioremediation). These processes involve the use of white-rot fungi or their ligninolytic enzymes to treat polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls and other hazardous xenobiotics. Whether to apply living fungi or isolated enzymes will depend on the specific properties of the material to be treated. For example, biopulping takes advantage of the fact that white-rot fungi not only produce a complete set of enzymes but can also transport these enzymes into wood chips and create the appropriate physiological conditions for enzymatic reactions. However, processes involving living organisms are relatively difficult to control. Thus, the use of isolated enzyme systems would be preferable in those cases, where the compounds to be treated are freely accessible such as in soil extracts. This presentation will give a brief overview of biotechnological applications of white-rot fungi and their ligninolytic enzymes and discuss in more detail the use of laccase for the bioremediation of PAH.

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202 - Some approaches to the evaluation of the white rot basidiomycetes ligninolytic activity

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Ligninolytic activity of basidiomycetes from different taxonomic groups has been extensively. The production patterns and levels of laccase and Mn-dependent peroxidase (MnP) differed among species and strains of the

same species studied. Production of MnP was generally optimal in SSF of grapevine cuttings sawdust (GCS), but was repressed by agitation of fungi grown in submerged liquid culture. Conversely, basidiomycetes laccase activity was generally enhanced by agitation. The highest laccase activity (up to 400-800 nkat/ml) was revealed in a submerged fermentation of mandarin juice production wastes by the strains of genera *Cerrena*, whereas extremely high Mn-dependent peroxidase activity (50-180 nkat/ml) was found in a SSF of the GCS by *Pleurotus ostreatus* and *Lentinus edodes*. Among the factors tested, only carbon sources and low-molecular-weight aromatic compounds in the media have been shown to significantly influence ligninolytic activity of basidiomycetes. However, the response of different fungi strains is greatly depending on chemical nature of the carbon source. Concerning nitrogen source only increased basidiomycetes growth at presence of additional nitrogen accounts for the higher levels of enzymes activity. This work shows that different approaches might be used to regulate basidiomycetes ligninolytic activity in general or activity of certain enzyme, however, caution is necessary when evaluating ligninolytic capabilities of the white rot fungi.

203 - Role of reticulation events in the co-evolution of *Epichloë* grass endophytes

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The *Epichloë* (anamorph = *Neotyphodium*) species are fungal endophytes of grasses spanning an evolutionary continuum from antagonistic sexual species to mutualistic asexual species. The asexual endophytes are strictly seed-borne (vertically transmitted), whereas the sexual endophytes can transmit contagiously (horizontally). Most of the sexual species are highly host specific, but their occasional jumps between host tribes can generate strictly seed-borne lineages. The fact that the resulting endophytes express no male or female reproductive structures, and no contagious spores on their new hosts probably reflects their relatively short history of coevolution with those grasses. Although such endophytes are asexual, they remain capable of parasexual interactions with horizontally-transmitted *Epichloë* spp., giving rise to aneuploid or polyploid hybrids. Out of 32 phylogenetically distinguishable asexual lineages, 21 were hybrids of two or three biologically and phylogenetically distinct sexual species. Among endophytes in certain host genera the likely host jump and subsequent hybridization events can be discerned. For example, *Epichloë bromicola* apparently jumped from *Bromus* into *Hordeum*, then underwent several hybridizations with *Epichloë typhina;* both the non-hybrid and hybrid descendants occur in extant *Hordeum* populations. Likewise it appears that an asexual endophyte in a *Lolium* ancestor underwent at least five hybridizations during the emergence of three host species.

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204 - Fitness costs in hybridization events between allopatric *Tilletia* species

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A closely related group of *Tilletia* species infects wild and cultivated hosts in the grass subfamily Pooideae. This group of smut fungi includes the wheat common and dwarf bunts, *T. tritici*, *T. laevis*, and *T. controversa*. A study of genetic relationships among isolates of common and dwarf bunts based on RAPD analysis supported natural hybridization as an explanation for the existence of isolates with reciprocal characteristics of spore morphology and germination. Nuclear rDNA sequence and RAPD analyses showed *T. bromi*, a bunt that infects brome grasses growing as weeds in wheat fields in the Pacific Northwestern USA, to be the species most closely related to the wheat bunts. The ability of *T. controversa* to form interspecific hybrids with *T. bromi* was examined. *Tilletia controversa* was hybridized with *T. bromi* and *T. laevis* in a susceptible cultivar of wheat under greenhouse conditions. *Tilletia controversa* x *T. bromi* progeny exhibited reduced fitness relative to *T. controversa* x *T. laevis* progeny as measured by basidiospore mortality, basidiospore fusion, and pathogenicity in wheat. Additionally, no evidence of natural hybridization or introgression based on RAPD analysis was detected between allopatric populations of *T. controversa* and *T. bromi*. Our data suggest reproductive compatibility is retained among species of *Tilletia* infecting pooid hosts, but progeny produced between species with distinct host ranges are unlikely to survive under natural conditions.

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205 - Stumps as a new niche for interspecific hybridization in the *Heterobasidion* spp. complex. Ecological instability vs. the genetic stability of hybrids

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The five known taxa in the *Heterobasidion annosum* Coll. complex display a marked host preference. This niche partitioning has strengthened the genetic isolation among these species, in spite of their potential interfertility. Through field and greenhouse experiments, we show that host preference is lost on tree stumps as well as in pine trees exposed to high levels of inoculum. An analysis of *Heterobasidion* species composition in stumps shows significant sympatry between species. A comparative analysis in trees shows no shift in species composition. Logging has thus greatly enhanced the potential for contact between species. An analysis of *Heterobasidion* species composition from the airspora, shows ratios resembling species composition from stumps rather than from trees. This finding underlines the impact of stumps on *Heterobasidion* species composition. Effects of stump creation is variable. Where mating barriers between species are strong (e.g. the Alps), little evidence of hybridization and gene introgression have been found. Where partial interfertility is present (e.g. California), hybrids and evidence of past and present gene introgression can be found. Although hybrids do not appear to have enhanced pathogenicity we show through inoculation experiments that they can be as fit as one of the parental species, especially on tree stumps. We finally show that hybrids form diploid hyphae, thus ensuring long-term genetic stability.

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206 - Influence of selection pressure on the outcome of current interspecific gene flow between *Ophiostoma* species

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The Dutch elm disease fungus *Ophiostoma novo-ulmi* has recently invaded areas of the northern hemisphere previously occupied by another DED pathogen, *O. ulmi*. This has enabled interactions between the two species, resulting in a series of unusual evolutionary events. *O. novo-ulmi* has physically replaced *O. ulmi*, forcing the latter into extinction. While doing so, *O. novo-ulmi* has spread as a series of single mating type single vegetative compatibility type (vic) clones. Locally, these clones have then become rapidly genetically diverse (c. 5-10 years), with many new vic and other phenotypes appearing. The mechanism for this rapid change from clonality to heterogeneity was unknown. Evidence based on co-segregation of AFLP markers and cloning and sequencing of mating type loci indicates that *O. novo-ulmi* has acquired both the new *vic* genes and the A-mating type locus from *O. ulmi* via horizontal gene flow. The *O. ulmi vic* and *mat A* genes appear to have been acquired by *O. novo-ulmi* under selection pressure imposed by deleterious viruses. Other 'less useful' *O. ulmi* genes acquired by *O. novo-ulmi*, such as genes for pathogenicity or for cerato-ulmin (toxin) production, appear not to be selected, probably because they render *O. novo-ulmi* less fit. Furthermore, only c2% of 'foreign' DNA appears to be acceptable if an introgressed *O. novo-ulmi* genotype is to survive.

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207 - Evolutionary processes and genome organisation among emerging interspecific hybrids in *Phytophthora* species

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Whilst common in plants, reports of interspecific hybridisation amongst fungi are scarce. The risks, however, should not be underestimated. Hybridisation may accelerate the evolution of plant pathogenic fungi resulting in rapid adaptation to new plant species or environments. Increasing global trade in plants and, inadvertently, their pathogens, creates opportunities for such events. The genus *Phytophthora* comprises over sixty plant pathogenic species, many with broad host ranges and some responsible for severe crop epidemics or for destabilising terrestrial ecosystems. This, alongside their global distribution, cryptic nature and flexible diploid genetic system serves to increase the risks and threats of hybridisation within the genus. Cytological behaviour, additive nucleotide bases in the ITS region and AFLP and isozyme profiles of a new aggressive Phytophthora pathogen of alder trees spreading in Europe have shown that it comprises a swarm of heteroploid interspecific hybrids between a *P. cambivora*-like species and an unknown *P. fragariae*-like taxon. Developmental and cytological instability and marked phenotypic and genotypic variation all indicate a recent hybridisation. Moreover, mtDNA typing suggests that this occurred more than once. Evidence for the continuing evolution (or stabilisation) of the hybrid genomes will be presented and the challenge of delimiting such hybrid taxa in a way that is practicable for quarantine legislation and diagnosis will be considered.

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208 - Evolution of interspecific *Melampsora* hybrids exhibiting new host specificities

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Interspecific hybridization has been an important tool in plant domestication. Domestication in *Populus*, and other forest trees, is just in its infancy, and hybridization has been the primary means by which poplar breeders have produced desirable plantation phenotypes. Are the pathogens of *Populus* undergoing parallel domestication? The answer appears to be yes for *Melampsora*, and no for *Venturia*. For *Melampsora*, interspecific hybridization of *M. medusae* and *M. occidentalis* now matches that of the respective hosts (i.e., *Populus deltoides* and *P. trichocarpa*). Multiple, exapted genes for rust resistance from both host species have been suddenly overcome in *P. trichocarpa x P. deltoides* hybrids yielding new and complex pathogenic variation. *Venturia*, and other ascomycetous pathogens of *Populus* are similar to a point. Multiple, exapted genes for resistance to *Venturia* (and *Taphrina*, and *Linospora* as well) are also known (from a common-garden study of *P. trichocarpa x P. deltoides* and *P. trichocarpa x P. maximowiczii* pedigrees). However, although European and native species of *Venturia* do co-occur in the Pacific Northwest of the U.S., they have not hybridized. Instead, they remain confined to their respective hosts (i.e., the European *Populus nigra* and the native *P. trichocarpa*, respectively).

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209 - Phylogenetic relationships in Homobasidiomycetes

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The time since IMC6 has seen a dramatic increase in molecular phylogenetic studies of homobasidiomycetes. This talk offers the opportunity for a general overview of recent results from our labs as well as published and unpublished results from other research groups. The eight major clades of homobasidiomycetes identified by Hibbett et al. (1997, PNAS 94:12002) have generally held up to more recent analyses but, despite the increased phylogenetic information of additional sequence data, support for basal divisions, particularly the polyporoid clade, remains weak and placement of some terminal taxa remains equivocal. A picture is emerging of rapid radiations among the basal lineages, echoed by rapid radiations among more recently evolved groups such as the secotioid and gasteroid members of agaric and bolete clades. The consequences of the former are the difficulty of firmly establishing the relationships among major clades and the evolution of fundamental ecological characters such as ectomycorrhizal habit and rot type. The consequences of the more recent radiations in fruiting body morphology include the potential for havoc in our classification system and great fun in the mycology classroom. In any case, molecular phylogenetic studies of homobasidiomycetes are both answering questions of systematics and ecology, and raising many new ones.

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210 - Phylogenetic relationships in the euagarics (Agaricales): insights into morphological and ecological evolution in mushrooms

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Molecular phylogenetics support the view that gilled mushrooms have evolved multiple times from morphologically diverse ancestors, making the Agaricales polyphyletic. Molecular data also show that poroid, sequestrate (e.g., puffballs and secotioids) and reduced (e.g., cyphelloids) forms have evolved several times from gilled basidiocarps. Here we present results from molecular phylogenetic analyses that sampled about one tenth of the total number of species known in the largest natural group of homobasidiomycetes, the euagarics clade (ca. 8400 known species). In many cases, it was possible to resolve natural relationships of several gilled fungi for which taxonomic position has been controversial in the past, and also to unambiguously resolve among the euagarics the systematic placement of many gasteromycetes and reduced forms. The mapping of characters onto phylogenetic trees indicates that ecology, biochemistry and/or physiology rather than morphology often support natural groups of euagarics. Newly discovered phylogenetic affinities include for instance relationships of the true puffballs (Lycoperdales) with the Agaricaceae, of *Panellus* and the poroid fungi *Dictyopanus* and *Favolaschia* with *Mycena*, and of the reduced fungus *Caripia* with *Gymnopus*. Examples of newly discovered monophyletic groups of euagarics include the clades resupinatus, lentinuloideae, Tricholomataceae, Mycenaceae, Agaricaceae, psychedelia, and Pleurotaceae.

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211 - Phylogeny and ecology - what can primitive homobasidioid fungi tell us?

E. Langer

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With special emphasis on Aphyllophorales, this study was carried out to verify hitherto published phylogenies of Hymenomycetes by nuclear large subunit rDNA sequences and comparative morphology. Preferably Aphyllophorales with dolipores having the imperforate parenthesome-type were used to remedy the imbalance in taxa composition of former analyses. The results support the hypothesis of a monophyletic origin of homobasidiomycetes having dolipore parenthesomes perforated by many small holes, including gilled, non-gilled mushrooms and gasteromycetes. Only a minor part of the formally settled orders or families could be verified by this analysis in full respect. Especially the Agaricales ss Singer contain many different related Aphyllophorales. A monophylum comprising non-gilled mushrooms from certain Corticiaceae and Hymenochaetales, having solely dolipores with imperforate parenthesomes is the sistergroup of the homobasidiomycetes having dolipores with perforated parenthesomes. The borderline between heterobasidiomycetes and homobasidiomycetes was detected to be unsharp, because of repeatedly loss of secondary ballistospores in a monophylum nesting the Tulasnellales, Botryobasidiales and cantharelloid taxa.

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212 - Phylogenetic relationships among corticioid fungi

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Basidiomycetes with effused, skin-like basidiomata are often classified together as corticioid fungi. Recent molecular phylogenetic studies have shown that homobasidiomycetes have evolved into at least eight lineages. In these earlier studies only a few corticioid representatives were included but already from a restricted sampling it is obvious that corticioid fungi are phylogenetically highly diverse. We performed a phylogenetic analysis of the homobasidiomycetes with a strong emphasis on corticioid forms. As characters we used nucleotide sites from 5.8S and 28S in the nuclear ribosomal DNA repeat. We recovered the eight clades earlier identified and in addition four new clades composed primarily of corticioid species. We hypothesise that increased sampling of corticioid fungi will reveal still other unique lineages and potential examples are given. The implications for the interpretation of homobasidiomycete evolution and for the design of future studies are discussed.

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213 - Phylogeny of corticioid fungi with russuloid characteristics (the genus *Gloeocystidiellum* in a phylogenetic perspective)

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The classification of fungi has traditionally relied on macro- and microanatomical features of the basidiome. The genus *Gloeocystidiellum* is characterized by the corticioid basidiomata, the presence of SA+ gloeocystidia and amyloid often ornamented spores. Phylogenetic analyses, based on nuclear rDNA, of 127 species within the russuloid lineage recovered 13 supported clades and six single branches. Species that have been combined to *Gloeocystidiellum* occur in 7 of the 13 clades. Other results support monophyly of the russuloid clade, and our data suggest that the presence of a gloeoplerous system, primarily observed as gloeocystidia, is a synapomorphy of russuloid taxa. In addition, compatibility studies were performed to discern biological species within the *Gloeocystidiellum porosum* / *clavuligerum* complex and to characterize the genus type *G. porosum*. Four compatibility groups were detected. Phylogenetic analysis of the complex revealed a great sequence divergence between *G. porosum* and *G. clavuligerum*. Based on morphological data, mating tests and phylogenetic analyses, three species in the complex could be characterised; *G. kenyense*, *G. clavuligerum* and *G. porosum*. *Gloeocystidiellum bisporum* was shown to be a parthenogenetic haploid species, evolved from *G. clavuligerum*. The results indicate that *Gloeocystidiellum* includes only *G. porosum* and a few closely related species.

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214 - Phylogenetic study of Thelephorales - the story of explanatory power of systematics

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Molecular phylogenies of Thelephorales and its genera will be presented. Based on these phylogenies the explanatory power of systematics to answer specific questions on autecology of Thelephorales will be demonstrated. In order to make systematics more powerful and attractive, database-driven Web sites of different taxa should be compiled which include all types of biological information. Such databases will provide facility to study taxon ecology, phylogeny, etc. *in silico* by other fields. The information facility for thelephoroid fungi is built using the PHP scripting language and the MySQL relational database. Access to database and a database administration is done over the Web. Currently the database-driven Web site includes molecular identification of resupinate thelephoroid fungi using ITS1, 5.8S and ITS2 regions of nuclear rDNA and BLAST similarity search. An example how it may be used for the identification of root mycobionts will be shown. In future the database will provide additional facilities for interactive identification of taxa based on morphology, provide information on nomenclature, phylogeny, ecology, etc.

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215 - Phylogenetic taxonomy of *Hymenochaete* and related genera (Hymenomycetes)

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Studies by Wagner and Fischer (2001, 2002) have demonstrated that 19 species of *Hymenochaete* s.l. studied (except *H. tabacina*) form, together with 2 *Hydnochaete* and 2 *Cyclomyces* species, a monophyletic genus in the order Hymenochaetales. We examined the taxonomy of this group adding 16 more species of *Hymenochaete*, *Coltriciella tasmanica* and *Hydnochaete olivacea*. Parsimony analysis of molecular (LSU nucrDNA sequences), morphological and combined datasets was carried out, including representatives of other genera of Hymenochaetales s.str. As an outgroup, 6 corticioid and polyporoid (non-hymenochaetoid) species, characterized by dolipore septa with continuous parenthesomes, were used. About 35% of the *Hymenochaete* species have been included in this study. Most hymenochaetes comprise a clade of closely related species; a separate clade (a new genus?) is formed of 4 species with dendrohyphidia or

denticulate setae. Two species of *Hymenochaete* and *Hydnochaete olivacea* can be added to the monotypic genus *Pseudochaete*. *H. villosa* is different from the other *Hymenochaete* species and belongs to a clade together with *Coltricia* and *Coltriciella*. As a rule, a clade ought to be named a genus only when there are morphological characters making the species group recognizable without molecular analysis. Within *Hymenochaete* s.str., such groups are lacking. Avoidance of schizotaxia (splitting) is advisable irrespective of the use of morphological or mainly molecular characters.

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216 - Building ecology: Influence of distribution, detection and control of biodeterioration

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Under normal circumstances do mould and wood-decaying fungi not have favourably condition for growth in buildings, mainly due to restricted access of water. However, it might occur local areas in constructions where the physical conditions are favourable enough for microbiological activity. Understanding the building ecology with respect to influence of distribution and activity of different fungi, gives possibilities for a successful detection, evaluation and control of the damages. By laboratory and field experiments, it has been proven what physical conditions that are necessary for fungal activity in buildings. The main factors for growth are humidity, temperature and the time of exposure. Other aspects might also have impact, but often to less extent. Due to the great variations of microclimate in a construction, it can be a significant change of the building ecology in few millimetres. This variation often leads to difficulties in the work of building survey. Since the damage development might be caused by small variations in building ecology, the detection and understanding of the physical conditions are important in order to control if remediation has desired effect. Subsequently, the risk of failure in handling damages caused by mould fungi is high - often with a significant impact on the indoor air quality. Guidelines for accomplishing building survey are needed in order to give a better understanding of the building ecology and thereby ensure an optimal result.

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217 - Studies on the dry rot fungus, *Serpula lacrymans*

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Molecular and morphological parameters of *Serpula lacrymans* isolates from various sites in the built environment in Europe and Australia were compared to similar parameters of 'wild' isolates from India, the Sumava Mountains (Czech Republic) and Mount Shasta (U.S.A.). The Indian, Czech Republic and all of the building isolates bar one showed identity in both molecular and morphological features. The Australian and the U.S. isolates (BF-050 and USA'94 respectively) showed specific morphological differences and could be separated on the basis of RAPD-PCR with the U.S. isolate being least closely related to the *S. lacrymans* type strain of FPRL12C. ITS sequence data revealed 2 base differences between FPRL12C and BF-050 in the 673 sequenced, 9 differences between FPRL12C and USA'94 and 16 differences between USA'94 and the closely related organism *Serpula himantioides*. The possible evolutionary relationships between the various isolates are discussed along with suggestions for the origin of *S. lacrymans* as a scourge of the built environment in many temperate areas of the world. We will also report on the use of Indian, US and Czech Republic Isolates of *Trichoderma* in the biocontrol of decay by *S. lacrymans*.

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218 - Moulds in Buildings 1998-2002

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Results of a multidisciplinary research programme: "Moulds in buildings, 1998 - 2002" are presented. The purpose of the programme was to gain more knowledge concerning conditions for mould growth in buildings and the ensuing health effects on people. Furthermore the aim was to create basic knowledge about safe and cost efficient solutions for solving problems and for preventing mould growth in buildings during planning, operation, maintenance and renovation. - A clear correlation was demonstrated to exist between the level of moulds in floor dust and a number of irritative symptoms from eyes and upper airways including the general symptoms such as headaches, dizziness and difficulties in concentration. - The correlation between exposure to moulds from floor dust nd symptoms was typically stronger among pupils with asthma and hay fever. This condition was especially pronounced for irritation of the mucous membranes of the eyes. - Experiments have shown that wood and wood-based materials; wallpaper and materials with glue (starch) have the lowest resistance to mould infestation. - Plaster and concrete, which have a very modest content of organic material, can be infested with mould growth, provided that the materials have a high humidity (water activity) preferably 95% RH. *Danish Moulds in Buildings-Consortium.

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219 - Mycotoxins

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Mycotoxins produced by the building associated funga have been proposed as one of the agents, which are responsible for the adverse health effects, observed among people living or working in damp and water damaged buildings. The vehicle for exposure to mycotoxins in the indoor environ-ment is presumable the fungal spores and fragments of these. The quantities of thee mycotoxins present on building materials are quite low (ng-µg/cm^2 range), and air concentrations generally so low that that they can not be measured. Penicillia do not produce many metabolites when growing on building materials compared with agar-media and foods. *Aspergillus versicolor* is a complex species, with at least 3 sub-groups and produces sterigmatocystins up to 30 µg/cm^2. However generally the concentrations are in the ng/cm^2 range. Interestingly 4-7 alkaloids previously seen from *A. ochraceus* are often produced. For *Stachybotrys chartarum* some evidence of building associated mycotoxicosis exists, as it in buildings produces high quantities of a number of metabolic families. These includes spiriocyclic drimanes (spirodihydrobenzofuranlactam) which are complement inhibitors (stachybotrys lactones and lactams) and have, fibrionolytical activity, neurotoxic effects etc. Additionally 30% of the iso-lates produces macrocyclic trichothecenes which are highly cytotoxic to mammalian cells and are known to give severe haematoxic effects.

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220 - Biodiversity of allergenic indoor fungi colonising dust particles and surfaces in New Zealand households

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Fungal species isolated from indoor environments have been shown to produce toxic volatile organic compounds (VOC) as well as produce allergenic spores, which can trigger respiratory and related illnesses. A series of mycological surveys of households were undertaken in four climatic regions of New Zealand to determine the biodiversity of fungi present in indoor environments, in particular, the presence of mycotoxic and allergenic fungal species. Fungi were isolated from vacuumed floor dust samples and from wall surfaces using 3M Petrifilms. Isolation results showed ubiquitous taxa, such as *Aspergillus* spp., *Cladosporium* spp., *Epicoccum nigrum*, *Alternaria* spp., and *Penicillium* spp., were frequently obtained in both dust and surface samples. An overall higher species diversity was obtained from dust samples than was observed from wall surfaces, however, the dominance of *Cladosporium* spp. on surfaces across all households may have adversely reduced the sensitivity of the Petrifilm method to detect many slow growing genera. Significant biodiversity differences were observed between the fungal species isolated from dust in different climatic regions. For example, *Aspergillus* spp. were only dominant in houses situated in regions with a dry warm climate. Many households were found to be contaminated with mycotoxic and VOC producing fungi such as *Aspergillus fumigatus*, *A. ochraceus*, *Penicillium verrucosum*, and *Stachybotrys chartarum*.

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221 - Chiral MVOC's - a new tool for studying moulds in building constructions?

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MVOC-measurements can be used as a non-destructive way to detect moulds in closed spaces in buildings. Most of the VOCs produced by moulds can also be found in emissions from building materials and various household products. It is therefore hard to find MVOCs that have microbiological activities as the only emission source. In order to find a good tracer for moulds in sick buildings, chiral volatile organic compounds emitted by microbiological activity (c-MVOC) have been studied in indoor air. For a chiral VOC-compound, there will be at last two enantiomers ('mirror images' that have equal chemical properties, but different physical properties). Man-made VOCs most often occur in racemic ratio (50:50). When there is microbiological activity involving enzymic reactions, one of the enantiomers will dominate, giving a non-racemic ratio (for instance 80:20 or 100:0). Air samples were adsorbed on Tenax TA, followed by thermal desorption and combined gas chromatography/mass spectrometry (GC/MS) and separation of enantiomers on suitable chromatographic columns. In this study six of the most common moulds in buildings with water damages were studied both at controlled conditions in the laboratory (grown on gypsum board and plywood) and in mould-infected buildings. This study has shown that some chiral-MVOC can be used as a tracer for microbiological activity (mould) in buildings.

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222 - Ectomycorrhizal fungal community structure in soil as identified from soil DNA extracts

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The various functional roles of ectomycorrhizal fungi in ecosystems can only be recognised when the mycelial distribution of individual species in soil is revealed. Collectively, the structure, function and activity of the ectomycorrhizal mycelium determine the nutrient mobilisation and translocation capacities of different fungal species. Though its ecological significance was recognized by many mycologists, identification of mycelium in soil remained impossible due to technological limitations. At present however, molecular identification techniques based on total DNA extracts can be used to detect and identify fungal hyphae in various substrates. In order to detect and identify ectomycorrhizal fungal mycelium in soil, molecular identification techniques were applied to sieved soil samples from which all root tips were removed. A basidiomycete-specific primer pair was used to amplify fungal ITS sequences from total soil DNA extracts. Amplified basidiomycete DNA was cloned and sequenced and a selection of the obtained clones was analysed phylogenetically. Through molecular identification of the soil mycelium, the ectomycorrhizal fungal diversity was determined in three vertical soil columns taken from a podzol profile. Latest results will be presented on the application of molecular identification as well as quantification techniques to determine fungal presence in soils.

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223 - Spatial structure in ectomycorrhizal fungal communities: a look belowground

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Ectomycorrhizal fungal (EMF) communities are highly diverse at the stand level. Although many communities have been characterized, little work has been done to examine their spatial structure. Analysis of spatial patterns might point toward processes structuring communities. We examined EMF community data from studies carried out in mature and post-fire conifer forests. To characterize the distribution of communities and populations, we used various measures of spatial pattern: indices of dispersion, mantel tests, and standardized variograms. Mantel tests indicated higher community similarity with decreasing distance, but this pattern was only evident at small spatial scales (usually < 2 m). Indices of dispersion and variograms indicated that most species showed significant clumping, but the degree and scale of clumping varied widely. There was evidence of clumping in both mature and post-fire communities. The former could be the result of genet size, contagious establishment effects that are either endogenous (e.g., local spore rain) or exogenous (e.g., patchy resources or disturbance), or patchiness in initial inoculum. The latter might be the result of patchiness in the inoculum source, given the presence of resistant propagules in the species exhibiting patchiness. To distinguish among alternative mechanisms for patch formation, further investigation is needed of genetic structure of EMF populations at the mycelial level.

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224 - Community composition of arbuscular mycorrhizal fungi in a tropical forest

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It has been proposed that arbuscular mycorrhizal (AM) diversity may be linked to, and even influence, plant community structure. In intact tropical ecosystems little is known of the AM fungal population, so it has not been possible to assess the role of these fungi. Therefore we have used molecular techniques to investigate the diversity and distribution of the AM fungi colonising tree seedling roots in the tropical forest on Barro Colorado Island (BCI), Republic of Panama. We used differences in small subunit ribosomal RNA genes to identify groups of AM fungi colonising different host species of various ages at different sites. The overall AM fungal diversity was found to be very high although the majority of types belong to a single family. The mycorrhizal population showed significantly high spatial heterogeneity and non-random associations with the different hosts. Moreover there was a strong shift in the mycorrhizal communities over time. The high diversity and huge variation detected across time points, sites and hosts, implies the AM fungal types are ecologically distinct and thus may have the potential to influence recruitment and host composition in tropical forests.

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225 - Organization of genetic variation within glomalean individuals

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Understanding the organization of genetic variation within individuals of arbuscular mycorrhizal fungi (Glomales) is a prerequisite for the study of population genetics in this group. Polymorphism of rDNA arrays within individual glomalean spores inspired generally believed yet unproven speculation that these fungi are largely heterokaryotic. We tested this hypothesis using monoxenically cultured *Glomus etunicatum* representing a natural population from a maize field in California. In addition to the rDNA ITS1-5.8S-ITS2 region, a putative gene encoding catalytic subunit of DNA polymerase alpha (*POL*) was identified as a variable genetic marker. To test for homo- vs. heterokaryosis, we examined variant sorting of these two markers in spores formed clonally in cultures initiated from single spores. The markers were PCR-amplified from individual spores, cloned and sequenced. In the initial screen of the *G. etunicatum* experimental population, we detected four variants of the rDNA ITS region and 16 *POL* variants. We analyzed 16 to 200 clones per spore in 42 spores representing five single-spore cultures. The patterns of the rDNA ITS and *POL* variant sorting in these cultures were consistent with the homokaryotic model of nuclear organization. The presence of distinct rDNA variants within individual nuclei implies that concerted evolution, which is responsible for homogenization of dispersed rDNA arrays in genomes of other organisms, does not operate efficiently in Glomales.

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226 - Diversity and distribution of ericoid mycorrhizal fungi in a Mediterranean forest

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Ericaeous plants are widespread on the globe and colonize substrates ranging from humid mor-humus substrates to arid sandy soils. This variety of habitats opens intriguing questions on the biodiversity of their mycorrhizal associates. Ericoid mycorrhiza (EM) has been regarded for long time as a highly specific plant-fungus interaction, featuring a very restricted number of fungal species. More recent studies by research groups worldwide have challenged this view as molecular analyses of EM fungi suggest a greater genetic diversity and a larger number of fungal species than previously thought. An interesting feature of several EM fungi is the occurrence of Group I introns in the small rDNA subunit, which further increases their genetic diversity but which may also represent a potential problem for RFLP analyses. The molecular analysis of ericoid fungi has also lead to deeper understanding of their ecology and relationships with plants, and has revealed that ericaceous plants can be very promiscuous, with multiple occupancy of their thin roots. In addition, some EM fungi seem also able to colonise plants from very distant taxa. We have studied EM fungus-plant relationships in Mediterranean forests, which are complex environments where high biodiversity in plant species and mycorrhizal types (arbuscular, orchid, ericoid, ecto- and ectendo-mycorrhiza) occur. In these environments, ectomycorrhizal and EM plants were found to share similar root endophytes.

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227 - Evolution of secondary metabolite pathways: non-ribosomal peptide synthetases and polyketide synthetases

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Our aim is to identify signaling factors (in pathways required for hyphal network formation, sporulation, vegetative growth and environmental sensing) present in filamentous ascomycetes, and virulence factors necessary for pathogenicity in plant pathogens. We hypothesize that independent lineages recruited similar genes from their non-pathogenic ancestors for novel roles in plant pathogenesis. To test this hypothesis, we are performing comparative genomics of saprobes (*Neurospora crassa* and *Aspergillus fumigatus*) and plant pathogens (*Cochliobolus heterostrophus*, *Botrytis cinerea*, *Fusarium verticillioides* and *F. graminearum*). The first analyses identified genes that encode polyketides (PKs) and non-ribosomal peptides (NRPs). *N. crassa* has 7 polyketide synthases (PKSs) and 3 non-ribosomal peptide synthetase (NRPSs), whereas the other filamentous ascomycete genomes have many more of these genes. Phylogenies of PKSs and NRPSs each feature a large clade that includes genes previously described as virulence factors. However, *N. crassa* has six PKSs and two NPRSs that group with the virulence clade, indicating that these genes have other roles. Based on these comparisons, we will select genes in *N. crassa* for mutational analysis. In parallel with these mutational analyses, metabolite profiling between wild-type *N. crassa* and mutants will be performed to match PKS- and NRPS-encoding genes with secreted metabolites, which will be characterized and tested for biological activities.

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228 - Evolution of mating type genes in filamentous ascomycetes

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To gain a deeper insight into the evolution of reproductive life-cycles from filamentous ascomycetes, a comprehensive sequence analysis of PCR-amplified sequences corresponding to *A*- and *a*- specific mating-type sequences was undertaken. The study included nine homothallic (compatible) and eight heterothallic (incompatible) members of the genera *Neurospora* and *Sordaria*. Distance and parsimony trees based on gene fragments from the *mat a-1* and *mat A-1* genes were compared with trees derived from partial DNA sequences of the *gpd* glyceraldehyde-3-phosphate dehydrogenase gene. In contrast to the sequences from the gpd gene, mating-type genes show striking sequence differences suggesting that these genes evolve very rapidly. Strong inter-relationships were found among homothallic, as well as among heterothallic members of both genera, indicating that in each genus, a change from one reproductive strategy to another might result from one single event. Pair-wise comparisons between pheromone genes and pheromone receptor genes of the heterothallic species *Neurospora crassa* and the homothallic *Sordaria macrospora* revealed an extremely low degree of nucleotide conservation in these genes, indicating that these, like mating-type genes, evolved also very rapidly.

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229 - Phylogeny of Zygomycetes: multi-gene approaches and the putative role of horizontal gene transfer

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The Zygomycetes encompass microscopic fungi which form zygospores in sexual interactions. The most prominent and largest order are the Mucorales comprising saprotrophic and facultatively parasitic species, among those, the soil fungi *Chaetocladium brefeldii* and *Parasitella parasitica* which parasitize on other mucoralean fungi during the establishment of plasma bridges and the unidirectional transfer of genes to the host. This parasexual interaction utilizes the pheromone trisporic acid for the identification of compatible hosts, similarly to sexual interactions. One of the last steps of trisporic acid biosynthesis is catalysed by the 4-dihydromethyltrisporate dehydrogenase. The gene encoding this aldo-keto reductase were found in all families of the Mucorales, Mortierellales, Entomophthorales and Kickxellales. The sequences were analysed and used for the reconstruction of phylogenetic trees. In order to study the evolution of Zygomycetes in multi-gene approaches sequences for the nuclear-encoded genes actin and beta-tubulin were also determined and applied in concatenated analyses of tree constructions. Phylogenetic analysis in the context of available sequence data (approx. 6300 nucleotide positions per species) revealed that current classification schemes for the mucoralean fungi are highly unnatural at the family and, to a large extent, at the genus level.

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230 - RAPD and microsatellite analysis of the local and global population structure of aflatoxigenic *Aspergillus* species

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The mycotoxigenic species *Aspergillus flavus* and *A. parasiticus* have been well characterised with regard aflatoxin biosynthesis. Comparatively little is known, however, on their natural population structure in the environment. We have developed new molecular markers based on RAPDs and microsatellites to analyse large populations of these fungi on crops and in soils. Investigation of an Australian field population revealed 1) a new species of aflatoxigenic fungus, related but genetically distinct from *A. flavus* was common in this region. This is the same as the "Group 2" *A. flavus*, previously reported by Geiser et al. (PNAS 94: 388-393,1998); 2) recombination has occurred in the *A. flavus* population, but the A. parasiticus population and the population of the Group 2 *A. flavus* species are clonal; 3) no clear distinction between toxigenic and nontoxigenic strains in any of the species. Seven microsatellite markers were then used to analyse a large, worldwide collection of isolates. Globally, *A. flavus* was found to be cosmopolitan and there was no geographic substructuring within this species. In contrast, *A. parasiticus* was geographically restricted, being very rare throughout all of Asia. Group 2 *A. flavus* isolates were likewise restricted, and all but two came from the southern hemisphere. The microsatellite data also indicated that genetic diversity was higher in *A. flavus* than in either the Group 2 or the *A. parasiticus* populations.

231 - Phylogeny of arbuscular mycorrhizal fungi (Glomales)

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Approximately 150 species of arbuscular mycorrhizal fungi (AMF) have traditionally been placed in the order Glomales in the Zygomycota. Recently the phylum Glomeromycota was established for them. Although this ubiquitous group of symbiotic fungi was shown to be at least 460 million years old, its diversification apparently is relatively low. According to rDNA phylogenies, the Glomeromycota are a sister group to Asco- and Basidiomycota. Taxonomy and identification of AMF has traditionally relied on spore morphology, but it has become increasingly clear that spore morphology is a relatively poor predictor of AMF species diversity. DNA sequences and sequence signatures have become important characters to define new taxa. Several lineages of AMF were shown to be deeply divergent within the Glomeromycota. Two new genera *Archaeospora* and *Paraglomus* were established for them, but the polyphyly of the remaining genus *Glomus* necessitated further taxonomic changes. The genetics of arbuscular mycorrhizal fungi is remaining mysterious, even more after they were shown to be heterokaryotic and predominantly non-recombining. Therefore it is problematic to apply evolutionary species concepts that have been successfully used in other fungi.

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232 - Fungi and the Northwest Forest Plan

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During the late 1980s and early 1990s a series of lawsuits severely decreased the harvest level of old-growth forests in the Pacific Northwest. At the center of the controversy was the old-growth dependent northern spotted owl (*Strix occidentalis*), a rare species protected by the Endangered Species Act. A regional forest conference to address the problem led to the development pf the Northwest Forest Plan. The plans goal was to provide for a sustainable timber harvest as well as the persistence of old-growth forest dependent species. Many rare species requiring old-growth forest habitat were analyses for protection under the guidelines and recommendations of the plan. Four hundred presumptive old-growth dependent species, including 234 fungal species, were identified that needed additional protection under a legally binding document called the Record of Decision that defines how the Northwest Forest Plan was to be implemented. We will briefly review the process that led to creation of the Northwest Forest Plan, then explore the challenges of implementing it and examine the early impact it has had on research of forest fungi in the Pacific Northwest United States.

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233 - Putting Australian fungi on the map

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Relatively few of Australia's numerous fungi have been thoroughly documented. Many species are yet to be described, yet there are very few Australian mycologists and comparatively few specimens of fungi in Australian herbaria. However, three current government initiatives are stimulating progress in cataloguing and understanding the diversity of the Australian mycota. Fungi have been included in the program of the Australian Biological Resources Study, which publishes the *Fungi of Australia* series. A second key initiative which embraces fungi is the current effort to comprehensively database specimens in state botanical herbaria (the Australian Virtual Herbarium). Thirdly, Fungimap, a partnership between government and community groups, is drawing on recorders across Australia to rapidly advance knowledge of the distribution and conservation status of selected macrofungi. Research on fungi can also result from their myriad interactions with other biota. A prominent example is the need to effectively manage endangered mammals that subsist largely on native truffles. This has stimulated taxonomic and ecological research on Australian truffles, which have been revealed as among the most diverse in the world. Continued progress in documenting Australia's mycota depends on government support for the maintenance and creation of mycological positions within herbaria, and the further integration of fungi into national biodiversity and conservation programs.

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234 - Fungi within the UK Biodiversity Action Plan

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The development and inclusion of fungi in the UK Biodiversity Action Plan (BAP) is briefly reviewed. The extent to which the conservation of fungi has been advanced by this process is considered. The inclusion of some fungi (27 taxa) represents a significant achievement for a taxonomic group which is traditionally under-represented in the setting of conservation priorities. Nevertheless, fungi remain the least well represented in comparison with other taxonomic groups. The action taken since the publication of the plans is summarised. Implementation of the plans includes a review and expansion of survey work on these target species which has resulted in an increase in our knowledge about their UK distribution. It has also facilitated scientific studies into the taxonomy and ecology of targeted species. The progress to date enables some reflections to be made on the appropriateness of the taxa chosen for action plans and the criteria used to select them. Observations are made on how the priorities and the process might be improved and adjusted to changing circumstances and continually improving knowledge. The influence of the process on the support given to the conservation of fungi by the government and non-government sectors is considered. However, the benefits arising from the inclusion of fungi in the UK BAP have not been confined solely to the target species. A much higher priority for, and a greater partnership approach to, the conservation of fungi is now evident.

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235 - The importance of fungi in IUCN Programmes

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IUCN (World Conservation Union) represents the world's oldest and largest global conservation body ; it is a powerful and well-structured organisation, devoted to nature conservation all over the world, seeking to influence, encourage and assist societies throughout the world to conserve the integrity and diversity of nature and to ensure that any use of natural resources is equitable and ecologically sustainable. Activities involve several thousands of people, distributed in about 150 countries. Fungi have been rather recently considered in IUCN scope, especially in establishing a Specialist Group for Fungi (within the Species Survival Commission), which aims to list the threats to Fungi, to consider the conservation strategies and to promote actual conservation actions for threatened Fungi. Testing and improving the red list criteria and categories have been another recent priority. The current investigations will be browsed and some example of current actions, some of them in collaboration with other organisms or councils (for example ECCF, European Council for the Conservation of Fungi) will be given. The Bern Convention case (for Europe), involving relations between several European states and demonstrating some difficulties in such international actions will illustrate this purpose. Finally global perspectives for future conservation of Fungi will be considered.

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236 - Conservation based on genetic diversity

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Conservation biology requires knowledge about biological diversity. Classic estimates of biological diversity use species (or genera) as proxies. The development of large-scale molecular phylogenies provides a unique opportunity to estimate biological diversity at the genetic level by using the branching order and branch lengths from phylogenetic trees. Both among-taxa and among-areas phylogenetic diversity can be estimated and compared with the use of the 'phylogenetic index of diversity' (PD). We will contrast PD with classic estimates of biological diversity in various groups of mushrooms for which extensive geographic sampling and molecular phylogenies are available, including genera *Amanita*, *Pleurotus*, *Xeromphalina*, and *Ganoderma*. There are at least two advantages of PD over traditional measures of estimating biological diversity: 1) PD does not require a priori knowledge of taxonomic circumscription (which is generally subjective in fungi), and 2) PD takes into account genetic distances, therefore emphasizes genetic breath when estimating diversity.

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237 - The Convention on Biodiversity triggers biodiversity research programs?

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Both member and non-member states have responded in varied ways to the Convention on Biodiversity. This paper presents the influence of the CBD in triggering biodiversity programs, and explores if regulatory conditions and expertise exist for their implementation. To date the Thai government has been exemplary in setting up biodiversity research and competence building programs, and drafting regulations on biodiversity even though it is not a signatory to the CBD. Now it plans to form a Committee on National Biological Diversity Conservation and Utilization to oversee and harmonize all biodiversity activities in the country. The cross-sectorial and participatory approach employed by Thailand is exemplary, but not devoid of conflicts. Malaysia and the Phillippines, a signatory to the CBD, have had specific research grant programs for biodiversity launched after ratifying the CBD. The Philippines has now enacted one of the most stringent biodiversity laws in the world. The enforcement of regulations in all three countries is weak and not all government, academic, and private institutions are well informed of protocols required for biodiversity studies. To a large extend both Thailand and the Philippines rely on foreign collaboration skills to catalog species. Nevertheless all three countries have adopted distinct modes of addressing CBD objectives and are much ahead of most nations in doing so.

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238 - Capturing mycological data, the Caribbean experience

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Since 1997 a team of mycologists, mainly from Cuba and the UK (but with additional contributors from Belgium, Puerto Rico, Trinidad & Tobago and Venezuela) participated in the UK Darwin Initiative project 'Fungi of the Caribbean'. This project aimed to: establish infrastructure necessary for a regional fungal identification service in the Caribbean; carry out field work in Cuba and Trinidad & Tobago, augmenting mycological reference collections in both countries; produce a computarized checklist of Caribbean fungi based on all this information. To achieve the last of these aims, it was necessary to capture information from living and preserved reference collections, and from bibliographic sources, and to enter that information into a suite of previously established relational databases (Biological Records, Nomenclature & Taxonomy, Bibliographic). The main software used for data entry, data storage and output was the SMARTWAREII database system. The resulting publication, Fungi of the Caribbean, of almost 1000 pages, was based on almost one hundred and fifty thousand (150,000) different biological records of fungi and associated organisms. Many practical problems can be encountered during capture of mycological data. To ensure high quality keyboarding, curation and output, suitable training is therefore necessary for all the team, and a clear set of data entry protocols must be supplied before work starts.

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239 - PLANTCOD - to the database formation in the Komarov Botanical Institute on biodiversity of plants and fungi in Russia

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Practically all the databases created in the Komarov Botanical Institute RAS have the taxonomical blocks. In the majority of these blocks the modification of ZOOCOD3 (Lobanov et al., 1999) concept is used to reflect the hierarchical classifications of plants and fungi in the flat relational tables. The field used for detailed representation of classification are the following: LATNAM (Latin name of a taxon), RANCOD (taxonomic code of a rank), SYSCOD (digital systematic code), SYN (code of synonymy), ABBR (unique mnemonic code of a taxon). The fields KEY (unique digital code of a taxon) and PARENT (value of KEY for the nearest parent) work with the Tree View Windows control to represent a hierarchy of taxa in a form of tree. Though these two fields adequately reflect multilevel hierarchy they are inconvenient for fast search of all descendants belonging to the same taxon. To decide the problem, the filling rules are changed for the SYSCOD field. The field SYSCOD is filled by the special utility, but the search of descendants of the given taxon is executed (with lightning speed and extremely simple) by superposition of the filter with a completely optimised condition. (This study was supported by grant 02-07-90439, Russian Foundation for Basic Research; scientific program 'Information system on biodiversity' 43.073.1.1.2510, Ministry of Industry and Science, Russia; Darwin Initiative awards, UK Government).

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240 - Information systems can improve the efficiency of biodiversity research, promoting collaboration and sharing of information

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Given the extent to which basic biodiversity information is missing - organisms being undescribed or described so poorly that identification is impractical - we urgently need to improve the efficiency of biodiversity research. To some degree biodiversity informatics can contribute, providing more efficient workbench applications and efficient sharing of information through web services and (xml-based-)interoperability data standards. - Information sharing covers sharing basic infrastructure resources like nomenclatural or reference data (which can be combined with project-oriented work on taxonomic assessment, descriptions, or reference indexing tasks). Significantly, it also covers sharing assessment and analysis data between generations of researchers, leading mycology into an age where updates of previous monographic works concentrate on new results rather than laboriously repeating work already done. Thought has to be given to intellectual property rights and copyright issues. The newly introduced database copyright could endanger such efficiency improvements and stifle future research. - The development of information systems capable of fulfilling this promise is a huge task itself. Information models need to be modular to simplify analysis. Applications should be developed as independent components that cooperate in a framework. Such a framework, the DiversityWorkbench suite of applications, is currently being conceptualized and developed (see http://www.diversitycampus.net/).

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241 - Use of nomenclatural and bibliographic mycological data

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Nomenclatural and bibliographic databases are important tools in mobilizing mycological information, especially within the subject area of biodiversity information. The nomenclator is fundamental to biosystematics research, listing names which have been published in the literature. The service it provides to biosystematists, as both producers and users of names, is obvious but most users of names have a somewhat different requirement. The two types of databases can be conveniently and favourably linked through the 'potential taxon' model of Berendsohn. This can deliver the service that users of names require; not the association of a name with a specimen (the type of the name) but the association of a name with an accepted taxon, usually a species. The global database of fungal names (http://www.indexfungorum.org/) originated as a nomenclator but a partial implementation of the potential taxon model allowed for the inclusion of taxonomic opinion, with heterotypic synonyms retrieved through a single-cycle recursive join. This simple and pragmatic approach has been very successful. The services it can and does provide will be demonstrated and possible enhancements and linkages will be explored. Any database today should seamlessly deliver to ink-on-paper and the Internet; the latter is the future.

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242 - Use of mycological data for producing a checklist, US experience

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The Systematic Botany and Mycology Laboratory has been a pioneer in the use of computer databases to manage mycological information. We found that success depends on several factors: clear goals, efficient user interfaces for data entry and continuous management of personal resources. Once the big push to complete a computer database project is over, the organization is faced with the need to maintain the database. To be successful over the long term database projects need to form an important component of the organization's reference materials and collection's management tools. Now that many mycological databases are available on the web we need to begin to look at ways in which a single user interface can be used to retrieve and integrate information from various Web sites. One way that this could be done is to extract data from a remote database on an as needed basis. The term 'Web Services' is being applied to a standard set of procedures that are being developed to handle this type of interaction. These procedures can greatly simplify the exchange of information between remote databases and it is important that mycologists make use of these procedures to further enhance the utilization of the databases that we have developed.

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243 - NZFUNGI - a biodiversity information system for fungi in New Zealand

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Landcare Research is New Zealand's foremost institute in systematics and environmental research in terrestrial ecosystems, and is the custodian of the national collections and associated databases of plants, insects, fungi, bacteria and nematodes. NZFUNGI is part of an ongoing project to unlock our extensive data on biodiversity. It comprises a cross-indexed information facility which integrates a number of existing and new resources on taxonomy, pathology, literature, specimens, cultures, descriptions, images and keys. Integration of these data was facilitated using a taxonomic framework provided by the extensive authority files of CABI Bioscience, together with a number of standard authority files and database structures (e.g. TDWG data standards, dictionaries, and the IOPI taxonomic information model). Software systems for these data are being developed to maximize the opportunities provided by the recent and rapidly development enabling technologies of XML/SOAP and web services. Adoption of this technology is allowing us to address some important issues. For example, it allows us to provide secure and sophisticated application interfaces that will operate anywhere on the net, and to consider the virtual integration of distributed data resources and functionality. The presentation will provide a demonstration and discussion of work to-date.

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244 - MycoKey - a database and a mycological expert system

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MycoKey is a user friendly genus identification system for fungal genera. It is based on the relational database software 'Forth Dimension' and will be distributed in both mac and PC versions. A beta version will be available at the congress. At present MycoKey includes 530 genera of Basidiomycota (excluding rusts and smuts) known from Northern Europe. MycoKey is based on a character matrix of more than 1.000 characters, 17 interrelated files and thousands of lines of custom programming. The result is an application with floating menues and a fully illustrated interface hiding the underlying complexity for the user. It contains information on authors, synonyms, type species and references. It also includes generic descriptions and not least numerous photographs of key taxa. With the '4D engine' MycoKey is compiled into a multi-platform, stand alone application for sale. MycoKey is also served by 4D and Webstar for use on the internet, see http://www.mycokey.com/.

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245 - A phylogenetic overview of the groups of fungi and fungus-like organisms that attack nematodes

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This talk will provide an introduction to the groups of nematode-destroying 'fungi', a guild of fungi and fungus-like organisms that attack living nematodes and consume them as food. These organisms may be important in regulating nematode populations in nature and, in certain circumstances, may act as biological control agents of nematode pathogens and parasites of crop plants and livestock. Over 400 species of predatory and parasitoid nematode-destroying fungi are known, including approximately 150 species of Basidiomycota (Hyphodermataceae and Pleurotaceae), 130 species of Ascomycota (Clavicipitaceae, Hypocreaceae and Orbiliaceae), 100 Zygomycota (Cochlonemataceae, Helicocephalidaceae, Meristacraceae and Zoopagaceae), 10 Chytridiomycota (Catenariaceae), and 40 Oomycota (Stramenopila: Myzocytiopsidaceae). In predatory species, one spore germinates to form a mycelium capable of attacking many nematodes; in parasitoids, each propagule attacks a single nematode. In the nematode-destroying Ascomycota and Basidiomycota, possession of a sexual state is apparently correlated with the predatory habit. Although the ability to attack and kill nematodes has arisen multiply (as has a marvellous diversity of means to achieve this end), this has not been a random process. Where well known, groups of nematode-destroying fungi are apparently monophyletic.

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246 - Teleomorph-anamorph connections in Orbiliaceae

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With the establishment of the teleomorph anamorph relation between certain of the hyphomycetous nematode trappers and the Orbiliaceae several phylogenetic and biological questions have come to light. Using molecular phylogenetic methods it has been suggested previously that the Orbiliaceae occupies a phylogenetic postion near the base of the Euascomycota tree along with the Pezizales. While the nematode trapping life style exists in several fungal groups there is no evidence of nematode trapping in the groups most closely related to the Orbiliaceae. We will present further molecular sequence data, particularly from the LSU rDNA and the RPB2 genes supporting the placement of the Orbiliaceae in this basal position. The implications of this specialized type of life strategy as represented in a basal member of the Euascomycota will be discussed.

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247 - Beyond Saccardo: a new generic system for nematode-trapping orbiliaceous hyphomycetes

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Perfect states are unknown for the majority of orbiliaceous nematode-trapping fungi. Therefore, generic concepts for anamorphs have been developed in which the morphology of conidia and conidiophores were preferentially used for generic delimitation. The mode and morphology of the trapping device, however, was only considered for species delimitation. A new concept is proposed with the mode of trapping device as the main morphological feature for generic delimitation. This new concept includes four genera, viz. *Arthrobotrys* Corda em. forming adhesive networks, *Drechslerella* Subram. em. forming constricting rings, *Dactylellina* M. Morelet em. forming stalked adhesive knobs, and *Gamsylella* gen. nov. producing adhesive columns and unstalked knobs. The concept corresponds well with molecular (rDNA sequences:18S, ITS I, II) and some ecological, physiological, and biological features. Also, chlamydospore formation, distribution, and host range are, to some degree, genus-specific.

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248 - Nematode endoparasites with verticillium-like anamorphs

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Verticillium comprises many unrelated anamorphs. The type and the best-known plant-pathogens are Phyllachorales. *Verticillium*-like anamorphs of Clavicipitaceae, previously in sect. *Prostrata*, are distributed over several genera. Many are entomogenous or fungicolous, some discrete groups are nematode endoparasites. A phylogeny-based distinction separates

species that attack free-living nematodes with adhesive conidia and parasites of nematode eggs and cysts. The two most important genera, *Lecanicillium* and *Pochonia* (syn. *Diheterospora*) differ by formation of dictyochlamydospores, stronger cyanophily of the mostly short conidia, and absence of crystal formation in the latter. In *Lecanicillium* (teleom. *Torrubiella*, *Cordyceps*), only few taxa are mildly nematode-parasitic. *P. chlamydosporia* (teleom. *Cordyceps*) and related species are efficient egg parasites of *Heterodera* and *Meloidogyne* species, promising in biological control. Species of *Simplicillium* (teleom. *Torrubiella*), lacking verticillate phialides, fall phylogenetically outside *Lecanicillium*; they also parasitize nematode eggs. Species with adhesive conidia form a discrete genus, *Haptocillium*. Its species were so far known as *V. balanoides*, but at least 7 species are distinct, the most frequent being *H. sphaerosporum*. Contrasting with *Drechmeria* coniospora, *Haptocillium* species attack a wider array of free-living nematodes and are promising biocontrol candidates.

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249 - Novel biologically active metabolites from *Pochonia chlamydosporia* and preliminary studies on their distribution in *Verticillium*-like anamorphs

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Extracts from the fungus *Pochonia chlamydosporia* var. *catenulata* strain P0297 showed interesting antiviral and antiparasitic activities. As active principles, we identified the known fungal metabolites, Monorden and Pseurotin A, besides several novel natural products, for which we propose the trivial names Pochonins A-E. The isolation and characterisation of these compounds is summarised in a concurrent poster. The current paper deals with studies on the distribution of secondary metabolites in strain P0297 and further *Verticillium*-like anamorphs from public collections by HPLC-based secondary metabolite fingerprinting, aided by morphological comparisons and Minisatellite PCR, using the methodology recently developed in our studies of Xylariaceae [Stadler et al, Mycological Research 105, 1191-1205, 2001]. According to preliminary results, the production of resorcylic acid lactones is restricted to particular species of the genus *Pochonia* sensu Gams & Zare [Nova Hedwigia 72 (3-4), 329-337, 2001], while these compounds were not detected in several species now accomodated in *Lecanicillium* and *Haptocillium*. Therefore, the recent segregation of the genus *Pochonia* from other *Verticillium*-like anamorphs based on morphological and molecular methods by Gams, Zare and co-workers also appears justified from a chemotaxonomical point of view.

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250 - Rhizosphere biology of nematophagous fungi

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Nematophagous fungi can infect, kill and digest living nematodes. Most of these fungi can also live saprophytically and some even have mycoparasitic abilities. Since most plant-parasitic nematodes attack plant roots, the rhizosphere biology of nematophagous fungi is important from a biological control point of view. We previously knew that nematophagous fungi were more abundant in the rhizosphere than in bulk soil. In this study, barley and tomato axenic plants were inoculated with the nematode-trapping *Arthrobotrys oligospora* or the egg-parasitic fungus *Pochonia chlamydosporia* (=*Verticillium chlamydosporium*). Roots were sequentially sampled, cryo-sectioned, and observed under light- or cryo-scanning electron microscopes. Both fungi grew inter- and intracellularly, formed appressoria when penetrating plant cell walls of epidermis and cortex cells, but never entered vascular tissues. Using histochemical stains we could show plant defence reactions, e.g. papillae, lignitubers and other cell wall appositions, but these never prevented root colonization. Nematophagous fungi grew extensively especially in monocotyledon plants producing abundant mycelia, conidia and chlamydospores (*P. chlamydosporia*). Whether this endophytic growth induces systemic resistance in plants is yet unknown, but worth further investigations. The ability to colonize plant roots may also be a survival strategy of these fungi and could explain soil suppressiveness to plant-parasitic nematodes in nature.

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251 - Holocarpic biflagellate parasites of nematodes including *Chlamydomyzium* and *Myzocytiopsis*

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In comparison to related straminipilous fungi that are easily cultured, the obligate nematode parasites are a little known group that have been considerably understudied. Because of their host-dependent nature and the paucity in phylogenetically significant data, their position within the peronosporomycetes, and their relationship to one another, has yet to be established. The nematode parasites produce a holocarpic thallus inside the bodies of their bactiverous nematode hosts. The infective agents are either motile biflagellate zoospores or adhesive aplanospores, which attach to the nematode cuticle and penetrate with a germ tube to initiate infection. This presentation looks at the main diagnostic characteristics of *Chlamydomyzium* and *Myzocytiopsis* and at the diversity within the genera. Development of the thallus into sporangia or into resting spores is demonstrated using both light microscopy and transmission electron microscopy (TEM), and the process of zoospore release is followed in a video sequence. A phylogenetic tree (mitochondrial coxII gene) of representative peronosporomycetes has been compiled (Hudspeth & Hudspeth) and includes a few species of nematode parasites.

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252 - What makes a species? Redundancy, recombination, and reproductive isolation

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The *Saccharomyces* "sensu stricto" group of yeast mate with *S. cerevisiae*, but produce hybrids that yield spores of very low viability, such that speciation seems to have arisen via reproductive isolation. Chromosomal rearrangements might act as a post-zygotic isolating mechanism. We studied the distribution of chromosomal translocations in 5 *Saccharomyces* "sensu stricto" species (*S. paradoxus, S. bayanus, S. cariocanus, S. mikatae and S. kudriavzevii*) by comparing them to the *S. cerevisiae* genome. No correlation was found between the sequenced-based phylogeny of these species and the presence of translocations. Instead, bursts of rearrangements are seen between closely related species, while more distant ones have co-linear genomes. Thus, chromosomal rearrangements are not the primary cause of yeast speciation, but may intensify reproductive isolation once a species has arisen by another route. We are using a novel molecular approach to generate yeast strains containing specific chromosomal translocations. Strains of *S. cerevisiae* have been constructed that contain the translocations found in other *Saccharomyces* "sensu stricto" species. These are then mated, both to wild-type *S. cerevisiae* and to the "sensu stricto" species whose genome configuration they mimic. The data show that translocations make a significant contribution to the post-zygotic isolation of species and indicate a mechanism for genome duplication via allopolyploidy.

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253 - Genus relationships in the Saccharomycetales from multigene analyses

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As with most fungi, circumscription of yeast genera is based primarily on phenotype. Gene sequence comparisons, such as from 18S or 26S rDNAs, have shown that many presently defined yeast genera are not monophyletic, but single gene analyses seldom provide sufficient resolution to unambiguously circumscribe genera. Analysis of the ca. 80 known species of the *Saccharomyces* clade from EF-1, RNA polymerase II, cytochrome oxidase II and actin, as well as from 18S, 26S and mitochondrial small subunit rDNAs, individually gave congruent terminal lineages and, when analyzed in combination, provided strong genus-level support. Intergeneric relationships are less well resolved making family assignments uncertain. A comparison of species in the phylogenetically distant *Stephanoascus*/*Blastobotrys* clade gave similar results. Diagnostic phenotypes were recognized for many of the phylogenetically defined genera, but for some genera, there appeared to be no unifying morphological or physiological characters.

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254 - Molecular approaches to the re-appraisal of species diversity within the ascomycete genus *Taphrina*

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The dimorphic ascomycete genus *Taphrina* Fries comprises nearly 100 species recognised by their mycelial states parasitic on different vascular plants. Whereas the filamentous state occurs exclusively in plant tissue, the yeast state is saprobic and can be cultured on artificial media. *Taphrina* species are differentiated mainly on the basis of host range and geographic distribution, type and site of infection, and morphology of the sexual stage in the infected tissue. However, there has been little progress in the systematics of the genus in recent years mainly due to the scarcity of molecular studies and of available cultures. Here we report on the molecular characterisation of *Taphrina* spp. obtained from culture collections (yeast states) in order to address the following topics: (i) is *Taphrina* a well defined genus?; (ii) are species defined on the basis of phenotypic criteria genetically distinct?; (iii) does host specialisation underlie speciation in *Taphrina*?; (iv) do *Taphrina* spp. occur naturally as saprobic yeast forms?; (v) can molecular methods help in accurately diagnosing the different diseases caused by *Taphrina* species? The molecular methods used comprised PCR-fingerprinting using single primers for microsatellite regions (MSP-PCR), and sequencing of two ca. 600 bp long nuclear rDNA regions: the 5' end of the 26S rRNA gene (D1/D2 domains) and the Internal Transcribed Spacer (ITS) regions (including the 5.8S rRNA gene).

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255 - Biogeography of floricolous yeasts: is everything everywhere?

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The old dogma of microbial ecology, 'Everything is everywhere, the environment selects,' has had a profound influence on the study of yeast biodiversity. One outcome has been a widespread neglect of the habitat as a significant element of species descriptions. The traditional paradigm implies that a global 'yeast seed bank' is available to fill any niche that is made available. Biogeographic theory, on the other hand, predicts that species diversity should be higher in the tropics, lower in isolated localities, and proportional to the size of contiguous landmasses. To test these opposing models, the yeast communities of ephemeral flowers were studied in various Pacific islands and several sites in Australia, Brazil, Costa Rica, the southern United States, and the eastern Nearctic region. These yeasts are vectored and maintained by insects such as bees and beetles. The yeast species composition is greatly but not exclusively affected by the nature of the vector insect species, and much less so by the plant species. Most biogeographic factors have a significant influence when not confounded by human interference. Different yeast species have different ranges on the global scale: some can be viewed as cosmopolitan and others as endemic. The emerging pattern is that indeed, the environment selects. However, geography plays a major role. The notion that 'everything is everywhere,' as least at it applies to floricolous ascomycetous yeasts, is misleading.

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256 - Molecular ecology of basidiomycetous yeasts in tropical marine habitats

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The current model of yeast systematics includes ^ 1000 species in >100 genera, numbers that are rapidly increasing with the discovery of new species and the description of new genera due to increased knowledge of the biology and phylogenetics of these unicellular ascomycetous and basidiomycetous fungi. Estimates indicate that this number of species may only represent 1% of the species in nature. Yeasts in marine environments are widespread from the tropics to polar regions and intertidal habitats to the deep sea floor. The ecological role of yeasts ranges from species that are host specific saprophytes to species with diverse habitats and appetites. Our specific knowledge of these roles is, however, meager. A major reason has been the lack of ability to specifically identify these organisms to the species level. The advent of molecular techniques has provided the necessary cure to the systematic problems. Research in our laboratory with the basidiomycetous yeasts has centered on the ribosomal DNA, exploring the ITS, D1D2 portion at the 5' end of the large subunit, and the IGS regions. Based on these data bases, the phylogenetic position and identity of yeasts can be determined. The sequence differences between strains and species has spawned methods for the rapid identification of culturable and uncultured species directly from the environment. These methods will be discussed.

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257 - Systematics of the human pathogen *Cryptococcus neoformans* in the genomics era

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Cryptococcus neoformans is a basidiomycetous yeast causing life-threatening infections in immunocompromised hosts. The species is known in both the asexual and sexual states. Since long two varieties *C. neoformans* var. *neoformans* and var. *gattii* were known. The observation of mating and gene flow resulted in the description of a separate genus *Filobasidiella*. A third variety *C. neoformans* var. *grubii* (= serotype A) was described recently. Molecular studies on the IGS and ITS of the rDNA, the mtLrRNA, URA5, laccase and phospholipase genes, as well as AFLP and PCR-fingerprinting showed that the three varieties belong to different phylogenetic lineages and may represent species. Novel genotypes could be distinguished, thus further questioning the species boundaries. The biological species concept was tested in an analysis of a mating between variety *neoformans* and variety *gattii*. Most of the descendants possessed the genotypes of either parental isolate. Gene flow could not be demonstrated under natural circumstances. Therefore, we proposed that both varieties represent at least two species. Hybrid serotype AD strains of *C. neoformans* originated from different parental strains. The rare serotype A MATa allele occurs in part of these hybrids. An analysis of virulence related phenotypic traits and antifungal susceptibility revealed that virulence and susceptibility varied widely. Fluconazole resistance was observed in some environmental isolates, suggesting an innate resistance.

258 - Functional genomics in *Candida albicans* for drug target discovery

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Current anti-fungals used to treat human diseases are few in number or invariably toxic. Most new anti-fungals are chemically modified versions of existing compounds that act against the same fungal targets [ergosterol synthesis, for instance]. The complete sequence of the *Candida albicans* genome has made it possible to utilize new strategies in order to identify targets that can be exploited in drug discovery. How does one select those genes [ORFs] that are the most appropriate for target validation? Most of the important ORFs are decided so by mostly 'common sense' thinking, including, first, the target should be represented in a number [if not all] of the human fungal pathogens but not in the human genome, or, if present, there should be sufficient differences that can be exploited. Secondly, the target should be vital to the infection process. Third, the function of the gene product should be at least partially understood so that appropriate assays can be established. Fourth, the gene product should be essential for growth or viability of the fungus, although virulence factors that are not essential for growth may be candidates. The spectrum of a candidate target is limited in definition since, except for the *C. albicans* genome, sequences for other human pathogens are non-existent or incomplete. In this symposium, topics related to the identification of functional gene homologues, target identification and validation will be presented.

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259 - Carbon dioxide exchange, diffusion resistances and water content in lichens: Laboratory and field

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Jumelle in 1892, first commented on depressed net photosynthesis at high thallus water contents and Stocker, in 1927, the first to infer that it was due to diffusion limitations. The topic attracted attention after demonstrations of its occurrence by Kershaw in the 1970s. There followed a period of contention as to what actually caused the depression. Two possibilities were proposed, depression through increased respiration or through increased diffusion resistances for carbon dioxide. Evidence, including chlorophyll fluorescence analysing activity of the photosynthetic apparatus and helox for direct determination of resistances, has consistently supported the changed diffusion resistances theory. This evidence will be summarised as well as field results that demonstrate the ecological importance of the depression in carbon gain. Although first found and described in the laboratory, suprasaturation depression of NP is not an experimental artefact but an important ecological feature of many lichen species in many habitats. As an example, averaged over a total year, net photosynthesis of Leconora muralis was heavily reduced through suprasaturation during 38.5% of the time when photosynthesis was possible. Despite these confirmations of the role of diffusion resistances there is still little agreement as to where these diffusion pathways are actually located. An interpretation of the thallus will be presented based on standard water potential water vapour considerations.

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260 - Hydrophobins in lichen-forming asco- and basidiomycetes

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Main building blocks of heteromerous lichen thalli are hydrophilic, conglutinate pseudoparenchyma (peripheral cortex) and loosely interwoven plectenchyma built up by aerial hyphae with hydrophobic wall surfaces (medullary and photobiont layers). Wall surface hydrophobicity prevents the thalline interior from getting waterlogged at high levels of hydration and facilitates gas exchange[1]. This wall surface hydrophobicity was shown to be primarily due to class 1 hydrophobins. Several hydrophobins were biochemically and genetically characterised: one from the haploid vegetative thallus of each of 4 *Xanthoria* spp. (XPH1/*parietina*, XEH1/*ectaneoides*, XFH1/*flammea*, XTH1/*turbinata*)[2, 4] and three from the dikaryotic, lichenized basidiocarp of *Dictyonema glabratum* (DGH1, DGH2, DGH3)[5]. With *in situ* hybridisation techniques hydrophobin gene expression was located in medullary hyphae of *X. parietina*[3] or in the photobiont layer, the lower stratum and the boundary layer to the hydrophilic tomentum in *D. glabratum*, respectively[6]. An antibody raised against the recombinant DGH1 bound on ultrathin sections to the electron dense outermost wall layer of hyphae in the photobiont layer where rodlets had been resolved in freeze-etch preparations[6]. 1 Honegger (2001) THE MYCOTA IX: 165-188; 2 Scherrer et al. (2000) FGBI 30:81-93; 3 Scherrer et al. (2002) New Phytol. 154:175-184; 4 Scherrer & Honegger, unpubl.; 5 Trembley et al. (2002a) FGBI 35:247-259; 6 Trembley et al. (2002b) New Phytol. 154:185-196.

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261 - Rolling stones in a harsh environment. Morphology and ecophysiology of globular erratic lichens

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Erratic lichens, growing free without any attachment to the substrate, are widely distributed in many biomes all over the world. *Xanthoparmelia* and *Aspicilia*, have the largest number of vagrant taxa world-wide. Habitat supporting vagrant lichens is typically windswept, semi-arid, cold in winter and sparsely vegetated. In Eurasia, these habitat conditions correspond to the steppes and semi-deserts and account for almost one-fourth of the continent. Vagrant lichens of these regions typically show short and compact lobes or branches, thick scleroplectenchymatous tissues and low chlorophyll content. Accordingly, the thalli of all the investigated species showed very low water-holding capacity and low Amax. Studies among and within populations of vagrant *Aspicilia* species, both from the Iberian Peninsula and from Kazachstan, allowed us to consider the significance of thallus size for water economy and CO_2 exchange. We found that in these compact globular lichens size can be a limiting factor for net photosynthesis, since the mass of the fungi increases volumetrically while the algal layer is more or less restricted to the surface. However, the highly specialized thallus structure prevents increased diffusion resistances for the transport of CO_2, even at the highest level of water content. Moreover, the capacity for photosynthetic activity remained the same for the entire surface of these globular thalli, even though they were partially buried in the soil for several weeks.

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262 - Fungal compounds as sun screens for symbiotic algae in lichens

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Lichens, like leaves of plants, have a non-photosynthetic tissue located as a filter between the incident solar radiation and the photosynthetically active cells. Unlike the predominantly green members of the plant kingdom, many lichens are coloured by cortical secondary compounds, e.g. the orange parietin, the yellow usnic acid and the brown-black melanins. Since many compounds can be extracted in a non-destructive way by 100% acetone, their screening role can be directly assessed. The upper cortex of lichens screens a substantial part of the irradiance, particularly UVB, even in the absence of extractable secondary compounds. However, secondary compounds modify the transmittance spectra further by reducing short wavelengths more than longer wavelengths. For instance, an acetone-rinsed air-dry *Nephroma arcticum* cortex with the usnic acid removed, transmits 1% of UVB, which is ten times the transmittance before usnic acid extraction (0.1%). Whereas usnic acid only has a potential in screening UV radiation, parietin and melanin can additionally protect lichens against excessive violet-blue, and all visible irradiation, respectively. For some species, the protection is not always sufficient. The heat- and high-irradiance susceptible *Lobaria pulmonaria* is caught between the risk of being damaged by excess irradiances due to a pale, highly transmitting cortex, or by high temperatures due to a melanic, highly radiation-absorbing cortex in sun-exposed habitats.

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263 - Structural and functional aspects of water uptake and photosynthesis in lichens at subzero temperatures

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It is well known that lichens are able to photosynthesize at subzero temperatures. This ability might be of high ecological significance in cold environments such as Antarctica where snow is the major source for hydration. Due to their poikilohydrous nature water relations in the lichen thallus are strongly correlated with the ambient water regime. At subzero temperatures the lichen thallus is partly dehydrated due to the negative ambient water potential. We present data from a combined structural and physiological study on water relations and metabolic activity in green algal and cyanobacterial lichens at subzero temperatures. Structural investigations were carried out with LTSEM techniques, CO_2 gas exchange and chlorophyll *a* fluorescence indicated the status of the photosynthetic apparatus of the photobiont of the lichens. At subzero temperatures hydrated thalli were kept in equilibrium with water saturated air or dry thalli were allowed to rehydrate from snow. There was no major difference in water relations and activation at subzero temperatures in lichens from cold and temperate environments. However, a major difference was found between green algal and cyanobacterial lichens: while green algal lichens were able to reactivate PSII through water uptake from snow in cyanolichens this did not lead to measureable reactivation of the photosynthesis. This difference may contribute to the almost complete absence of cyanobacterial lichens in continental Antarctica.

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264 - Live-cell imaging of fungal hyphae at high spatial resolution

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A revolutionary new perspective of the cell biology of fungal hyphae is arising as a result of using live-cell imaging techniques to analyse organelle and molecular dynamics at high spatial resolution. A primary objective of the work in our group is to perform live-cell imaging at the highest possible spatial resolution that can be routinely obtained. For this purpose we have been primarily using laser scanning confocal microscopy although more recently we have also started to evaluate multiphoton microscopy and spinning disc confocal microscopy for imaging living fungal hyphae. A major focus of our research is in understanding the mechanisms and regulation underlying hyphal tip growth. Laser scanning confocal microscopy has enabled us to image living hyphae without significantly compromising hyphal growth. Much of this work has concentrated on studying vesicle trafficking and organelle dynamics in living hyphae using a range of vital fluorescent dyes (e.g. FM4-64, FM1-43, and Rhodamine 123) or recombinant GFP specifically targeted to these organelles. Organelles that we are routinely imaging in living hyphae include nuclei, mitochondria, ER and vacuoles. We are also imaging the dynamic localisation of various proteins fused to GFP. Examples of movies from our live-cell imaging studies can be found at the following website: http://www.fungalcell.org/.

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265 - Measurement and interpretation of biomechanical parameters relevant to fungal development

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Without biomechanical experimentation, mycologists have no hope of understanding mechanisms of fungal growth, reproduction, or pathogenesis. Having said this, it must be tearfully-acknowledged that most biologists view the study of fungal mechanics as an eccentric pursuit whose findings can be ignored without grievous consequences. There are two major reasons for this impression, and for the consequent lack of biomechanical research on fungi: (i) an illegitimate conviction that scientific inquiry restricted to the molecular level can answer all of the important questions, and (ii) a legitimate fear that methods for measuring minuscule forces from microscopic cells can be very difficult. If successful, this presentation will alleviate both problems by offering a balanced appraisal of the value and challenges of biomechanical investigation. Methods for determining cellular turgor pressure and the invasive pressures exerted by hyphal apices will be discussed, along with recent efforts to explore the mechanical performance of multicellular fruiting bodies.

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266 - Analysis of calcium signalling in living hyphae expressing a codon-optimised aequorin gene

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Analysis of the *Neurospora* genome indicates that that the calcium signalling machinery is more complex than that found in budding yeast. However, calcium signalling is little understood in filamentous fungi largely because easy and routine methods for calcium measurement in living hyphae have previously been unavailable. We have developed the recombinant aequorin method for this purpose. High levels of expression of the calcium-sensitive aequorin photoprotein have been obtained in a range of filamentous fungi, including *Neurospora crassa*, *Aspergillus nidulans*, *A. niger* and *A. awamori*, by codon optimisation. Three external stimuli (mechanical perturbation, hypo-osmotic shock and high external calcium) transiently increase cytosolic free calcium. The calcium-signatures associated with each of these physiological treatments have been quantified and are highly reproducible under any one set of conditions. Each of the three stimuli generates a unique calcium-signature suggesting that the involvement of different calcium-mediated signal transduction pathways. This is supported by results using calcium modulators. However, we have also found that various parameters (e.g. amplitude and full width half maximum) of calcium-signatures are dependent on temperature and whether the fungus is grown on solid or in liquid medium. Future directions for research on calcium signalling using aequorin will be described.

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267 - Dynamics of organelles and the cytoskeleton in the plant pathogen *Ustilago maydis*

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In contrast to vertebrates, fungi are surrounded by a rigid cell wall that limits motility of fungal cells. However, substrate invasion and pathogenic development of fungi require migration of the cell that is achieved by hyphal tip growth. This process is based on delivery of growth supplies to the expanding apex of the hypha involves the cytoskeleton and associated motor molecules. Recently, the use of advanced light microscopy methods has enabled us

to monitor dynamics of organelles and the cytoskeleton itself in *Neurospora crassa* and *Ustilago maydis*. These experiments have revealed remarkable levels of motility within the fungal cell and it has become obvious that intracellular motions are essential for spatial organization, growth and morphogenesis of fungi.

268 - Using light and electron microscopy to explore hyphal cytoplasmic order, behavior and mutation mode of action

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To assess the impact of single mutations on cell morphogenesis, it is essential to explore the effect of the mutation on cytoplasmic organization and behavior. Video microscopy provides a real time record of organelle behavior and morphological changes in living cells. Such images can provide clues about the mode of action of a mutation that can be tested by transmission electron microscopy of sectioned specimens. One good example is in the study of the *ro-1* and *nudA* mutations in hyphae of *Neurospora crassa* and *Aspergillus nidulans*, respectively. These genes encode subunits of cytoplasmic dynein. In wild-type cells a well-defined Spitzenkörper (Spk) dominated the cytoplasm of the hyphal apex. Vesicles exhibited active motility in subapical regions. Hyphae contained abundant microtubules (MT) that were mostly aligned parallel to the growing axis of the cell. Mitochondria and nuclei maintained a near constant position in the advancing cytoplasm. Dynein deficiency causes disruption of MT organization and function. Beside the overall perturbation to cytoplasmic organization and organelle motility, these mutations disrupt the organization and stability of the Spk, which, in turn, leads to severe reduction in growth rate and altered morphology. The combined use of light and electron microscopy has lead to a more complete understanding of MT disruption and other cytoplasmic phenotypes that result from dynein deficiency.

269 - Four-dimensional laser scanning microscopy of fungal plant pathogens expressing fluorescent proteins

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The three-dimensional mapping of host-pathogen cell interfaces over time, or 4D analysis, represents a challenging but important prerequisite for understanding pathogenesis. We generated fluorescent transformants of two different pathogens, *Fusarium verticillioides* and *Magnaporthe grisea*, and used them for real-time imaging by confocal and multi-photon microscopy. Driven by strong constitutive fungal promotors, expression of spectral variants of green fluorescent protein, as well as the recently identified reef coral fluorescent proteins derived from several *Anthozoa* species, had no detectable effect on either growth rates or abilities to cause disease. Cytoplasm-targeting of fluorescent proteins, coupled with fast image capture rates, allowed discrimination of many subcellular organelles by differential exclusion and facilitated monitoring of rapid changes in permeability of the nuclear envelope. Alternatively, use of fluorescent markers as fusion proteins, for example with tubulin, made it possible to image specific proteins/structures/organelles during cell growth, development, and pharmacological treatment. The intense brightness of some strains expressing fluorescent proteins in the cytoplasm permitted documentation of pathogen cells during invasion of plant host tissues. AmCyan and ZsGreen reef coral fluorescent proteins were sufficiently excited at 855 and 880 nm, respectively, to facilitate time-resolved in planta imaging by two-photon microscopy.

270 - Studying cell biology of arbuscular mycorrhizal fungi by combined high resolution biochemical, molecular biology and microscopy techniques

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Glomeromycotan or arbuscular mycorrhizal (AM) fungi are widespread mutualistic symbionts whose taxonomic position, ecological distribution, reproductive strategies, not to mention cell biology is still poorly understood. At the basis of this lack of knowledge is probably the fact that these fungi are obligate biotrophs, i.e. they cannot complete their life cycle unless they have successfully colonized a host plant. Unsuccessful attempts of culturing AM fungi axenically have been made in the last forty years, this greatly hindering progress in our knowledge of these ecologically important fungi. Moreover, the difficulties to study the intraradical fungal phase withouth disturbing symbiosis functioning, and the extraradical mycelium while maintaining intact their soil-growing hyphae requires of non-destructive, in situ techniques. In this presentation we will briefly review recent advances in our understaning of the cellular biology of these symbiotic fungi. This has been possible by combining techniques such as monoxenic AM cultures, NMR spectroscopy, image analysis and multiphoton microscopy.

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271 - Integrating scientific research and information systems

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The GLOPP project (Global Information System for the Biodiversity of Plant Pathogenic Fungi, http://www.glopp.net/) started in the summer of 2000 and will continue until spring 2003. It aims to provide a unified view of data about fungi parasitizing higher plants. The information system will integrate data on host plant specificity, geographical distribution, and a core set of descriptive characters that allow interactive online identification. European and especially German pathogens are treated with priority to guarantee high data quality and a certain completeness. We plan to continue our work within the framework of international, collaborative successor projects. - The GLOPP system is presented as a highly useful exercise combining basic organismic research with research in biodiversity informatics. The system is presented from the perspective of (a) system development and information modeling, (b) contributing research scientists, and (c) users in applied fields like plant pathology extension services. Ultimately such efforts will be successfull only if they are as valuable to content generators as they are to information consumers. Contributing researchers should be able to work more efficiently within the information system than otherwise. This is currently not the case, but the progress made is substantial (see http://www.diversitycampus.net/).

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272 - Fundamentals of biodiversity research or: What we still do not know!

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Our knowledge of fungal biodiversity is limited not only because of numerous undiscovered species, but we also have many unresolved taxonomic and systematic questions (incomplete descriptions, bad type material, unclear synonymy), the morphology of many species is not well documented, we ignore host ranges of many species, areas of distribution, numerous aspects of life cycles, and effects of interactions with other organisms. For the documentation of existing knowledge of fungal diversity in the internet, we include morphological descriptions in a database. Therefore, we first need a list of morphological characters and character states, which requires a reasonable selection of adequate descriptive vocabulary. The description of a species in literature might be satisfying at first sight, its inclusion into the database, however, in addition to problems of 'translation' due to the use of different terminology, usually shows that the description is far from complete. Often, the germination of spores and other morphological details are not well documented. During the input of data into the database we are forced to ask numerous questions, of which many usually can not be easily answered. Only by field work we obtain fresh material which allows us to elaborate complete sets of character states for the database, and contribute to our knowledge on morphology, host range, distribution, and ecology of the fungi.

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273 - Connecting traditional and modern data sources: Towards an integrated view on the biodiversity of powdery mildews (Erysiphales)

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The Erysiphales form a well delimited order of phytopathogenic ascomycetes with about 650 taxa occurring worldwide. As they represent a group of strongly related fungi, which is relatively well-investigated, they are selected to realise an integrated view on biodiversity data of different kinds. Within the project, descriptive, ecological, distributional and molecular data are collected and linked with data available from traditional and modern data sources. Information is gained from the material of two major collections of Erysiphales in the herbaria of Halle and München (label and specimen data including sequence data) as well as from literature (descriptive data). For the storage and maintenance of collected data the database modules of DiversityWorkbench, the online-information system LIAS and the sequence data management system ARB are used. Subsequent analysis of data from the various sources is achieved via export to various applications. The employment of new database systems and informatics tools will simplify scientific studies on the correlation and evolution of characters both in a distributional and historical context.

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274 - Reconsidered species and genus boundaries: How molecular data influence the content of the database

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The GLOPP (Global Information System for the Biodiversity of Plant Pathogenic Fungi, http://www.glopp.net/) project does not aim at a simple compilation of data, so critical appraisal of the data found in taxonomic literature is necessary. As a rule, this entails experimental re-examination of the published data. Unfortunately, the morphological data so far used for Oomycete taxonomy are often ambiguous. This is especially important where only few morphological characters are available, as it is the case in taxon delimitation in most plant parasitic Oomycete groups. Even the total number of species in Peronosporales and Sclerosporales stated in the literature can vary very much depending on the underlying species concept used by different authors. To cope with these uncertainties, the combination of morphological with modern molecular methods is required. The content of the database will depend to a high degree on the results from this approach.

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275 - Discovering the crunches: the use of the GLOPP databases in rust systematics, ecology and biogeography

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The knowledge about rust fungi (Uredinales) is still patchy: there are some well-studied pests of economically important plants, and there is the bulk of species growing on hosts with no obvious economic importance. The latter group of rusts has received much less attention accordingly. Another striking disparity of knowledge exists between tropical and subtropical areas and extratropical regions. Whereas floras or monographs on rust fungi are widely available in extratropical regions, the student of tropical rusts most often has to confine to scattered original work that may not be handy or accessible. In our project we aim to compile a database on rust fungi with a focus on tropical and subtropical species. The database is geared to (1) facilitate the determination of rust fungi and to provide up-to-date taxonomic information (2) to supply data on the family affiliation of the host plants and on the known geographic range of the fungi in question. Additional information on relevant literature and personal observations are also included. The present database allows to retrieve data that are not readily available on the internet: - host family-rust fungus search - geographical search - synonymy of rust fungi - complex queries with several parameters Such options enable the user to reduce the number of possible determinations of a given rust fungus and to find hidden coincidences and contradictions in regard of host spectra, systematics, and geographical distribution.

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276 - Phytopathogenic fungi from South Africa

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The appointment of I.B. Pole-Evans as mycologist of the former Transvaal Department of Agriculture in 1905 heralded the beginning of phytomycology in South Africa. As diseases of wild and cultivated plants became more well-known, several lists were published of these pathogens. The early lists were prepared by Doidge (1924) and Doidge and Bottomley (1931). The most significant one, however, was published by Doidge et al. (1953), which was primarily based on information contained in her voluminous Index of South African Fungi and Lichens to the end of 1945 (Doidge, 1950). In later years this list was updated again, and several new records added in a series of three science bulletins published by Gorter (1977, 1981, 1982). These data formed the basis for the book, Phytopathogenic fungi from South Africa, which was later published by Crous et al. (2000). An abbreviated version of these data can be viewed on the USDA web site in Beltsville. Members of the Southern African Society for Plant Pathology required a more adaptable database, however, that could easily be updated and changed to fulfill the needs to the local plant pathological community. For this reason a new database template was developed that would support a CD-Rom and web-driven version of the database, incorporating illustrations, notes, all local and relevant publications, culture collection and DNA sequence data.

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277 - The polypores of the world - a useful database or are we still in the alpha phase?

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Over a period of 30 years mycotas of East Africa, North America, Europe and North East Asia have been published, while those of New Zealand and the neotropics are in manuscript and will be published shortly. The generic keys in these books will this autumn be available on the Internet for free downloading. In 1987 360 poroid species were registered in Europe, and since then, i.e. over 15 years, about 5 species have been added, some of them probably tropical imports. 12 species have been described as new, almost all of them based on splitting of previously to widely accepted species, such as *Antrodiella semisupina*. This indicates clearly that the alpha phase is finished in Europe. The situation is similar in North America and North East Asia. In tropical Africa 320 species are registered excluding critical genera like *Ganoderma* and *Phellinus* while at least about 20 species are ready to be

described based on recent collections. Still the Congo-basin is strongly under collected and will certainly yield a good number of new species, especially those with resupinate basidiocarps. Thus, there is still some time before the alpha-phase can be said to be finished in this continent. In the neotropics the situation is similar and recent intensive collecting in the Amazonas basin has revealed a number of new species, clearly indicating that we still not have a clear picture of the biodiversity in this group.

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278 - Taking your data online: Database experiences at the USDA Systematic Botany and Mycology Laboratory

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As the Web becomes increasingly pervasive as a primary source of information, one might suggest that if the information is not on the Web it does not exist. A primary role of systematics is to provide a basic framework that society can use to communicate about the natural world. While there are many potential users of systematic information, these data are most readily available to those with access to the large libraries associated with universities and research institutions. As systematists, we need not only to develop and synthesize information, but to ensure that it is available to those who need it. The web is a visual and interactive medium, which opens up many exciting ways for systematists to distribute their knowledge. Greater thought should be given to providing information on the Web if we want to remain a viable part of the information age. At the same time it is important to understand the differences between the Web and print media and to develop a presentation formats that organize and deliver content is a usable way. To assist systematists in facilitating their use of the Web, an online tutorial has been developed. This tutorial explores the techniques for presenting and relating the descriptive text, images, specimen data and keys that make up the systematists' publication. It is hoped that these examples will stimulate systematists to include Web publication as one of their research products.

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279 - Foliicolous diversity: the Caribbean example

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Flora and vegetation in the Caribbean Islands have a great diversity and richness, therefore, it is a nice place for fungi, particularly for foliicolous, leaf-surface fungi. As treated here, they are those which have the mycelium and fruit bodies on the leaf surface and are in contact with the leaf and the atmosphere, and subject to the influence of both. The Caribbean is a good region for leaf-surface fungi because: • There are more than 20 fungal families and hundreds of species • There are more than 1,000 host plants belonging to 125 families They might be divided, since an ecological or practical point of view, in 3 main groups: Saprobic, Parasite and Hyperparasite. Interesting examples may be shown: • *Trichomerium grandisporum*: it has a world-wide distribution and has been found on more than 50 host plants belonging to more than 20 families. It grows since cultivated areas at sea level to rainforests • *Meliola opuntiae*: on Cactaceae in dry places, in Cuba and Jamaica with only 2 records • *Meliola orchidacearum*: on Orchidaceae in humid vegetations, only in Cuba and Dominican Republic with 3 records Some outstanding examples among host plants are: *Mangifera indica*, *Syzygium jambos*, *Rondeletia canellaefolia*, *Sideroxylon jubilla*, *Acrosynanthus trachyphyllus*, associated with many saprobic species, or *Cupania americana* and *Ocotea leucoxylon* with parasitic ones. The present paper offers a list of leaf-surface fungi in the Caribbean and the related host plants.

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280 - The ecology of foliicolous fungi

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Foliicolous fungi require leaves to make a living in a forest, yet remarkably little is known of such fungi where most leaves are available - in the forest canopy. The 3-dimensional structure of diverse tropical forests creates strong vertical gradients and horizontal patchiness in suitable environmental conditions and host availability for leaf-surface fungi. Using canopy cranes in Australian and Panamanian moist tropical forests, we have explored the roles of host plant availability, fungal dissemination, and microclimate in determining the spatial distribution and diversity of foliicolous fungi. Fungi were found in all strata of the forest at Cape Tribulation, Australia, but the more than 30 genera of non-lichenized leaf-surface fungi were much less common on exposed canopy leaves than in the understory. Understory environmental conditions were darker, relative humidity greater, and temperature variation less extreme, all factors that may promote fungal growth. For three focal plant species, the density of hyphal growth on the leaf surface was greater in the understory than in the canopy. Nevertheless, within the understory in both Australia and Panama, the probability of fungal colonization increased with increase light availability. The types of fungi found varied greatly among plant hosts. The spatial pattern of fungal colonization in the understory was not strongly associated with presence of fungi in the canopy.

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281 - Amber fossils of sooty moulds

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Fungi are unlikely candidates for fossilization and thus relatively few fungal fossils have been described. However, perfectly preserved amber fossils have shown that many modern genera of fungi, and possibly even species, were already present in the Tertiary. For example, several well preserved specimens of sooty moulds were recently found from European amber dating back to 22-54 million years ago. The fossils represent fragments of superficial subicula composed of brown moniliform hyphae with markedly tapering distal ends. The subglobose cells are quite identical to those of extant *Metacapnodium* (Metacapnodiaceae, Dothideales) species. Also the production of two distinctive conidial states supports a placement in this extant genus. The fossils demonstrate that the hyphae of some sooty moulds have remained unchanged for tens of millions of years. As there is little reason to believe that the fossilizations would have been immediately preceded by a period of more rapid evolution, the initial divergence of the fungi must have happened in the distant past, probably in the Cretaceous. The fossils also indicate that hyphal morphology and conidial states can be given considerable classificatory significance in this group of fungi. Eventually, after more detailed phylogenetic hypotheses have been generated for different groups of sooty moulds, the available fossils can become invaluable for timing branching events and calibrating molecular clocks.

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282 - Molecular systematics of some sooty molds

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Sooty moulds are a community of several hundred darkly pigmented mitosporic and ascosporic species of fungi, living on tropical plant surfaces in association with honeydew from insect infestations. All phases of the life cycle of these fungi retain a melanised cell wall creating a sooty layer on leaf surfaces. Mitospores of many of the species are formed on a variety of pycnidial or coremial structures, all of which appear to be adaptations to the leaf surface environment that maximise spore dispersal after wetting. This guild of fungi was extensively studied on citrus and other tropical plant species during the late 19th C, and at that time was given the common epithet *Capnodium citri* (Farlow, Underwood). It is now realised that this an ambiguous name for a polymorphic group. The systematics of these fungi and their interactions with plants and aphids is of interest as the economic effects of sooty mould colonisation of citrus, pecan and other horticulturally important plants begins to become apparent. The taxonomic status of some of the sooty moulds has recently received some clarification based on morphological and physiological characters. However, the complexity of the associations mean that identification based on morphological and biochemical features is unsatisfactory and molecular approaches reported in this work, including RAPD, microsatellite fingerprinting and ITS sequence analysis, provides better data for taxonomic and cladistic analysis.

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283 - Foliicolous ascomycetes in the canopy of a lowland rainforest in the Orinoco Basin, Venezuela (Surumoni Crane Project)

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The fungi were investigated from the forest floor to the upper canopy (till 35 m height) in an area of 1.5 ha of an evergreen forest using a mobile crane system. Data are available from 1996 to 1998 for rain and dry season and for leaves of different ages. The studied fungi, teleomorphs and selected characteristic anamorphs, belong to saprobic, plant pathogenic or fungal pathogenic species and were collected from trees of 25 plant families. Fungi of 15 genera in 12 families were identified in the canopy. Typical genera for tree crowns are e.g. *Atichia*, *Brefeldiella*, *Brooksia*, *Microthyrium*, and *Myriangiella*. The most common genus is *Micropeltis*, which occur on all tree species with a sufficiently thick leaf cuticle and produce first fruitbodies on leaves of an age of about 2 months. The fungal diversity (including saprobic fungi) is very different for individual tree species. Trees particularly in the Annonaceae and Caesalpiniaceae have a high fungal species number. Diversity and quantity decrease from the lower and medium canopy to the top. Probably unfavourable climatic conditions (till about 35 °C and 40% rel. humidity) are responsible for it. This assumption is supported by the fact that in the crane site at the end of rain season foliicolous ascomycetes are best developed. Some of these have a preference for the undersurface of leaves, e.g. *Schizothyrium*, which is a further indication of the strong influence of temperature and humidity on leaf inhabiting fungi.

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284 - Foliicolous lichens: Evolution and ecology of an unusual growth habit

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Foliicolous lichens inhabit leaves of vascular plants in tropical rainforests. They have to adapt to the short longevity of their substratum, as leaves are mostly shed after 12 to 36 months. Furthermore, they must not interfere with leaf functions of gas exchange and photosynthesis. In spite of these limitations, foliicolous lichens evolved a surprising diversity and unique phenotypic features. Fifty species can be found on a small leaf, and 300 taxa within a single rainforest spot. Following their needs for rapid dispersal in a semi-aquatic environment, foliicolous lichens developed particular, convergent dispersal strategies, such as disc-shaped isidia (vegetative), derived conidiomata (asexual), and facultative parasitism on other lichens (sexual reproduction). Based on different evolutionary models of foliicolous lichen growth, and using phenotype-based phylogenetic analyses of selected groups, we tested whether individual features fulfill the criteria of 'key innovations' and led to subsequent radiation. It is concluded that radiation occurred only when sexual and asexual reproduction were maintained as principal dispersal modes within a clade, and the nature of radiation, whether specific or generic, depended on the inherent plasticity of the putative 'key innovation'. Thus, clades characterized by facultative parasitism or derived conidiomata show a high degree of radiation, while speciation is virtually absent in clades with specialized vegetative dispersal organs.

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285 - The phylogeny of *Roccella* (Roccellaceae) with emphasis on the Macaronesian species

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This paper presents a phylogenetic analysis of the lichenized fungal genus *Roccella* (Roccellaceae, Arthoniales), focussing on the Macaronesian species. The ITS rDNA region, including the 5.8S region, ITS1, and ITS2, was sequenced for 14 *Roccella* species, including two putative species pairs. The sequence information was analyzed under a range of alignment parameters, and with gaps coded as missing data. The genus *Roccella* is found not to be monophyletic, since two species, *Roccella hypomeca* and *Roccella portentosa*, are more closely related to another genus, *Roccellina*. *Dirina* is the sister group of the core *Roccella*, as found in earlier studies. The Macaronesian species form a monophyletic group in some, but not in all analyses. Our study also indicates that the two putative species pairs *Roccella allorgei*/*Roccella africana* and *Roccella tinctoria*/*Roccella canariensis* each should be treated as conspecific.

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286 - The *Sphaerophorus globosus* species complex - morphology re-interpreted with molecular phylogeny

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The *Sphaerophorus globosus* species complex (Lecanorales, lichenised Ascomycota) shows a surprisingly large morphological variation, and several relatively distinct morphotypes can be distinguished over the distribution area. We have sampled populations from most areas where the species complex occurs, with a particular focus on the populations in the North American Pacific Northwest, where the morphological variation is very large in the group. In this study, we utilise a multi-gene based maximum-parsimony approach (nITS+LSU rDNA, mtSSU rDNA, ß-tubulin, actin) to investigate the phylogeny of this complex. If the different morphotypes constitute distinct monophyletic groups in the multi-gene phylogenies, this would support the hypothesis that the morphotypes are different phylogenetic species. Monophyletic groupings corresponding to geographical origin would, on the other hand, support the alternative hypothesis that the different morphologies are likely to be caused by the environmentally induced phenotypic variation, rather than by common descent. The results point at the presence of several distinct phylogenetic species in the group.

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287 - Phylogeny of Ostropales and Gyalectales - evidence from molecular and ontogenetical data

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Despite various morphological and anatomical similarities, the two orders Gyalectales (lichenized ascomycetes) and Ostropales (lichenized and non-lichenized ascomycetes) have been considered to be distantly related to each other, and their position within the Ascomycota was unsettled. To estimate relationships within these groups and their respective phylogenenetic placement within the Ascomycota, we investigated the ascoma ontegeny and analyzed DNA sequences from the SSU and LSU nrDNA using Max. Parsimony, Max. Likelihood, and Bayesian statistics with Markov chain Monte Carlo algorithms. We report here that the Ostropales, in their current circumscription, are paraphyletic, and that the Ostropales s.l. include the Gyalectales and the Trapeliaceae. *Dimerella* and *Coenogonium* are congeneric, and *Petractis thelotremella* and *P. hypoleuca* are reunited with members of the genus *Gyalecta*. The ontogeny of the ascomata is variable within the Ostropales and can be used to further subdivide different lineages within this large and challenging order. Ascoma ontogeny, apothecial anatomy and molecular data support the separation of *Coenogonium* (incl. *Dimerella*) as family Coenogoniaceae from the Gyalectaceae. In addition to requiring less computational time, Bayesian inference of phylogeny recovered the same topology as a conventional heuristic search using Max. Likelihood as the optimization criterion, and seems superior to bootstrapping in estimating support for short internal branches.

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288 - Molecular evaluation of the generic concepts in the Pertusariaceae (lichenized ascomycota)

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The Pertusariaceae is a rather large and diverse family of crustose lichens. Its members are characterized by hemiangiocarpous ascoma development, thick-walled amyloid asci and relatively large ascospores. The group boasts a great diversity of secondary lichen compounds. Because several taxa are quite heterogeneous, morphologically and anatomically variable and often sterile, there has been a lot of discussion on the taxonomy of the family. In the present study we analysed nuclear LSU, mitochondrial SSU and LSU sequence data of 45 species to re-evaluate the generic concepts in the Pertusariaceae. We ran maximum parsimony and Bayesian (MCMC) analyses and tested for monophyly of the genus *Pertusaria*. Our results suggest that the genus *Pertusaria* is polyphyletic comprising the *Pertusaria* s.str.-, the Monomurata- and Varicellaria-groups. The Monomurata-group is a sister taxon to *Ochrolechia*; both are closely related to the Varicellaria-group. The genera *Loxosporopsis* and *Melanaria* are included in *Pertusaria* s.str. in the molecular genealogies. Morphological, anatomical and chemical characters are re-evaluated in the light of the molecular study.

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289 - Character evolution in *Phacopsis* inferred from nrDNA sequences

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Phylogenetic analyses of SSU nrDNA sequence data of two species of the lichenicolous genus *Phacopsis* (*P. huuskonenii* and *P. oxyspora*) revealed their membership of the lichen family Parmeliaceae. Studies of the ITS nrDNA of the family additionally included *Phacopsis vulpina*. While the monophyletic origin of the three lichenicolous species could not be rejected by the 'approximately unbiased test', phylogenetic trees calculated with parsimony and likelihood algorithms indicate no closer relationship among the investigated species of *Phacopsis*. Based on these facts, the evolution and adaptive value of selected morphological characters within the Parmeliaceae in the enlarged concept is discussed. Aside from characteristics of asco- and pycnospores, the 'cupulate' hypothecial layer is notable because it is present in Parmeliacean taxa and in *P. oxyspora*, but not in *P. vulpina* and *P. huuskonenii*. Furthermore, the hypothetical ways of thallus reduction and delichenisation are addressed. The loss of lichenisation (in the sense of a mutualistic interaction between a fungal and an algal partner) does most likely not occur in members of the genus *Phacopsis*.

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290 - Evaluation of morphological characters in the genus *Usnea* with the help of molecular data

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A molecular phylogeny of a group of *Usnea* species was constructed utilising two independent gene loci, nITS-LSU rDNA and ß-tubulin. This phylogeny was used to 1) test if the traditionally used morphological species-characters actually characterize monophyletic groups, and 2) to analyse the species delimitation of the closely related pendant *Usnea barbata*, *U. chaetophora*, *U. filipendula* and *U. rigida*. The morphological characters most commonly used for species identification were mapped onto the molecular tree to assess their usefulness for characterizing monophyletic groups. Other species included were: *Usnea articulata*, *U. ceratina*, *U. florida*, *U. hirta*, *U. longissima*, *U. subfloridana* and *U. wasmuthii*. The species in focus form three monophyletic groups. *Usnea barbata* and *U. rigida* form one group with intermixed specimens. Most of the *Usnea chaetophora* specimens form a well supported group. The third group contains all *U. filipendula* specimens and several *U. barbata* and *U. chaetophora* specimens. There are some morphological characters that support these groups (such as branch shape and branching type in the *U. chaetophora*-group, and isidiomorph and isidia occurrence in the *U. filipendula*-group). Many character, though, e.g. the shape and number or papillae and fibrils, are inconsistent with the molecular phylogeny.

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291 - A new look at the Pezizales

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Recent molecular phylogenetic studies have shown that the Pezizales are positioned at the base of the Euascomycota tree. These studies have confirmed the phylogenetic importance of a series of characters, such as ascus construction, ascus cytology and septal pore configuration, that have been used both at ordinal and family levels. Within the order molecular phylogenetic studies also have provided useful information on relationships among taxa. At least three major lineages have been recognized which include families and/or groups previously delimited based on morphological studies. I will review the characters currently in general use at family and genus level and suggest areas for further investigation. It is important at this juncture in the development of the classification of the Pezizales to direct attention to studies of morphology, life histories, ecology, population dynamics and biogeography. This is essential to the development of a useful and biologically informative classification. The idea that the Pezizales, with more than 1,000 described species, are relatively well known taxonomically will be discussed in light of recent field studies and those showing genetic diversity within even morphologically well characterized taxa.

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292 - A closer look at truffle phylogeny

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Small subunit ribosomal RNA (18S rRNA) sequence analysis has proven effective in resolving phylogenetic relationships among pezizalean families. However, due to the limited number of informative sites, a low statistical support is usually obtained for branch topologies at the intra-family and intrageneric level. Even though 18S rRNA topology in Tuberaceae has been corroborated by a parallel analysis of rRNA internal transcribed spacer regions (ITS), an extended sampling of species was required for a robust assessment of some unexpected relationships evidenced among *Tuber* and between *Tuber* and related genera. Moreover, the lack of convenient outgroup sequences prevented a correct rooting of the truffle phylogenetic tree. The results of a study based on a comprehensive set of *Tuber*, *Choiromyces* and *Paradoxa* ITS sequences and suitable outgroups such as *Dingleya* and *Redellomyces* will be presented. Comparisons of molecular phylogenies with morphological character trees and the convenience of a standard encoding system of truffle descriptors (TuberKey, http://www.truffle.org/tuberkey) will also be discussed.

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293 - Phylogeny and biogeography of *Morchella*

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True morels classified in the genus *Morchella* are among the most highly prized and widely collected macrofungi by mycophiles. In order to infer their phylogeny, biogeography and the evolution of reproductive mode, multigene genealogies were constructed to investigate species limits within this systematically challenging genus.

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294 - Progress toward a phylogenetic classification of the Pyronemataceae

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Of the families of the Pezizales the Pyronemataceae (including Otideaceae) remains the least well studied. The family has been considered a default family for pezizalean taxa with uninucleate spores and iodine negative asci, which lack distinguishing anatomical characters by which they could be segregated into putative natural families. Standard treatments of the Pyronemataceae include taxa with a wide diversity of both morphological features and nutritional modes. Recent molecular phylogenetic studies indicate that the Pyronemataceae is part of a lineage composed of Sarcoscyphaceae, Sarcosomataceae, Ascodesmidaceae, and Glaziellaceae. Within these analyses however, the Pyronemataceae is only poorly sampled. The goal of this investigation is to generate a multiple gene phylogeny of the Pyronemataceae and closely related taxa using sequence data from three unlinked nuclear loci (large subunit ribosomal DNA, ß-tubulin and RNA polymerase II) to infer the evolutionary relationships of the family and the genera it contains. Initial phylogenetic analyses of a large subunit ribosomal data set will be presented and discussed.

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295 - Phylogenetics in the Pezizaceae / Ascobolaceae lineage

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Together Pezizaceae and Ascobolaceae appear to be a well-separated lineage within the Pezizales supported by nuclear rDNA sequence data, iodine positive asci (unique to these families within the Pezizales), uninucleate spores, presence of an internal, circular indentation of the ascus apical apparatus, and some similarities in the type of ascus septum pore plugging. The family boundaries, however, remain questionable and will be addressed here, particularly with regard to the placement of *Iodophanus* and *Thecotheus*. Delimitation and relationships among genera within the Pezizaceae are highly controversial. Our previous analyses of 90 partial nuclear LSU rDNA sequences suggest that the circumscription of the large genus *Peziza* and other genera of the Pezizaceae, e.g. *Plicaria*, *Amylascus*, *Scabropezia*, *Hydnotryopsis* and *Sarcosphaera* should be re-evaluated. *Peziza* is composed of at least 6 major lineages, most of which include other genera of the Pezizaceae. In this study we will investigate the evolutionary relationships within the Pezizaceae using analyses of an expanded LSU rDNA data set, in combination with analyses of sequences from a portion of the protein coding nuclear genes β-tubulin and RPB2 for subsets of the taxa. A phylogenetically based classification of Pezizaceae is in progress.

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296 - Ophiostomatoid fungi associated with the Eastern Himalayan spruce bark beetle *Ips schmutzenhoferi* and other bark beetles in Bhutan

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The bark beetle *Ips schmutzenhoferi* infests Eastern Himalayan spruce (*Picea spinulosa*) and Himalayan blue pine (*Pinus wallichiana*) and has been known to cause destructive outbreaks in conifer forests of Bhutan, Eastern Himalayas. We have recently conducted a preliminary investigation of the assemblage of ophiostomatoid fungi associated with *I. schmutzenhoferi*. The mycobiota of two other Himalayan bark beetles (*Polygraphus* sp. and *Dryocoetes* sp) was also considered. Nine, two and four ophiostomatoid fungi were found to be associated with *I. schmutzenhoferi*, *Polygraphus* sp. and *Dryocoetes* sp., respectively. In total, 13 ophiostomatoid fungi, including 1 *Ceratocystis* sp., 2 *Ceratocystiopsis* spp., 6 *Ophiostoma* spp., 3 *Leptographium* spp. and 1 *Pesotum* species were collected. The spectrum of fungi associated with *I. schmutzenhoferi* included *Ceratocystis* sp., *Ceratocystiopsis minuta*, 2 *Leptographium* species (sp. 1 and sp. 2), *Pesotum* sp., *Ophiostoma* cf. *ainoae*, *O.* cf. *cucullatum*, *Ophiostoma* sp. 1 and *Ophiostoma piceae*. *Polygraphus* sp. was associated with *Ceratocystiopsis* sp. and *Ophiostoma* sp. 3, while *Leptographium* sp. 3, *Ophiostoma* sp. 1, *Ophiostoma* sp. 2 and *O. piceae* were associated with the *Dryocoetes* sp. The majority of these fungi are suspected to belong to hitherto unknown taxa and investigations on their taxonomic placement are continuing. This study has improved our knowledge on the occurrence and taxonomy of ophiostomatoid fungi and their vectors in the Himalayas.

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297 - Taxonomy, host relationships and breeding systems in *Ophiostoma minus* and *O. pseudotsugae*

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Ophiostoma minus is widely recognised both as a bluestain fungus causing discoloration of harvested lumber and as a pathogen of standing pines. On pine, *O. minus* is usually associated with bark beetle vectors: with *Tomicus piniperda* in Europe and with *Dendroctonus frontalis* in the USA. There is another *Ophiostoma* species, *O. pseudotsugae*, that is morphologically very similar to *O. minus* and so the two species have been considered to be synonomous. However, analysis of behavioural traits, molecular markers and breeding systems have demonstrated that *O. minus* and *O. pseudotsugae* are separate biological species. Moreover, it is also apparent that isolates currently identified as *O. minus*, comprise two distinct taxa that can be distinguished on the basis of their mating behaviour: one is homothallic and the other it is heterothallic. The extent to which these taxa should be considered as separate species is considered.

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298 - Hitch-hikers with invasive *Tetropium fuscum* (Fabr.) (Coleoptera: Cerambycidae) in Atlantic Canada

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Species of *Ophiostoma* are well-known as causal agents of blue-stain in living trees and lumber, and as serious pathogens, and occur in close association with insects, especially bark beetles, which often function as vectors of these fungi. In 1999, dying red spruce (*Picea rubens*) in a small area of the Halifax Regional Municipality in Nova Scotia, Atlantic Canada were infested by *Tetropium fuscum*. In Europe, *T. fuscum* is usually a secondary pest of Norway spruce and rarely attacks healthy trees. In this area of Nova Scotia, *T. fuscum* is infesting and killing red spruce, whose native range is restricted to northeastern North America. Several ophiostomatoid fungi occur with this beetle. The aims of this study were to identify the fungi associated with *T. fuscum* in Halifax and compare them with ophiostomatoid fungi associated with this insect in Europe. Fungal associates of *T. cinnamopterum* (indigenous to and transcontinental in Canada) were also studied and compared to those of *T. fuscum*. Species were identified based on morphological and molecular characters. *Ophiostoma tetropii* is the most common species associated with *T. fuscum* in Europe and Canada, probably introduced into Atlantic Canada by this insect. *O. tetropii* was only isolated from trees initially attacked by *T. fuscum*. Other species isolated from trees attacked by both *T. fuscum* and *T. cinnamopterum* include *O. piceae*, a secondary colonizer of conifer sapwood, and the rarely reported *Pesotum fragrans*.

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299 - Surviving life in the fast lane: sex and clonality in *Ophiostoma novo-ulmi*

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On several continents the Dutch elm disease pathogen *Ophiostoma novo-ulmi* is the fungal equivalent of an adventurous tramp. Having left its original home or centre of origin and 'jumped aboard a moving express train' (a spreading series of Dutch elm disease epidemics) it has had to cope with a many new and challenging events, processes and opportunities. 'Positive' opportunities include less distraction from competition between genotypes (a consequence of founder effects and selection), escape from natural enemies, an abundance of beetle vectors combined with a highly susceptible host, easier access to the saprotrophic phase and easier access to the vectors. 'Negative' changes include increased risks from threats such as virus infections and sibling species and, potentially, a reduced opportunity for genetic variation if the environment changes. The impact these lifestyle changes appear to have had on the pathogens fitness, behaviour and population structure will be discussed, with special reference to sexual and asexual reproduction, clonality, the influence of fungal viruses and the role of outcrossing and horizontal gene transfer.

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300 - Hosts, insects, sex, aleurioconidia and the evolution of *Ceratocystis*

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Phylogenetic analyses of rDNA sequences and a portion of the *MAT-2* gene show *Ceratocystis* to be monophyletic and most closely related to *Gondwanamyces* and more distant to the Microascales. Five major clades are found within *Ceratocystis*. The *C. fimbriata* complex and *C. albofundus* form a basal clade. Species in the *C. paradoxa* clade are pathogens on monocots. The *Thielaviopsis basicola* clade includes four soilborne, asexual species. The *C. coerulescens* clade lacks aleurioconidia and includes a subclade adapted to the Pinaceae. The remainder of the species group loosely in the *C. moniliformis* clade, which includes *C. adiposa*, *C. fagacearum*, and three asexual symbionts of ambrosia beetles: *Ambrosiella xylebori*, *A. hartigii*, and *A. ferruginea*. Characters found in the basal *C. fimbriata* clade and in one or more of the other clades were likely present in the most recent ancestor of *Ceratocystis*. The hypothesized ancestor was a wound-colonizer of woody dicots, insect-dispersed, and homothallic (unidirectional mating type switching); had hat-shaped ascospores accumulating at the tip of long-necked perithecia; and formed both endoconidia (*Chalara*-like) and aleurioconidia (pigmented, thick-walled spores that can survive in insect frass and soil). Shifts to heterothallism, loss of sexual reproduction, loss of aleurioconidia, adaptations to monocots or the Pinaceae, and bark beetle or ambrosia beetle symbiosis appear to have occurred in one or more lineages of *Ceratocystis*.

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301 - Relationships amongst *Ceratocystis polonica*, *Ceratocystis laricicola* and their bark beetle vectors in Europe and Asia

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The pathogenic blue-stain fungus *Ceratocystis polonica* occurs on various spruce species (*Picea* spp.) and is associated with the bark beetles *Ips typographus*, *Ips amitinus* and *Ips duplicatus* in Europe and *Ips typographus* f. *japonicus* in Japan. *Ceratocystis laricicola* is the dominant pathogenic fungus vectored by the bark beetle *Ips cembrae* that infests larch (*Larix* spp.) in Europe and Japan. These two fungi are morphologically identical. However, recent studies have shown that they can be clearly separated using a number of molecular characters

including isozymes and sequences of the ITS region of the ribosomal DNA operon, the beta-tubulin gene and the HMG box of the MAT-2 gene. Our recent data also show that the Japanese isolates of *C. laricicola* represent a distinct taxon. This is consistent with DNA-based comparisons of its vectors, which are presently also viewed as two separate species occurring in Europe and Asia. Reciprocal inoculations in a number of experiments including our own in central Europe, have shown that *C. polonica* is preferentially pathogenic to spruce. In contrast, *C. laricicola* is more pathogenic to larch than to spruce. These and other studies comparing populations of isolates using microsatellite markers, show that *C. polonica* and *C. laricicola* represent discrete biological entities. They have evidently evolved closely with their respective insect vectors, on different host trees.

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302 - *Pleurotus* species as medicinal mushrooms in health care and nutrition

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Pleurotus mushrooms, popularly called oyster mushrooms are distributed all over the world. Modern scientific analysis has shown that *Pleurotus* mushrooms are rich in proteins (especially those containing essential amino acids), vitamins, essential fatty acids and minerals. In past three decades in the United States of America, attention is increasingly been drawn to the studies of oyster mushrooms for its nutritional values and medicinal benefits. There are reports on biologically active compounds, lectin and different polysaccharides isolated from oyster mushrooms that have been tested for their medicinal effects. Polysaccharides from different oyster mushroom have been shown to have 50-95% reduction in cancel cell growth in vitro and animal models in some cases. The scientific and clinical studies of the medicinal properties of oyster mushrooms are still limited. Generally, the problem of standardization and a uniform approach to the studies on medicinal benefits of mushrooms also apply to oyster mushrooms. This report is aimed at bringing to light the traditional uses of oyster mushrooms and the current state of knowledge on the scientific studies of oyster mushrooms in food and medicine. The limitation in methodology and approach to scientific studies and application of oyster mushroom in nutrition and health care delivery, as well as solutions and better approach to this subject are discussed.

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303 - *Pleurotus* sporocarps - a hypocholesterolemic nutraceutical?

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Coronary artery diseases and hypercholesterolaemia are the main cause of morbidity in developed countries. The initial step in their prevention and treatment is the modification of the nutritional regime (diet low in fats and saturated fatty acids; rich in crude fibers). The oyster mushrooms are a good source of nonstarchy carbohydrates, have a very high dietary fiber content and a low caloric value. Therefore their hypolipidaemic efficiency was for a long time attributed only to the interaction of crude fibers and chitosan with bile acids in the small intestine, causing increased excretion and reduced enterohepatal circulation of bile acids and accelerating cholesterol catabolism by a feedback mechanism. Later it was discovered that addition of dried oyster mushroom to high cholesterol diet effectively reduces cholesterol accumulations in the serum and liver of rats, redistributes cholesterol in favour of high density lipoprotein (HLDL) cholesterol, reduces production of very low density lipoprotein (VLDL) and low density lipoprotein (LDL) cholesterol. It also reduces cholesterol absorption and the activity in liver of the major rate limiting enzyme in cholesterol biosynthesis pathway, 3-hydroxy-3-methylglutary CoA (HMG CoA) reductase. By the combined action of crude fibers and specific inhibition of HMG CoA reductase, a considerable reduction in VLDL and LDL cholesterol levels can be explained.

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304 - Antioxidant activity of several basidiomycete mushrooms cultures

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The active metabolites of medicinal basidiomycete mushrooms have different medicinal and preventive properties. However, the data concerning their antioxidant activity (AOA) is rare. The ability of mushroom-derived compounds to prevent oxidative damage was observed within cultures mostly from the genera *Agaricus*, *Coprinus* and *Ganoderma*. The cultural liquid (CL) was proposed for preventing cancer, nephritis, hypertension, and for delaying senility. The higher peroxidase inhibition potential of fungal melanin pigments was also reported. Mycelial melanin is promising for elaboration of new biotech-compounds with AOA. In our experiment 17 mushroom cultures from 13 genera (*Coprinus*, *Coriolus*, *Flammulina*, *Lentinula*, *Lepista*, *Marasmius*, *Hypholoma*, *Pholiota*, *Pleurotus*, *Schizophyllum*, *Stropharia*, *Suillus* and *Volvariella*) were tested on AOA. The CL and mycelium suspension (MS) obtained on days 7 and 14 haven't possessed AOA. The all samples showed different degree of activity on 21 day. The CL samples were more active than MSs. The highest AOA in CL of *Pholiota alnicola* (44%) and in MS of *Volvariella bombycina* and *Lepista personata* (26%) has been revealed. No correlation between AOA and ecological belonging of tested species has been observed. Further AOA-screening, study of mechanism of action and dosa-effect correlations of active compounds will assist in obtaining new biopreparations or mushroom dietary supplements with antioxidant effect.

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305 - Mushrooms and weight loss: a randomised, double blind study

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We investigated the impact of mycelium shiitake or *Agaricus bisporus*, when associated with fibers, on body weight. Material and methods Biscuits A were prepared with 6% of mushroom powder (70% of Shiitake mycelia grown on wheat and 30% of fibers) and Biscuits B 3% only (70% of dried *Agaricus bisporus* and 30% of fibers). The micture was finalised according to the patent PCT/FR01/00577. Consumers were randomized. Each had to eat 5 biscuits (50g) per day for 4 weeks. Body weight, fat index, food intake, physical activity and global caloric balance (caloric intake - physical activity) were compared. Results 38 consumers were included: 19 (18 women) in group A and 19 (14 women) in group B. 26 consumers completed the form on food intake and physical activity.

Table 1: Populations A and B were comparable

	Group A	Group B
Mean age	30 ±10	35 ±10
Body weight (kg)	67 ±10	71 ±11
% of fat	30 ±6	32 ±10

For group A, caloric balance - for 4 weeks - was equal to +998 kcal and +1562 for group B.

Table 2: Difference for body weight and fat weight after 4 weeks.

	Group A	Group B
Body weight difference	-0.2 ±1.1*	-1.3 ±1.3*
Fat weight difference	+0.3 ±1.0*	-1.4 ±2.1*
*p<0.001		

One consumer completed the form and lost weight in group A, 9 in group B ($p<0.001$). We concluded that *Agaricus bisporus*, when enriched with fibers, reduces overweight at the dose of 1.5g per day for 4 weeks. Shiitake mycelia was probably destroyed by heat and fibers alone were not efficacious.

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307 - Wild growing medicinal mushrooms of Ukraine: Radiocaesium contamination

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Concentrations of radiocaesium in 135 species of mushrooms and substrates from their habitats in 1990-2001 have been studied using gamma-spectrometric methods. Radiocaesium content varied within a wide range. Maximum levels of Cs-137 were found in samples from the Chornobyl Exclusion zone in *Gomphidius glutinosus* 17117 kBq/kg, in *Lactarius turpis* 13203 kBq/kg and in *Boletus edulis* 1560 kBk/kg d.w. Interspecific differences in levels of radiocaesium accumulation among species from the same habitat reached 10^3 times. Level of radionuclide accumulation in mushroom depends both on radioecological situation in the sampling sites (composition, forms of radionuclides, moisture, pH, type of soils, climatic factors etc) and on biological specificity (depth of mycelial location, systematics, ecological belongness). It is those factors in association with mosaic pattern of contamination and processes of radionuclide migration within the components of ecosystems that condition extremely geterogenous picture of mushroom radiocaesium contamination. Results obtained made it possible to single out species-indicators for long-term radioecological monitoring. Among them there are representatives of mycosymbiotrophic families Russulaceae, Boletaceae, Paxillaceae, Cortinariaceae. The information on the species contamination also allowed to some extent to predict a degree of safety of their usage in medicine and nutrition.

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308 - Inhibiting effect of some medicinal mushrooms on aflatoxin production by *Aspergillus parasiticus* Speare

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The inhibiting effect of mycelial or fruit body extracts or liquid culture filtrates from *Lentinus edodes* (Berk) Sing. and other medicinal mushrooms has been tested on aflatoxin production by *Aspergillus parasiticus* Speare. The mushrooms tested were: *Lentinus edodes*, *Trametes versicolor* (L.:Fr) Pil., *Auricularia auricula-judae* (Bull) Wettstein and *Pleurotus ostreatus* (Jocquin ex Fr.) Kummer. The aflatoxin producer strain was *Aspergillus parasiticus* NRRL 2999. The medicinal mushrooms were inoculated on Potato Dextrose Broth (PDB, Difco) and incubated at 25 °C till they have reached plateau. The cultures were than filtered, and the culture filtrates were lyophized and tested at the concentration of 2% w/v in the presence of *A. parasiticus* conidia and incubated at 28 °C up to 6 days. The fruit bodies and spent compost of *L. edodes* were water extracted and assayed in the same way. The extracts from mycelia and liquid culture filtrate were also partially purified and the obtained fractions tested on the aflatoxin production. The results obtained showed that not purified lyophilised or liquid filtrates, fruit bodies and spent compost extracts from *L. edodes* inhibited aflatoxin production of 85-90% without interfering with *A. parasiticus* growth. Among the partially purified extracts some showed a strong inhibiting effect both on fungal growth and aflatoxin production (*T. versicolor*), while others inhibited only the aflatoxin production (*A. auricularia-judae*).

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309 - Botanical antifungal drug from lichen metabolites fight fungal infections

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During antifungal screening of some lichen metabolites (LM), tested at different concentrations against human pathogenic fungi (dermatophytes) *Epidermophyton floccosum*, *Microsporum audouinii*, *M. nanum*, *M. canis*, *M. gypseum*, *Trichophyton mentagrophytes*, *T. rubrum*, *T. violaceum* and *T. tonsurance*. The lichen metabolites of *Usnea longissima* Ach. was found to be most effective. The minimum inhibitory concentration of the LM was found to be 30 µl ml^{-1} at which LM showed fungistatic action. The minimum fungicidal concentrations (MCCs) of the LM were found to be 50 µl ml^{-1} against human pathogenic fungi. The LM at 50 µl ml^{-1} showed heavy doses of inoculum potential. Moreover, LM did not exhibit any adverse effect on mammalian skin up to 10% concentration. Further, the LM based ointment of *Usnea longissima* was trialed clinicaly in MLN Medical college, Allahabad. 30 patients were selected, showing positive potassium hydroxide (KOH) results at the start of the trial. At the end of medication, 30.0% of patients recovered complete cure, 45.0% showed significant improvement from the disease. No KOH negative cases of relapse were observed when patients were reexamined after two month following the end of treatment. The ointment was found cost effective and absence of any adverse effects. The commercial utilization of the ointment could be determined after undergoing successful multicentre clinical trial. Which is in progress.

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310 - Mycotoxins as ecotypic characters in *Fusarium* species

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Fusarium species can be found in many different ecological niches all over the world, including natural as well as man-made niches. Furthermore, it is well-known that every *Fusarium* species is able to produce several biological active metabolites, of which some are known as mycotoxins. However, this does not imply that every *Fusarium* mycotoxin can be found everywhere, as not all *Fusarium* species occur everywhere. Each ecological niche has its own associated set of *Fusarium* species, which can growth on the given substrate under the given conditions. Taking an ecological approach a link between *Fusarium* species, their mycotoxins, and the ecological niche will be demonstrated, indicating that known mycotoxins may have more functions than being toxic to humans and livestock. The presentation will focus on the profile of produced compounds originating from more metabolite families and not on individual compounds of the trichothecenes, zearalenones, fumonisins, moniliformin, and other well known *Fusarium* metabolites. The holistic interpretation of metabolite profiles is launched as a fruitful platform for future discussions on fungal chemistry and toxicology towards a better understanding of fungal ecology.

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311 - Ecology and genetics of loline alkaloid expression in grass endophyte defensive mutualisms

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Loline alkaloids (saturated 1-aminopyrrolizidine alkaloids with an oxygen bridge) are fungal secondary metabolites often present in grasses symbiotic with endophytes in the genera *Epichloë* and *Neotyphodium*. Endophytes producing loline alkaloids provide grass hosts with enhanced protection from herbivory, drought and pathogens. *Epichloë festucae* has proven to be an excellent genetic model system for ecological and genetic studies of lolines. In an earlier study, segregation analysis supported a single locus (*LOL*) contributing to a naturally occurring lolines expression polymorphism in *E. festucae*. Responses of two aphid species (*Rhopalosiphum padi* and *Schizaphis graminum*) to meadow fescue symbiota containing E. festucae progeny segregating for *LOL* (Lol+ vs Lol-) clearly implicate lolines as agents of protection against insects. Successful map-based cloning has resulted in identification of a >40kb gene cluster unique to the Lol+ parent. Subsequent sequencing of this region in *E. festucae* (and related gene clusters in *Neotyphodium uncinatum*; see poster by Spiering et al.) reveals relationships of the likely loline alkaloid synthesis genes with fungal genes known for synthesis of amino acids, polyamines and other secondary metabolites.

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312 - Inherent fitness advantage associated with progression along the AF/ST polyketide biosynthesis pathway in *Aspergillus*

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Sterigmatocytin (ST) is a polyketide-derived *Aspergillus* mycotoxin and penultimate precursor of aflatoxin (AF). Although the specific functions of many ST cluster genes have been elucidated with regard to their role in the ST/AF biosynthesis, the specific function of ST/AF for the fungi that produce them is not known. We are attempting to identify the fitness costs and/or benefits of ST expression. Isogenic and prototrophic, *A. nidulans* mutants interrupted at different genes in the ST biosynthesis cluster (delta-*aflR*, delta-*stcJ*, delta-*stcE*, delta-*stcU*) and the wild type (ST) were generated. These genotypes were grown on petri plates containing glucose minimal media or on live. Relative fitness was determined as a difference in the number of conidia harvested after incubation for seven days at 37°C.Two light conditions were used in these fitness tests. In both experiments completing the ST pathway was beneficial to fungal fitness. Rather than being attributable to the impact of a knockout mutation, the tight relationship between fitness ranking and position of each gene product in the biochemical pathway support a direct effect of these mutations on conidiation. These data suggest that ST (and presumably AF) production contributes to the fitness of *Aspergillus* under the environmental conditions tested.

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313 - Biology of secondary metabolites

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There are many perspectives on secondary metabolites of the marine fungi, conifer endophytes and agricultural fungi I have studied. The genes that code for secondary metabolites seem largely to be clustered together implying that their acquisition was not incremental but chaotic. Their role in determining population structure focuses on the benefit to species. Necrotrophic plant pathogens produce virulence factors that also discourage the consumption of the affected plant tissues by animals. Fungi that produce toxins that affect animal competitors typically produce several families of toxins. Some toxin families many be more or less active against different potential herbivores. Others result in additive toxicity with the important consequence that acquisition of resistance to the toxin is improbable. Despite this high degree of evolution, the production of secondary metabolites by filamentous fungi must first be thought of in terms of the biochemistry of the terminal few cells of the mycelium. It has been known since the invention of the microscope that it is only these cells that are active, with of course the most active being the hyphal tip. Control of the pathways of secondary metabolism reflects the regulatory processes of glycolysis, the citric acid cycle and electron transport. As nutrients become unavailable to the active cells, accumulation of acetate, citric acid cycle products and some amino acids become available for the production of secondary metabolites.

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314 - Liard Hotsprings hypsothermal relict commmunity: thermal impacts on fungi and plants in a boreal forest ecosystem

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The response of plants and fungi to temperature gradients was studied in Liard Hot Springs Provincial Park in the boreal forest of British Columbia. Within 50 m of the hot springs, the forest was characterized by the proliferation of understory shrubs such as bitter cherry (*Prunus emarginata*) and bunchberry (*Cornus canadensis*), and hardwood tree species such as balsam poplar (*Populus balsamifera*) and paper birch (*Betula papyrifera*). By contrast, the forest 150 m from the springs was occupied primarily by white spruce (*Picea glauca*). Basal area of tree stems declined with proximity to the springs and increasing proportion of hardwoods; basal area 50 m from the springs was half of the basal area 150 m from the springs. Diversity of macrofungal fruiting bodies also declined from an average of 33 species 150 m from the springs, to an average of 13 species 50 m from the springs. Fungi found in the warmer zones included species such as *Humaria hemispherica* and *Mycena delicatula*. *Hygrophorus* spp., *Marasmius* spp., and others were found in the colder zones, while *Cortinarius* spp., *Inocybe* spp., and others were found throughout the gradients. Plant diversity and the proportion of mycorrhizal species were unchanged with respect to distance from the springs. While

further study would be needed to extrapolate this information into general trends, boreal hot springs appear to offer unique opportunity to study the potential impacts of climate change on boreal fungi and plants.

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315 - Biomass of ectomycorrhizal mycelia at different soil depths and along nutrient gradients in the boreal forest

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Nutrient uptake by forest trees is greatly dependent on ectomycorrhizal (EcM) fungi. We quantified the production of EcM mycelia in the field by the use of in-growth mesh bags. Mesh bags were placed at different soil depths (5-15-30 cm) in spruce and mixed spruce/deciduous forests in southern Sweden and in the humus layer along a natural nutrient gradient in northern Sweden. Spruce stands produced 590 kg EcM biomass ha^{-1} while mixed forests produced 420 kg ha^{-1}. The delta-13C value of mycelia collected from mesh bags was similar to values of EcM fruitbodies, and it was not influenced by soil depth, indicating that the mycelia were of EcM origin. The delta-13C value in mycelia from mixed forests suggested that the mycelia received more carbon from spruce trees than from oak trees. The production of EcM mycelia decreased with soil depth. The decrease was more accentuated in mixed stands compared to pure spruce stands. In the natural nutrient gradient (low N and low pH changed gradually to high N and high pH) the production of EcM biomass decreased when moving from the nutrient poor to the nutrient rich end. We identified some of the EcM species that colonized the mesh bags with PCR/RFLP analysis of ribosomal DNA. We found a low level of similarity between EcM species found on root tips outside the mesh bags and EcM species found as mycelia or rhizomorphs inside the mesh bags. Fast growing species such as *Paxillus involutus* were common in mesh bags but rare on root tips.

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316 - New perspectives on the ecological distribution of epiphytic hair lichens (*Bryoria*) in northern forests

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Caribou biologists working in the high-elevation conifer forests of inland British Columbia have long observed that hair lichens in the genus *Bryoria* attain much heavier loadings in oldgrowth stands than in adjacent younger stands. In an attempt to account for this phenomenon, I examined *Bryoria* species composition in regenerating forests along successional and within-stand vertical gradients. *Bryoria* appears to enter regenerating stands in two phases. In the first phase, to about 70-100 years, *B. fuscescens* and *B. glabra* dominate throughout the canopy. These are small, sorediate lichens with a relatively high tolerance for prolonged wetting. After ca. 100 years, these species are increasingly replaced, at least in the upper canopy, by the non-sorediate *B. fremontii* and *B. pseudofuscescens*. These are large, relatively xerophytic hair lichens favoured by open, well-ventilated conditions. *B. fremontii* and *B. pseudofuscescens* have indeterminate growth; once established in the upper canopy, they tend to fragment, thereby being continuously 'parachuted' into the lower canopy. It is the gradual accumulation of these non-sorediate species at all levels of the canopy that ultimately leads to an elevated *Bryoria* biomass in oldgrowth forests. The ability of *B. fremontii* and *B. pseudofuscescens* to persist in the lower canopy does vary considerably from year to year, depending on the occurrence of 'die-back' events associated with prolonged dampness.

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317 - Implications of fungal translocation for nutrient cycling in boreal forest ecosystems

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Most current models of nutrient cycling have been developed with a vague concept of 'microorganisms' in mind, generally relating to bacteria. In boreal forest ecosystems, fungi probably play a more important role than bacteria in the decomposition of organic matter. Most fungi differ from bacteria in that they are multicellular and able to translocate resources throughout their mycelia. Current nitrogen cycling models are centred around mineralisation; as substrates with a low C/N-ratio are degraded, nitrogen-containing organic compounds are thought to be used as an energy source, leaving ammonium as an undesired biproduct that is released. Fungi may, however, degrade substrates with a low energy content, using carbohydrates that are translocated from external sources such as woody debris, litter or living roots in the case of mycorrhizal fungi. Obtained nutrients may be translocated back in the opposite direction to support colonisation and degradation of the nitrogen-poor wood or litter, or to support a mycorrhizal host plant. Translocation of resources thus enables fungi to utilise resources of various qualities more efficiently, conserving nutrients within their mycelia instead of exuding them. In light of the capacity of boreal forest plants to access organic forms of nitrogen via their mycorrhizal associates, it is likely that a significant fraction of the nitrogen recycling from organic matter back to plants takes place without the nitrogen ever being mineralised.

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318 - The natural abundance of ^{15}N in mat-forming lichens

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Natural abundance of ^{15}N and [N] was studied in thalli of mat-forming lichens collected from tundra and heathland sites in the Northern and Southern Hemispheres. The study includes samples of British *Cladonia portentosa* from sites in regions of high and low N-loading and in heathland growing on peat and independently of the soil substratum, in a canopy of prostrate gorse. In the mat-forming lichens examined, a non-random pattern in [N] and δ^{15}N was characterised by a minimum in δ^{15}N, which occurred most frequently at 40-60 mm below the thallus apex. [N] increased above this point, towards the apex, though remained invariably low towards the thallus base. We discuss the significance of the pattern in [N] and δ^{15}N for current theories describing the uptake and recycling of nitrogen by mat-forming lichens in oligotrophic habitats. Our data are incompatible with the suggested uptake of soil organic nitrogen depleted in ^{15}N, though are consistent with possible internal recycling and the development of a structural necromass. The study emphasises the internal fractionation of nitrogen isotopes and provides a caveat against the assumption that values of δ^{15}N provide an unequivocal indicator of source-sink relationships in nitrogen cycling.

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319 - Role of environmental conditions and birch tree genotype in endophyte-herbivore interactions

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We compared phenotypic and genetic frequency correlations for two endophytic fungal genera (*Fusicladium* and *Melanconium*) and bich rust (*Melampsoridium betulinum*) with the performance of six invertebrate herbivores growing on the same half-sib progenies of mountain birches (*Betula pubescens* ssp. *czerepanovii*) in two environments over a 3-year period. We found little support for causal association between fungal frequencies and performance of herbivore species. Instead, genetic correlations, particularly between autumnal moth (*Epirrita autumnata*) and birch rust, suggest that herbivore performance may be affected by (1) genetic differences in plant quality for fungi and herbivores, or (2) genetic differences in responses to environmental conditions. Genetic analysis (RAMS-PCR) of *Venturia ditricha* (anamorph *Fusicladium betulae*) revealed that (1) host genotypes and environment influence the probability of infection by particular endophyte genotypes, (2) genetic variation correlated negatively with infection frequencies of the fungus, and (3) the susceptibility of the host to a particular endophyte genotype may change when environmental conditions are changed (environment-host genotype interaction). Our results propose that the seemingly direct interactions between herbivores and fungi may actually indicate genetic differences in plant quality for fungi and herbivores or responses to environmental conditions.

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320 - Specificity of fungal-algal interactions on leaves

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Several types of algae are adapted to the growth on living leaves, coccoid green algae and members of Trentepohliales being most common and also serving as photobionts of lichen-forming fungi. Initials of these lichens need to form composite thalli with appropriate algae in a relatively short time. Therefore, particular strategies are pursued to ensure efficient dispersal and colonialization of leaves. Specific growth patterns of trentepohlioid algae, found in both lichenized and non-lichenized forms, suggest that foliicolous mycobionts have a high specificity for their photobionts. Adjacent free-living *Phycopeltis* colonies belonging to different morphotypes are not accepted as photobionts by the same fungus. Different ecological preferences of photobionts apparently shape the diversity of foliicolous lichens found in a given microhabitat. A particular strategy regarding photobiont selection of lichens is found in lichenized, foliicolous species of *Chroodiscus*, which grow facultatively parasitic on lichens of the genus *Porina*. *C. australiensis* and *C. coccineus* show a high degree of host specificity: *C. australiensis* parasitizes *Porina mirabilis*, whereas *C. coccineus* is specifically found on *P. subepiphylla*. In genera of lichens, which include both lichenized and non-lichenized, lichenicolous species (e.g. *Arthonia*), the latter take advantage of the host photobionts.

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321 - Fungal endophytes in a neotropical forest: ecological, systematic, and physiological insights

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Fungal endophytes associated with woody angiosperms are abundant and diverse; yet especially in tropical forests, they are poorly known. In particular, the scale of endophyte diversity, and the nature of endophyte-host interactions, are not well understood. Drawing from extensive field studies at scales ranging from individual leaves to disparate sites across lowland Panama, I will discuss evidence for spatial structure, temporal variability, host affinity, and horizontal transmission among tropical forest endophytes. Drawing from experimental results, I will describe a potential mechanism for host affinity based on defensive chemistry of host leaves. Using molecular sequence data (nrDNA: ITS1, ITS2, 5.8s), I will show that tropical endophytes are diverse at both low and high taxonomic levels, and will discuss phylogenetic patterns of diversity. Finally, I will discuss roles of endophytes in mediating host defense against foliar pathogens, and will assess general costs and benefits of endophyte infection in tropical woody angiosperms.

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322 - Effect of agricultural management on diversity of root endophytes: the role of dark septate endophytes

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Three mesotrophic grassland sites of similar physical characteristics but differing management histories were chosen to test the hypothesis that agricultural disturbance has a deleterious effect on the diversity of fungi inhabiting plant roots and the prevalence of potentially pathogenic species (e.g. *Fusarium* spp.). Species abundance data were collected for fungi isolated from surface sterilised root samples (>40 taxa). Shannon and Brillouin indices of diversity, TWINSPAN and detrended correspondence analysis were applied to the community data. Quantitative ordination separated the samples by site showing that the communities differed in fields of contrasting management. Species presence and absence appeared to be affected seasonally; site differences were manifested in relative abundance. Diversity did not appear to vary by site, but a methodological explanation for this is proposed. Sterile dark septate endophytes (DSE) were shown among the most abundant groups at all sites, and diversity among these fungi was investigated using a PCR-based approach. It is suggested that DSE, in addition to the overall diversity of root-colonising species, may be implicated in relating plant root communities and plant health. Microcosm systems have been used to study the dynamics of root colonization by DSE, as well as *Fusarium* spp.

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323 - Rock inhabiting fungi and lichen photobionts: symbiotic or antibiotic interactions?

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Phototrophs are considered to be the first land colonisers, although this ability is often supported by mutualistic associations with fungi. Furthermore bare rock surfaces are frequently dominated not by phototrophs, but by free-living and symbiotic ascomycetes. On desert rock surfaces lichens often yield and in the harshest environments only microcolonial fungi (MCF) are present. MCF - highly stress-tolerant free-living organisms - could represent the remnants of symbiotic associations, which implies their capability to interact with photobionts. Four typical MCF were cultivated with 4 lichen photobionts isolated from similar desert locations. The relations between the partners were investigated by electron microscopy and histological methods. After several months of cultivation a structure involving both partners has been developed. Photobiont cells were not changing size, but fungal branching was more expressed in the vicinity of green algal cells. Histological analysis of interwoven colonies exhibits spatial adjustment of the partners. After continued cultivation algae lost vitality. Only one MCF strain enabled survival of the algae tested. The absence of antibiotic influences in the first stage of the experiment hints to an universal ability of MCF to form unstable mutualistic relations with lichen photobionts. The later loss of mutualistic balance may be connected to the diverse taxonomic position of the MCF strains in question.

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324 - Cyanobiont diversity in ecological lichen guilds

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Many cyanolichen species are organized into guilds around groups of phylogenetically related cyanobacteria. In a study of old-growth associated cyanolichens in central Finland all bipartite epiphytes were found to house closely related Nostoc strains and most of the thalli contained one specific strain. While the cyanobionts of all these lichens were closely related, the lichen-forming fungi were necessarily not. Moreover, some related terricolous species associated with a different group of *Nostoc* symbionts and thus belonged to a different ecological guild. A comparison with cyanolichens from North America and East Asia revealed concurrent patterns. Thus, a similar specificity was evident on all spatial scales ranging from single tree trunks to global distributions. All lichen-forming fungi

within a specific guild can potentially share cyanobionts with each other. Further studies of guild structure will give us a better understanding of the dispersal ecologies and conservation requirements of old-growth associated epiphytes and other cyanolichens.

325 - Grass endophyte host specificity and evolutionary implications

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The *Epichloë* (anamorph = *Neotyphodium*) species are fungal endophytes of grasses, and span an evolutionary continuum from antagonistic to mutualistic symbionts. The antagonistic endophytes are sexual, horizontally transmitted (contagious), and suppress host seed production; the more mutualistic can transmit vertically in host seeds. Some vertically transmissible species are sexual and capable of occasional contagiously spread; others are strictly seed-borne and asexual. In general, each *Epichloë/ Neotyphodium* species occurs in a single host genus or tribe. However, one of the more antagonistic species, *Epichloë typhina*, can infect grasses in at least three diverse tribes. The high genetic diversity in *E. typhina* compared to other *Epichloë* species suggests that speciation has been relatively slow in *E. typhina.* This may be due to its dependence on horizontal transmission, because diversification by sexual recombination may enhance the potential of *E. typhina* to adapt to the diversity of available hosts. Among the seed-borne sexual species, host specialization appears to help drive speciation, such that there is good correspondence between the phylogenetic relationships among some *Epichloë* species and the phylogeny of their respective hosts. Curiously, the strictly seed-borne endophytes do not exhibit co-phylogeny with their hosts. Instead, they usually seem to have arisen by transfers between host tribes, and often have undergone interspecific hybridization.

326 - Exceptionally high nucleotide substitution rate differences between lichenized *Omphalina* species and their symbiotic green algae *Coccomyxa*

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To test the hypothesis of a low rate of genetic change in the inhabitant of mutualistic symbiotic systems, we investigated substitution rates in the nuclear ribosomal spacer region of the *Omphalina*/*Coccomyxa* lichens. Thallus fragments of six lichenized *Omphalina* species were collected in Greenland, Iceland, and in Eastern Canada. The average number of substitutions per 100 sites in the *Omphalina* ITS1 portion was 25.7, 2.8 in the 5.8S portion, and 25.5 in the ITS2 portion. The corresponding substitution rates for *Coccomyxa* were 1.6 (ITS1), 0.8 (5.8S) and 1.0 (ITS2). On average, rates in ITS1 were 23.4 times higher in *Omphalina* compared to *Coccomyxa*, 3.6 times higher for 5.8S, and 29.9 times higher in ITS2. This finding is in accordance with the hypothesis that the lichen inhabitants have lower rates of genetic change when compared to their exhabitants. A comparison of rates between lichenized and free living sister species revealed that the rate differences observed between the mycobiont and photobiont is explained in part by an accelerated rate in the lichenized fungi. No shifts in rates were detected between free living and lichenized algae. Based on the extremely low variation among *Coccomyxa* ITS sequences and on morphological evidence (Friedl, pers. comm.), we believe that the *Coccomyxa* isolates belong to a single species.

327 - Copper toxicity in *Saccharomyces cerevisiae*

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Copper is an essential metal, but can also exert toxicity. One proposed mechanism of Cu toxicity arises from its capacity to promote free radical generation. Several Cu resistance mechanisms have been described in *Saccharomyces cerevisiae*, including metallothionein (Cup1) and Cu,Zn superoxide dismutase (Sod1). These products confer resistance by diminishing free Cu in cells, though they also have antioxidant activity. To examine the roles of such factors in the Cu resistance of growing cells, we asked the question: when only ^50% of cells in a genetically-homogeneous yeast culture are killed by Cu, what dictates which cells die or survive? Such phenotypic heterogeneity is well known, but is poorly understood even though it can be critical for the fitness of organisms. Using flow cytometry to sort Cu-sensitive/-resistant cells within isogenic cultures, it was shown that heterogeneity was partly dependent on cell cycle stage. Other potential contributory factors include cell age, cell size, rhythms and stochastic variation. The actions of various potential Cu resistance genes in heterogeneous Cu sensitivity were examined. One antioxidant gene was identified that was responsible for cell cycle-dependent Cu resistance. The same gene may also underpin heterogeneity imparted by cell age, cell size etc. The study highlights the specific factors that can account for the variable Cu resistances of cells in non-manipulated natural yeast populations.

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328 - Fungal influence on metal mobility: mechanisms and relevance to environment and biotechnology

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In the terrestrial environment, fungi are of importance as decomposer organisms and plant symbionts (mycorrhizas), playing important roles in carbon and many other biogeochemical cycles. For example, the ability of fungi to solubilize insoluble metal phosphates is important for plant and microbial nutrition. Metals and their derivatives can interact with fungi in various ways depending on the metal species, organism and environment, while fungal metabolic activities can also influence speciation and mobility. Certain mechanisms may mobilize metals into forms available for cellular uptake and leaching from the system, e.g. complexation with organic acids, other metabolites and siderophores. Metals may also be immobilized by, e.g. sorption onto cell components, exopolymers, transport and intra- and extracellular sequestration or precipitation. The relative importance of such apparently opposing phenomena of solubilization and immobilization are key components of biogeochemical cycles for toxic metals, whether indigenous or introduced into a given location, and fundamental determinants of fungal growth, physiology and morphogenesis. Furthermore, several processes are of relevance to environmental bioremediation. This contribution seeks to highlight selected physico-chemical and biochemical mechanisms by which fungi can interact with and transform toxic metal species between soluble and insoluble forms, and to draw attention to the environmental significance of these processes.

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329 - Molecular mechanisms induced upon cadmium exposure in the ectomycorrhizal fungus *Paxillus involutus*

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The response of mycorrhizal fungi to toxic metals is of importance in view of their interest in the reclamation of polluted sites and their importance in tree growth. However, the molecular mechanisms underlying their response towards metals remain poorly understood. We have used the suppression substractive hybridization (SSH) procedure combined to cDNA array hybridization, as well as direct cloning to isolate genes which expression is modulated by cadmium (Cd) in *Paxillus involutus* (Pi). More particularly, we are investigating antioxidant and detoxification enzymes such as those involved in free oxygen radical scavenging, in glutathione and chelating agents biosynthesis. The increasing activity of the Pi superoxide dismutase as well as the fact that it can functionally substitute for the E. coli SODs under Cd stress suggests that this enzyme is involved in the cellular response of Pi to Cd. The transcript levels of g-glutamylcysteine synthetase, GSH synthetase, metallothioneins, thioredoxins and tyrosinase were measured. Of these, metallothioneins and tyrosinase were shown to be highly regulated by Cd. The intracellular pools of amino acids, glutathione, gamma-glutamate-cysteine and different phytochelatins in Pi were also subjected to changes under Cd exposure. The isolation of genes and proteins involved in the response to cadmium-induced stress open new perspectives in the understanding of molecular mechanisms that promote tolerance in mycorrhizal fungi.

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330 - Interactions of ericoid mycorrhizal and soil fungi with heavy metals: implications for tolerance and bioremediation

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Ericoid mycorrhizal fungi increase the ability of their host plants to colonize soils polluted with toxic metals, although the underlying mechanisms are unclear. We investigated the molecular and cellular responses of ericoid mycorrhizal fungi to heavy metals, and found a novel range of extracellular proteins being produced in the presence of different soluble metal compounds. Some of these proteins were identified by N-terminus sequencing as antioxidant enzymes (e.g. superoxide dismutase, SOD). SOD enzymes play a pivotal role in stress responses and are involved in metal tolerance. We also found that ericoid mycorrhizal fungi can solubilize insoluble metal compounds thanks to the release of organic acids. Solubilization activity was not the same for all the strains tested, with isolates from unpolluted soils being more efficient in the solubilization process than those from metal polluted soils. We are also exploring the potential of ericoid mycorrhizal and other soil fungi to mobilize iron from a very specific insoluble substrate: asbestos fibers. These fibers contain variable amounts of iron, one of the most relevant chemical features in the mechanism of asbestos carcinogenicity. Iron extraction from asbestos may thus decrease their cytotoxic potential. Our results suggest that some fungal species can remove significant amounts of iron from the fibers, thus opening new perspectives for the decontamination of asbestos-contaminated sites.

331 - A zinc tolerant ectomycorrhizal fungus protects zinc-stressed pine seedlings

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The effect of sublethal zinc concentrations on pines mycorrhizal with either a Zn-adapted or a Zn-sensitive *Suillus bovinus* isolate was studied. A dose response experiment was performed. During the zinc treatments the nutrient uptake capacity (NH_4^+ and P_i) was measured in a semi-hydroponic environment. The Zn sensitive plant-fungus combination already failed to sustain nutrient acquisition of the pines at 38 µM Zn. While nutrient uptake in plants inoculated with the Zn tolerant *S. bovinus* was not affected when exposed to the same Zn levels. Subsequently plants mycorrhizal with the tolerant isolate captured more P_i and were less iron deficient as plants mycorrhizal with the sensitive fungus. Plant biomass was not yet affected, but excess Zn significantly reduced the biomass of the Zn sensitive fungus. These results show that the genetic adaptation for increased Zn tolerance in *Suillus bovinus* is required for its own survival and to maintain nutrient acquisition in pines exposed to high Zn concentrations.

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332 - Arbuscular mycorrhizal fungi and stress by heavy metals and salts

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Plants from heavy metal soils can be colonized by arbuscular mycorrhizal fungi. A Glomus intraradices isolate has been obtained from the roots of the zinc violet, *Viola calaminaria* which confers heavy metal tolerances to diverse plant in diverse heavy metal soils. The genes which are differentially expressed in AMF colonized tomato roots are currently studied in our laboratory. The genus *Thlaspi* (pennycress) of the Brassicaceae contains heavy metal hyperaccumulating species. Some of them have now been found to be strongly colonized by AMF. The fungi colonizing *Thlaspi* in Nature have now been identified. Many plants of salt marshes are also good mycorrhizal plants. Up to 80% of all spores isolated from diverse saline habitats in Germany and Hungary belong to one single species, *Glomus geosporum*, as shown by molecular characterization. The role of this fungus in conferring salt resistance to plants will be discussed.

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333 - Gene order evolution and paleopolyploidy in hemiascomycete yeasts

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The wealth of comparative genomics data from yeast species allows the molecular evolution of these eukaryotes to be studied in great detail. We used 'proximity plots' to visualize gene order information from 14 hemiascomycetes, including the recent Génolevures survey, to *Saccharomyces cerevisiae*. Contrary to the original reports we find that the Génolevures data strongly support the hypothesis that *S. cerevisiae* is a degenerate polyploid. Using gene order information alone 70% of the *S. cerevisiae* genome can be mapped into 'sister' regions that tile together with almost no overlap. This map confirms and extends the map of sister regions that we constructed previously using duplicated genes, an independent source of information. Combining gene order and gene duplication data assigns essentially the whole genome into sister regions, the largest gap being only 36 genes long. The 16 centromere regions of *S. cerevisiae* form eight pairs, indicating that an ancestor with eight chromosomes underwent complete doubling; alternatives such as segmental duplications can be ruled out. Gene arrangements in Kluyveromyces lactis and four other species agree quantitatively with what would be expected if they diverged from *S. cerevisiae* before its polyploidization. In contrast, *S. exiguus*, *S. servazzii* and *Candida glabrata* show higher levels of gene adjacency conservation, and more cases of imperfect conservation, suggesting that they split from the *S. cerevisiae* lineage after polyploidization.

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334 - A major role for gene duplication and gene loss in the evolution of synteny and redundancy in yeast

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Based on Génolevures, a recent large-scale sequencing program of 13 yeast species (FEBS Letter, special issue, 487(1), 2000), a comparative sequence study showed that 90% of the synteny breakpoints characterized between *S. cerevisiae* and *S. uvarum* corresponded to micro-synteny rearrangements. So, as little as 10% of all the synteny breakpoints are attributable to gross rearrangements such as reciprocal translocations. Micro-synteny breakpoints resulted from ancestral duplications of one (or few) gene(s) onto two different chromosomes followed by the differential loss of the two copies between the two genomes. In few cases, traces of the ancient presence of one duplicate in the corresponding *S. cerevisiae* intergenic regions were identified (Fischer et al., 2001, Genome Res, 11, 2009-2019). These traces were called relics rather than pseudogenes because of the high number of mutations accumulated in these sequences that erased all characteristics of an ORF. A genome-wide search for relics in *S. cerevisiae* reveals the presence of at least 130 intergenic regions showing weak homology to functional ORFs. The combined chromosomal localization of the relics, the members of the multigene families and the trans-chromosomal series identified during the Génolevures program reveal some overlap between the different duplicated regions showing that at least part of the redundancy in the yeast genome results from a dynamic equilibrium between gene duplications and gene losses.

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335 - Comparison of genomic data of the cotton pathogenic fungus *Ashbya gossypii* with *Saccharomyces cerevisiae*

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Ashbya gossypii and *Saccharomyces cerevisiae* are very different ascomycetes with respect to growth form and habitat but their genomes share remarkable similarities. The comparison at the nearly complete map of the 4800 *A. gossypii* ORFs with the map of the 6200 *S. cerevisiae* ORFs reveals the following results: 1. For 96% of the *A. gossypii* ORFs homologs are found in *S. cerevisiae*. 2. The majority of these homologs are present in *S. cerevisiae* as duplicated segments with up to 50 genes displaying relaxed synteny with the *A. gossypii* gene order. 3. Most duplicated *S. cerevisiae* ORFs are present as single copy ORFs in *A. gossypii*, e.g. RAS1/RAS2, TOR1/TOR2, MYO2/MYO4, CLB1/CLB2 and many others. Several of these 'ancient twin ORFs' code for functionally different proteins in *S. cerevisiae* and it is an open question which function is encoded by the single *A. gossypii* ORF. 4. At least 300 functional *S. cerevisiae* ORFs are not present, or no longer present in *A. gossypii*. 5. Homologs for several of the *S. cerevisiae* ORFs annotated as questionable were detected at syntenic positions in *A. gossypii*. However for most of these ORFs no homolog was found in *A. gossypii*. 6. The *A. gossypii* genome contains many gene families, which are also, present in the *S. cerevisiae* genome, but often with fewer members. Implications of these results with respect to the evolution of both genomes will be discussed.

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336 - Evolution of the yeast genome

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The genetic material has often been rearranged during the evolutionary history of various organisms. For example, gene duplications and rearrangements of the gene order have been particularly frequent. The most plausible way to deduce the molecular mechanisms, which are responsible for these changes, is to compare the genomes of closely related contemporary species. Because yeast species are easy to manipulate in the laboratory and the yeast genomes are relatively small, these organisms represent ideal models to understand the molecular evolution of eukaryotic genomes. In collaboration with laboratories from France and U.S., several *Saccharomyces* species were recently analysed for the structure of their mitochondrial and nuclear genomes. Several mitochondrial DNA molecules were mapped for their genes and a couple of the mitochondrial genomes was recently totally sequenced to get an idea about the origin of the present mitochondrial gene order. A number of nuclear genes, homologous to the duplicated *S. cerevisiae* genes, were analysed for their phylogenetic relationship to deduce the timing of the duplication, as well as the gene differentiation event(s).

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337 - PHOREST: a web based tool for comparative analysis of EST data

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Large-scale sequencing of cDNAs prepared from specific tissue or material prepared under different growth conditions has proven to be an efficient way for gene discovery. For such applications, we present PHOREST, a web-based tool for managing, analyzing and comparing various collections of expressed sequence tags (ESTs). PHOREST is specifically designed to support EST data projects and for successively receiving and incorporating data as project grows. After entry of sequence data a search for homologous sequence information in publicly available databases is automatically conducted, followed by assembly of ESTs/clones into contigs. After manual annotation of contigs the redundancy and distribution of transcripts/contigs into functional categories can be analyzed. Several projects/databases can be managed in parallel and overall assembly and normalization of data display transcripts/contigs being differentially regulated in the different projects can be compared. Access can be set for multiple users to search and annotate the same dataset without interference. The tool has been used for two EST projects comparing three different stages of fungal growth and infection. The projects are comparing stages in a nematode-trapping fungus, *Monacrosporium haptotylum* and a mycorrhizal fungus, *Paxillus involutus*.

338 - Genetic Mapping of *Phytophthora cinnamomi* with Microsatellite Fragment Length Polymorphisms (MFLP)

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Phytophthora cinnamomi is a major oomycete pathogen of an extensive range of mainly woody plants. We are conducting genetic mapping of *P. cinnamomi* to localise genes involved in pathogenicity. Because we are restricted to analysing mapping populations of F1 hybrid progeny in this diploid species, choosing an informative (i.e. highly polymorphic) marker system for genetic mapping is critical. Microsatellite loci are ideal markers for this purpose. Their high polymorphism can be harnessed without prior DNA sequence information of the subject organism by a method called microsatellite fragment length polymorphism (MLFP) (1). MFLP are analysed in a very similar way to amplified fragment length polymorphisms (AFLP). In our analyses of four F1 mapping populations of *P. cinnamomi* progeny, we have detected between 1 and 4 informative loci per primer combination. We are in the process of constructing a genetic map of the *P. cinnamomi* genome with this data. 1. Yang, H., Sweetingham, M. W., Cowling, W. A., and Smith, P. M. C. (2001) DNA fingerprinting based on microsatellite-anchored fragment length polymorphisms, and isolation of sequence-specific PCR markers in lupin (*Lupinus angustifolius* L.). Molecular Breeding 7:203-209.

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339 - Introduction to the theory of metabolic (modelling and) control. Application to the citric acid production by *Aspergillus niger*

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Analysis, control (and optimization) of biochemical pathways requires mathematical modelling frameworks that are able to integrate different types of data and to capture the essence of complex systems. In this talk we will introduce a modelling approach that provides such a framework. It begins with the derivation of models from basic concepts of metabolic modelling; introduces the different types of system analysis, namely the stability, sensitivity (or control) and dynamics analysis; illustrate the parameter estimation techniques and finally, apply them to the citric acid production by *A. niger*. Once the model has been successfully tested and fine-tuned, we are provided with a full, quantitative and unambiguous description of the pathway behaviour, including its steady state control structure and dynamics. This description can be used for tasks that are otherwise difficult or impossible to execute. One of such tasks is the optimization of the pathway with respect to criteria that are not given by nature but defined by human demand. But this is another history.

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340 - Carbon metabolism in *Aspergillus* and *Penicillium*

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Filamentous fungi belonging to the genera *Aspergillus* and *Penicillium* are used extensively in the fermentation industry for production of a variety of products. These products fall in many different industrial sectors: 1) food additives, e.g. citric acid; 2) antibiotics, e.g. penicillin; 3) high-value pharmaceuticals, e.g. statins; and 4) industrial enzymes, e.g. amylases and xylanases. In the bioprocess industry there is a trend towards applying specific microbial strains for production of many different products. Hereby optimisation of the function of these specific strains can be harnessed for the production of many different products. These plug-bugs include *A. oryzae* and *A. niger*, which are both used extensively for production of industrial enzymes, where a strong and tailor made promoter structure is used to drive the production of the enzyme of interest. Recently it has also been demonstrated that *P. chrysogenum* can be used as plug-bug for production of different b-lactams, e.g. penicillins and adipoyl-7-ADCA. With the development of plug-bugs it is of significant industrial interest to obtain fundamental insight into the carbon metabolism of these organisms, as well as to engineer the strains in order to redirect the carbon fluxes towards the product of interest. In this presentation several different techniques for analysis of the central carbon metabolism in *Aspergillus* and *Penicillium* will be presented.

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341 - Regulation of catabolic fluxes and the energetics of adaptive responses in *Saccharomyces cerevisiae*

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The metabolic network of the living cell is endowed with an amazing capacity to cope with different environmental conditions. This capacity is due to a variety of signal transducing and regulatory systems designed to tune the cell's metabolic machinery to prevailing physico-chemical conditions e.g. nutrient availability, temperature, osmolarity and the nature of the energy source. Although the mechanisms that underly various adaptive responses may differ in many respects, they seem to have in common that the energetic and redox state of the cell is crucial to their functioning on the one hand and that they affect energy and redox catabolism on the other. In this context, it will be shown that the extent to which exponentially growing cells can withstand a heat shock is related to the ATP pools and to their potential to adapt the internal trehalose and glycogen metabolism. Further, a quantitative flux analysis of strains modified in the levels of proteins (hap4p and hexokinase 2) that take part in general glucose repression regulatory pathways, will illustrate the role of these pathways in the bioenergetics of yeast growth and maintenance. Finally, we will present an example of the transient transcriptional and physiological events which are invoked when steady state glucose-limited chemostat cultures are challenged with oleate as energy source. Here again, the initial adaptive response (prior to peroxisome biosynthesis) is gouverned by redox and energetic imbalance.

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342 - Uptake and catabolism of glucose

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Fungi are chemoheterotrophs found in a variety of habitats and their life cycles are similarly varied. Strategies adopted by fungi vary from typical 'r' strategists, with a high reproductive rate and a short life cycle, to 'K-'strategists which use resources efficiently and reproduce more slowly. Whatever their environmental niche most fungi can use glucose and do so with the same basic biochemistry. The uptake of glucose uses members of the major facilitator superfamily which mediate the thermodynamically downhill movement of glucose across the cell membrane. Free glucose within the cell is maintained at a low level by hexokinase, which also confines the subsequent metabolism to the cell by phosphorylation. Glycolysis is the most common pathway for the degradation of glucose. Although the basic biochemistry is the same in most cases, the regulation of the enzymes differs for different strategies. Faced with a surfeit of glucose, a situation that may occur naturally or in industrial processes, fungi grow rapidly, but this may not be enough to use all the glucose that enters the cell by simple diffusion. The rate of diffusion can exceed the maximum uptake rate of facilitated transport. When this happens facilitated transport appears to shut down by an unknown mechanism, and free internal glucose levels rise. In the opposite situation, with oligotrophic media, the problem becomes one of how to maintain internal metabolite levels when the entry rate is less than the flux.

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343 - Galactose metabolism in *Trichoderma* and *Aspergillus*: properties, regulation and identification of a second, reductive pathway

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D-Galactose metabolism via the Leloir pathway is a ubiquitous trait in pro- and eukaryotic cells. Its metabolic regulation has been extensively studied in yeast (*Saccharomyces*, *Kluyveromyces*) but not in filamentous fungi. A more detailed knowledge on the latter would be worthwhile, as lactose (1,4-0-β-D-galactopyranosyl-D-glucose) arising from whey - represents a renewable carbon source for several fungal fermentations, notably cellulase production by *Hypocrea jecorina* (anamorph *Trichoderma reesei*), but an effective exploitation of lactose for the biotechnical utlization is still hampered by its slow metabolism and a lack of basic knowledge on its utilization in *H. jecorina* and filamentous fungi in general. We therefore have functionally characterized the genes for the Leloir pathway in *H. jecorina*. Here we will report that they differ from the yeast counterparts with respect to genomic organization, protein structure and genetic regulation. We will also provide evidence for the involvement of the Leloir pathway in cellulase induction by lactose. Further, we will provide evidence for the existence of a second, so far unknown pathway of D-galactose breakdown in *H. jecorina* and *Aspergillus nidulans* which involves a NADPH-aldose reductase dependent reduction to D-dulcitol, and its subsequent oxidation by 2-arabinitol dehydrogenase to D-tagatose.

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344 - The practicalities of integrating anamorph and teleomorph taxonomies

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Article 59 of the International Code of Botanical Nomenclature enshrines a system that biases naming of pleomorphic fungi in favor of the teleomorph. The system maintains artificial separation of teleomorph and anamorph taxonomy resulting in loss of information and confusion in understanding species. DNA sequencing has eroded the significance of the teleomorph to taxonomy by integrating species and genera based on anamorph characters into the 'botanical' system. That all fungi may undergo outcrossing of some sort has further eroded the importance of the sexual morph in the life-cycle. Three scenarios are anticipated if Art. 59 is dropped. I. Retain primacy of the teleomorph but permit description of species for which no teleomorph is known as botanical species; permit inclusion of genera for which no teleomorph is known as botanical genera. In case of priority conflict, the oldest type based upon teleomorph material will determine the correct name. II. Permit names based on teleomorph material compete with names based on anamorph material strictly on the basis of priority. III. Selectively give priority to teleomorph or anamorph names based on defined criteria (e.g. common usage). The advantages and disadvantages of the scenarios are discussed. The authors conclude that despite its shortcomings, the current system is so firmly established that change based on any of the three scenarios presented would result in an unacceptably high level of disruption.

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345 - Anamorphs, teleomorphs and users of names

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The eventual abandonment of Article 59 is inevitable. The separate naming of anamorphs has provided a practical solution in some groups of fungi to the challenge of pleomorphism. Phylogenetic placement of anamorph taxa increasingly is possible as a result of data from molecular phylogenetic studies combined with morphological approaches. Systematists can now craft an alternative to the dual naming system, one which should be the least disruptive to communication and information retrieval. A practical strategy would be to consider groups on a case by case basis as monographic studies using morphological and molecular approaches are conducted. Harmonization and revision of the Code should be a gradual process in order to avoid total chaos and in order to learn of the pitfalls and problems that could not be predicted, as they come to light. Several case studies will be presented along with an analysis of nomenclatural options.

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346 - Dual nomenclature and classification in higher fungi: Six procedures to resolve it, with proposals to emend Art. 59

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Since the symposium, 'The Fungal Holomorph', the Deuteromycetes are considered group of form-taxa, the deuteromycetes (decapitalized), rather than a (capitalized) fungal Class. Integrated classification implies unification of the two parallel nomenclatures. The fundamentals of dual nomenclature lie in the distinction of Linnean or botanical nomenclature based on botanical (holomorphic) typification and anatomical nomenclature based on anatomical (anamorphic and teleomorphic) typification. Article 59 assigns holomorphic application to teleomorphic types, but denies it to anamorphic types, which are restricted to anamorphic application. The nature of the type of a name is thus distinguished from its application. Any process for nomenclature integration must envisage a change of anamorphic to holomorphic type applications, ie. the change of form-names into botanical names, and the suppression of alternate names. This can be achieved using six different procedures, from conservative to revolutionary, depending on the extent of the changes in type application, extent of retroactivity, the taxonomic ranks affected, and the choice of preserved name amongst alternate names of pleomorphic and pleoanamorphic fungi. Preservation and suppression of names, already implicit in Art. 15.2, cannot be processed by conservation and rejection, because they must be revisable when the organic connections between correlated names are disproven. See Hennebert & Gams on http://www.cbs.knaw.nl/ for full text.

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347 - The history of Article 59 and anamorph definitions - and a new twist: synteleomorphic names

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The Linnean system of classification dates back to 1753 (Species plantarum). Less obvious to young scientists is knowledge that the starting dates for fungal systematics used to be 1753, 1801 (Persoon's Synopsis), or 1821 (Fries' Systema) depending upon fungal groups. The latter two authors' named publications currently serve as Sanctioning

works. Lost in time are when the Codes of nomenclature were written, when pleomorphic fungi were allowed multiple names (i.e. Art. 49bis in 1910), or why? Murkier are the dates when it was commonly accepted that fungi had sexual and asexual morphs, or when teleomorphs of Basidiomycota could be distinguished from Ascomycota or from anamorphs. When were lichens and Zygomycota added as exceptions to the article on pleomorphic fungi, and why? While DNA sequence-based technology paves the way for merging anamorph typified names with teleomorph typified names, another nomenclatural and taxonomic issue emerges, that of merged taxa typified by grossly dissimilar teleomorphs (e.g. false truffles within agaric or bolete genera and visa versa). At the generic level, the nomenclatural problems presented by anamorph- versus teleomorph-typified names mirrors the problems of teleomorph versus teleomorph name conflicts that now spawn ideologies leading to creation of a PhyloCode. Future changes to the Botanical Code should consider these two conflicts jointly and not separately because they are the results of data generated by the same technology.

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348 - Consequences of deletion of Article 59 for economically important fungi

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Modification of the wording of Art. 59 has been the preferred avenue of change for governing the application of anamorph names. We will examine the consequences of complete deletion of this article from the ICBN, and the consequent loss of sanctioned status for teleomorph-based holomorph names at all taxonomic ranks. In this scenario, the oldest name of a species would be available for the actual name in combination with the oldest generic name, in line with priority, irrespective of what morphs were described in the protologue. We consider this essential for many groups of economically important fungi, where the number of described anamorph taxa often greatly exceeds the number of teleomorphic-holomorphs. For example, the ratio of anamorph:teleomorph species is about 10:1 in the Glomerellaceae, 5:1 in the Pleosporaceae, 3:1 in the Trichocomaceae, 2:1 in the Chaetosphaeriaceae and 1.5:1 in the Hypocreales, Tubeufiaceae, and Microascaceae. This approach will result in far fewer changes in binomials than the alternative of retaining teleomorph priority. These changes will have negligable effects on the names of families or higher taxa. Since 969 of 1361 genera of hyphomycetes and 196 of 602 genera of coelomycetes currently lack known teleomorph connections, deletion of Art. 59 would protect the status of these names even if teleomorphs are eventually discovered.

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349 - Has dual nomenclature for fungi run its course? The Article 59 debate. Arguments for retaining Article 59

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Fungal taxonomy is mainly morphology-based. Understanding the morphology of a fungus is part of understanding its function. Many modern studies illustrate the indispensability of morphology. Replacing identification methods by molecular routines is a form of reductionism to be opposed. The users' needs must not be ignored. Permitting separate names for the morphs of pleomorphic higher fungi conflicts with the principle of 'one species - one name'. No higher-rank classification of anamorphic fungi is recognized and integration of anamorph taxa in the teleomorph system is essential, but anamorph morphology is inadequate to do this. Retaining a separate anamorph nomenclature (particularly at the generic level) has advantages in morphological identification. The provisions of Art. 59 have grown through time to satisfy the needs of identifying mycologists. In many cases, the anamorph absolutely dominates in culture and often also in nature; it often is more differentiated morphologically than the teleomorph. Complete integration of teleomorph and anamorph nomenclature with all its consequences could work only if a 1:1 relationship of anamorph and teleomorph genera were possible. If the correlation between anamorph and teleomorph species is not 1:1, retaining both systems is of particular advantage. Abandoning Art. 59 immediately will cause nomenclatural chaos; any solution will require very complicated procedures (see detailed texts on CBS website!).

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350 - Has dual nomenclature for fungi run its course? The Article 59 debate. Arguments to reject dual nomenclature

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Dual nomenclature, the practise of giving separate scientific names to teleomorphs and anamorphs of pleomorphic fungi, predates the governance of fungal nomenclature by formal Codes. While there may be some practical benefits of having identification systems and separate names for the anamorphic fungi that are often collected or cultured independently of any teleomorph, having two names confuses non-mycologists and can cause problems in communication. There has always been discomfort at the existence of an artificial taxonomic system for anamorphs that imperfectly parallels the supposedly phylogenetic holomorphic system. Calls for the abolition of dual nomenclature have become more frequent with the advent of molecular systematics, which allows all fungi to be included in a unified phylogeny irrespective of the morphology expressed. Dual nomenclature for some groups of fungi is governed by Article 59 of the International Code of Botanical Nomenclature, but this does not apply to all and no such provision exists for any other kinds of organisms. We will argue for the eventual rejection of formalized dual nomenclature and a major revision of Article 59.

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351 - Biogeography and species complexes in some corticioid fungi

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Species diversity has by tradition been based on a morphological species concept. As shown in numerous studies such morpho-species among corticioid basidiomycetes are frequently complex, consisting of morphologically indistinguishable biological species. Phylogenetic analyses based on ITS sequences support the biological species concept. Moreover, from such analyses it may be possible to estimate genetic divergence within and between geographical regions as well as evolutionary relationships between biological species. For a few species an extensive sampling has been done, originating from temperate areas around the world. From datasets in *Hyphoderma praetermissum* and *Hyphoderma setigerum* it is shown that representatives from some of the different biological species have very wide distribution areas at least. The different groups revealed in a complex are distinctly separated in their sequence divergence but, on the other hand, more closely related to each other than to morphologically distinguished relatives. Homothallic isolates group together with heterothallic ones, indicating that homothallism must be looked upon as a life strategy which repeatedly have come into existence within certain populations.

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352 - Genetic and molecular evidence for vicariance events in the hymenomycetes

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Many Northern Hemisphere basidiomycete fungi have intercontinental distributions (North America, Europe and Asia). For the taxa we have studied, most collections retain the ability to interbreed in vitro throughout their geographical range (e.g., *Auriscalpium vulgare*, *Flammulina velutipes*, *F. rossica*, *Panellus stypticus*, *Lentinellus castoreus*, *Clavicorona pyxidata*, *Collybia cookei*, *C. cirrhata* and *C. tuberosa*). We have examined the genetic relationships among collections of selected taxa from widely separated geographical regions based on ribosomal ITS DNA sequences. For *Panellus stypticus*, *Flammulina velutipes* and *F. rossica*, the data suggest that western NA and Asian populations are most closely related, perhaps via Beringia. Eastern NA populations of these species are clearly distinct but their origins are obscure, perhaps derived from western NA populations, perhaps from Europe. For *Clavicorona pyxidata*, the data suggest that collections from eastern NA and Europe are most closely related, possibly through a North Atlantic land bridge open during the Eocene. For species with both Northern and Southern Hemisphere distributions, migration may have occurred via the Malaysian Archipelago (*Panellus stypticus*) or via South and Central America (possibly *Lentinellus castoreus*). The finding of distinct but contaxic fungal populations across broad geographical areas suggests that fungal species may be very old.

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353 - Natural biogeography and movement by humans of host-specialized forms in the *Ceratocystis fimbriata* complex

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There is little morphological diversity but a wide range of hosts in the wilt pathogen *Ceratocystis fimbriata*. Sequences of rDNA and the MAT-2 gene have shown a surprising amount of variation among more than 200 isolates studied. Phylogenetic analyses group all isolates into three major geographic clades, each comprised of host-specialized lineages. The center of diversity of the complex is found in the USA, and the North American clade (NAC) includes lineages on *Prunus*, *Quercus*, *Populus* and *Carya*. The Asian clade (AC) includes a *Ficus* lineage and a *Colocasia* lineage. The Latin American clade (LAC) appears indigenous from southeastern USA to South America. The LAC has a wide range of hosts, many of which are exotic crop plants, but it is less genetically diverse and appears younger than either the AC or NAC. Host specialization was demonstrated in LAC populations on *Ipomoea*, *Platanus*, the family Araceae, *Coffea*, *Theobroma*, *Gmelina*, *Eucalyptus*, and *Mangifera*. The LAC appears to be comprised of relatively small and geographically isolated populations in the process of host-specialization and speciation. Genetic isolation of LAC populations may be due to limited geographic dispersal by insects. However, there is genetic and historical evidence for dissemination by humans of *C. fimbriata* strains on plant materials: *Colocasia* (AC) to Hawaii, *Populus* (NAC) to Poland, and LAC forms on *Ipomoea* to Asia, *Syngonium* to Hawaii, *Platanus* to Europe, and *Theobroma* to Brazil.

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354 - Disjunct distributions of some tropical agarics and boletes

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The agarics and boletes in Neotropical forests exhibit several different patterns of origin and distribution. The patterns and diversity are directly related to the types of habitats in which these fungi occur and have been influenced by geological history, native and introduced phanerogamic vegetation, climate and, ultimately, human impact. Specifically, the aforementioned factors affect the distribution of those agarics and boletes that occur naturally in the montane oak forests of Latin America. Documentation and personal observations of ectomycorrhizal associations and distributions during the last two decades have revealed some distinctive patterns: (1) relictual disjunct distributions; (2) generic and specific distributions along a cline; (3) local endemism; (4) high generic similarity; (5) low species similarity; and (6) high levels of macromycete diversity.

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355 - Biogeography and possible origin of *Armillaria* species

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Armillaria (Fr:Fr.) Staude is a globally distributed plant pathogenic basidiomycete that causes root rot on a variety of hosts. The phylogeny of *Armillaria* species from the Northern Hemisphere and Australasia has been relatively well studied in recent years. However, very little is known regarding the phylogenetic relationships between species from the Northern and Southern Hemispheres. In a series of studies, we have attempted to elucidate these relationships and to develop a hypothesis regarding the origin of *Armillaria* species. Isolates that we have studied included those from Africa, Asia, Australia, New Zealand, South America, North America and Europe. Phylogenetic analyses were based on distance analysis using DNA sequences from the large subunit (LSU) ribosomal RNA operon. Resulting phylogenetic trees separate species from the Southern and the Northern Hemisphere into two strongly supported monophyletic clades. *Armillaria* species that grouped within the Southern Hemisphere clade showed a higher interspecific diversity than the Northern Hemisphere species. Results suggest that the Southern Hemisphere *Armillaria* group is older than its Northern counterpart. Furthermore, our results lead us to believe that *Armillaria* probably has a Gondwanean origin.

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356 - Phylogeography of the widely disjunct lichen *Cavernularia hultenii*

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Up to now, almost nothing is known about the population structure and history of lichenized ascomycetes. The large-scale infraspecific disjunctions displayed by many species have alternatively been explained by high evolutionary age or exceptional dispersal abilities of the species. In order to infer pleistocene and holocene population history of the widely disjunct lichen *Cavernularia hultenii*, we sequenced the ITS and part of the IGS region of 300 samples from 62 populations across the species' range. While four ancestral haplotypes are found in all areas, none of the observed descendant haplotypes is present in more than one of the part ranges. Although this is evidence for a past fragmentation event, nested clade analysis fails to unambiguously infer allopatric fragmentation due to the shallow gene genealogy and widespread ancestral haplotypes. Long-distance dispersal is not inferred either. Mismatch distributions yield evidence for exponential population growth, probably in connection with postglacial invasion of *C. hultenii* into formerly glaciated areas of Western North America. The presence of a southern and at least one northern glacial refugium in south Central Alaska is inferred from the data. Nested clade analysis does not infer range expansion from our dataset either. This and the failure to infer fragmentation is explained by slow action of genetic drift, which has not completely removed ancestral haplotypes from the post-fragmentation and post-expansion areas.

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357 - Biogeography of mushrooms: Finding Wallace's line for the fungi

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The combined study of phylogeny and biogeography provides a framework for understanding the relationship among different components of speciation, including population genetic structure, isolation mechanisms, vicariance, morphological evolution, and ecological adaption. Examples of fungal groups with rich phylogeographic histories include *Pleurotus*, *Ganoderma*, *Schizophyllum*, and *Xeromphalina*. In these groups, patterns of genetic variation among geographically isolated populations indicate that speciation is primarily allopatric

in mode. However, gene flow between widely isolated locations is also evident, and repeated dispersal to distant provenances may provide opportunities for biodiversification through repeated rounds of allopatric speciation. Older lineages may also be more likely to have broader distributions than recently evolved ones. This presentation will review recent advances in understanding diversification in fungi through phylogeographic studies.

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358 - *In vitro* fruiting of *Cordyceps militaris*

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Every year, 200-400 specimens of *C. militaris* are collected mainly from Kangwon Province of Korea and their isolates preserved in EFCC, KNU, Korea. *Cordyceps* species are highly regarded as medicinal mushrooms in East Asia, including Korea. Stromata of *C. militaris* could be produced in vitro when its liquid suspensions were inoculated in brown rice medium supplemented with silkworm pupae and incubated in 20-25 °C under light and high humidity conditions. But, the isolates showed unstable variation in fruiting. Most of the isolates failed to produce stromata or produced only few deformed stromata. Other isolates, which produced good fruiting, could not produce the same quality of fruiting when their subcultures were used, while other few isolates produced good fruiting when their subcultures were used. This was the main problem in commercial cultivation of *C. militaris*. In order to understand the fruiting character of *C. militaris*, several single ascospore strains were crossed with each other and observed for their fruiting. Out of eighteen strains, ten strains produced stromata profusely with the remaining eight strains in all the combinations, but not among themselves and vice versa. When two opposite strains were repeatedly inoculated together in large scale, they produced good quality of fruiting continuously. This paper intends to report that *C. militaris* is a bipolar heterothallic fungus and crossing between two opposite types is a good method of fruit body production.

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359 - Breeding of mushroom by using cell fusion techniques

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Twenty-two homokaryons were isolated from one commercial fruiting body of *P. ostreatus*. Then, all homokaryons were crossed each other. In crossing tests of homokaryotic mycelia, only two sexual phenomena were observed: clamp formation and "Barrage" reaction, in contrast with *Schizophyllum commune* (Raper 1966). We determined the mating type of *P. ostreatus* by this two reactions. As a result, *P. ostreatus* was determined to have a bifactorial incompatibility; KM 4 (A1B1), KM 7 (A2B2), KM 18 (A1B2) and KM 22 (A2B1). All compatible dikaryons formed their fruiting bodies on commercial sawdust media. By means of UV-irradiation to protoplasts of these homokaryons, five strains of auxotrophic mutant were obtained. The ratio of regeneration to mycelia from protoplasts of homokaryons was about 0.1%. Reversion rate of each mutant to prototroph was calculated from the number of colonies that appeared on the complete medium and that on the minimal medium against the number of protoplasts or regenerated protoplasts. And then, protoplast fusion between strain KM 4-1 (wet-) and KM 4-34 (ade-) was done in the same mating type. Fifteen strains which could grow on the minimal medium and possessed one nucleus per each cell obseved by fluorescent microscopy, were obtained with this fusion treatment. These strains were inoculated to MYA medium containing methyl - 1 - (butylcarbamoyl) - 2 - benzimidazole carbomate (benomyl). Benomyl is known as a regent of haploidization. Two strains (KM D-4-1 and KM D-4-2) occurred sector on this medium. Therefore, these strains were found to have one diploid nucleus per each cell. On the other hand, the other diploid strains having the opposite mating type were made by the same cell fusion technique. After getting the strains, crossing were done among the diploid strains. Finally, tetraploid mycelium were gotten. The growth rate of these strains became about two times of diloid strains and their fruiting also became faster than normal commercial strains.

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360 - The genus *Pleurotus* as a bioremediation tool

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The genus *Pleurotus* belongs to the white rot fungi, capable of rapid growth on lignocellulosics and with a preference for lignin. It lacks lignin peroxidase, but actively excretes other oxidative enzymes upon proper induction. Oxidised products arising from extracellular enzyme action are recycled by mycelium-bound reductases; enzymatic and/or non-enzymatic re-oxidation of such reduced molecules results in hydrogen peroxide production in the growth media. Some details of this particular mechanism have been recently elucidated whereas others are under study. *Pleurotus* acts as a biochemical hydrogen peroxide factory by means of quinone/polyphenol and benzyl alcohol/benzaldehyde redox cycles. The bleaching power of hydrogen peroxide as well as its production in autoxidation reactions could be enhanced by the ability of the fungus to raise the pH of culture media when stressed by xenobiotics. Hydrogen peroxide and excreted enzymes co-operate in the breakdown of a wide range of different molecules. Therefore the fungus efficiently grows on a variety of substrates. Chemicals that are toxic for other fungi are bleached by *Pleurotus* just out of the hyphae and cannot accumulate within the fungal cells. So safe and valuable sporocarps could be obtained when starting from some substrates that are unsuitable for direct use as human food and/or animal feed, while concomitantly destroying hazardous substances they eventually contain, and operating an efficient bioremediation.

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361 - Development of functional foods by use of basidiomycetal strains

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In this study, it was attempted to investigate the hepatoprotective effects of two fractions of methanol extracts, larger molecular fraction(above 20,000 MW) and smaller molecular fraction(under 20,000 MW), obtained from waxy brown rice cultured with *Phellinus igniarius*. After administering these sample fractions to rats orally for 2 consecutive weeks, rats were treated with CCL4. The determination of enzyme activities in sera of treated rats as well as biochemical analysis of liver were conducted and compared with those data from control group. Serum GOT and GPT of rats increased highly in the control group treated with CCL4 only. The treatment group fed with larger molecular fraction of the methanol extracts obtained from waxy brown rice cultured with *P. igniarius* showed a strong suppression on the GOT and GPT augmentation. In the group treated with only CCL4, all the activities of ALP, LDH, γ-GTP, SDH increased considerably compared to the normal rats without CCL4 treatment. However, both the groups fed with methanol extracts from brown rice cultured with *P. igniarus* prevented the increase in activities of these enzymes, almost to the level of normal rats without CCL4 treatment in some of the data. The larger molecular fraction demonstrated higher effectiveness in general.

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362 - High incidence of mycotoxin zearalenone in stored wheat from Mazandaran Province, north of Iran

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In Iran, wheat is one of the main cereals for human consumption. Wheat grains containing zearalenone can cause genital problem and reproductive disorders in domestic animals. In human, episodes of breast enlargement in young boys and sexual precocity have been reported. This toxin may therefore have a role in hormonal balance and mammary cancer in regions with high zearalenone ingestion. In this study the incidence of zearalenone in wheat samples from Mazandaran warehouses were investigated. A total of 118 wheat samples (1 kg) were randomly collected from all 12 warehouses of Mazandaran province. Competitive microtitre plate enzyme immunoassay for the quantitative determination of zearalenone was used according the manufacturer instructions (Sigma-Aldrich Laborchemikalien GmbH). Zearalenone was found in 81% of samples and 64.4% of samples contained >200 µg/kg of this toxin. The minimum levels of zearalenone were 29 µg/kg. All of the warehouses were positive for the presence of zearalenone. 50-100% of samples in each of warehouse had >200 µg/kg contamination of zearalenone. Our results showed the high percentage and levels of contamination of wheat samples with zearalenone. With regard to proposed standards given by the Standard Administration of Iran, under which the content of zearalenone should not exceed 200 ppb in food products, 64.4% of samples meet these criteria.

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363 - Mycelial growth of *Ganoderma lucidum* and *Pleurotus ostreatus* on the selenium-enriched media

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Ganoderma lucidum and *Pleurotus ostreatus* are mushrooms of high pharmacological value. 119 different kinds of triterpenoids and various polysaccharides from *G. lucidum* fruiting bodies have shown antitumor and immunostimulating effects. Polysaccharides isolated from *P. ostreatus* have shown antitumor, immunomodulating, antiviral, antibacterial activities. Selenium is one of the essential oligoelements, and it is indispensable for cell functions. Selenium adding in nutrition has been shown to have anticancer and antimutagenic effects. The aim of our research was to investigate the influence of selenium added in media on the growth rate and biomass changes of mycelia and them ability to accumulate this microelement. The medium used for analyzing the growth rate of the mycelia was Malt agar. The same submerged medium was used for measuring mycelial biomass and the accumulated selenium. The concentrations of selenium, used in a form of sodium selenite, were the following: 0.3 mg/l, 0.7 mg/l, 1 mg/l and 1.3 mg/l. The accumulation of selenium in mycelia was measured using the Atomic Absorption Spectrophotometer. The analyzed concentrations of selenium did not show any influence to the growth rate of both species mycelia in comparison to the control. Different concentrations of selenium showed influence on mycelial biomass in submerged culture. The results of selenium accumulation in mycelia have shown higher concentration in samples grown on selenium containing media.

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364 - Antiprotozoal activity and mitogen effect of edible medicinal mushroom *Lentinus edodes* (Berk.) Sing. (Shiitake)

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The *Lentinus edodes* is a worldwide-cultivated mushroom of high nutritional value and excellent pharmacological properties. It has been used in traditional medicine of Asian countries as elixirium ad longam vitam. In nowadays, different forms of dietary supplements with curative and preventive properties are obtained from *L. edodes*. The mitogen and antiprotozoal activities of *L. edodes* have not been investigated sufficiently. As part of our Medicinal Mushrooms Pharmacological Activity screening program, the presented results concern the study of antiprotozoal activity (APA) and mitogen effect (MGE) of *L. edodes* against *Paramecium caudatum*. The 25-day-old mycelium of L. edodes cultured on liquid malt-extract medium was used. The mycelium extract (ME, 0.03%, 0.03 ml) and cultural filtrate (CF, 0.03 and 0.06 ml; not diluted and diluted by Lozinskii solution in ratio 2:1, 1:1, v/v) were tested. All ME and CF samples during 76 hours showed 100% APA. The fastest effect was observed after 2 hours under the influence of 0.03 ml CF without dilution. The highest APA with 21% MGE showed 0.06 ml CF (2:1, v/v). The strongest MGE (221%) possessed ME. The revealed physiological activities of *L. edodes* allow us to conclude that its exo- and endo-metabolites can be used for formulation of different bio-preparations, with antiprotozoal and mitogen (wound-healing) activities. Experiments including pathogenic test-organisms *Entameoba histolytica* and *Balantidium coli* are in progress.

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365 - Knowledge and utilisation of edible mushrooms by local populations of the rain forest of south Cameroon

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Indigenous knowledge on edible fungi and their utilisation by local populations in the rain forest zone of southern Cameroon were investigated. Members of both major tribes, Bantu and Bagyeli (Pygmy), were interviewed. Mushroom usage was monitored on a daily basis for more than a year. Mushroom knowledge among both Bantu and Bagyeli is very high. More than 50 vernacular names were retrieved. At present, about 35 species have been identified to species level. Bantu sample most mushrooms in secondary forests, and their mushroom diet consists mainly of saprotrophic fungi. Bagyeli collect mushrooms more frequently in the primary rain forest, and ectomycorrhizal and termitophilous fungi constitute a larger part of their diet. Some species are also used in traditional medicine. Toxic mushrooms were not mentioned by local populations. Some differences in mushroom collection and consumption do exist between Bantu and Bagyeli, but actual rates of consumption are low for both groups, with resp. 1.4 and 1.1 kg fresh mushrooms per person per year. Consumption is much lower than in other parts of central and eastern Africa. Suggestions are given how the apparent discrepancy between widespread and extensive mushroom knowledge and rather infrequent mushroom use can be explained.

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366 - Evolutionary and co-evolutionary genomics in *Saccharomyces cerevisiae*

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I used laboratory populations of budding yeast maintained by daily transfers to compare rates of adaptation by haploid and diploid populations over 2000 generations, and tested whether adaptation occurred through many mutations or by a few major mutations. As predicted by recent theoretical work, haploids adapted more quickly than diploids, but lost this advantage when the experiment was repeated with much smaller population sizes. Genetic analysis of one haploid evolved genotype indicates that its 72% fitness increase is attributable to approximately 4 mutations. Adaptive changes are being further characterized by hybridization of genomic DNA and cDNA to microarrays. Despite the artificial simplicity of the environment, ecological diversity evolved from a single ancestral genotype in at least one population within 1000 generations. Two genotypes with contrasting growth curves are maintained by negative frequency-dependent selection (competitive advantage when rare). Interesting interactions also occur between the mitochondrial and nuclear genomes within cells. The population of mitochondrial chromosomes within a cell experiences conflicting selective pressures at different levels: selection among cells favors mitochondria that contribute to cellular fitness by encoding respiratory proficiency, while selection among mitochondrial chromosomes within cells favors less mutualistic mutants that encode no respiratory function but replicate faster than wild-types.

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367 - Selfish genes, sex and adaptation in yeast

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Most biologists agree that evolution occurs because those members of a population best adapted to the current environment will flourish at the expense of those less well adapted. But what makes certain members of a population better adapted? What is the link between genotype and phenotype? How does this adaptation arise? The short answer is by chance, random genetic mutations or novel gene combinations will confer a greater fitness. But what sort of mutations (e.g. duplications/point mutations), how many mutations, does one mutation always win out or are there many equally good solutions to a problem? I will describe an experiment with asexual yeast which looks at some of the mechanisms of adaptation to a saline environment. The vast majority of organisms are sexual to some degree, so how does sex affect adaptation? I will describe a second experiment which tests the function of sex - does it, as originally suggested, allow adaptation to occur more rapidly? Lastly, even though sex has presumed advantages, I will discuss some of the more unfortunate consequences of sex - namely how it allows selfish or parasitic genes to prosper.

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368 - Mutation and selection in the yeast *Saccharomyces cerevisiae*

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Fitness of organisms is to a large extent determined by quantitative traits, such as growth rate. The study of mutation affecting such traits is impeded by the fact that individual alleles often cause small and recessive effects. Fungal experimental systems, such as laboratory populations of baker's yeast, provide unparalleled opportunities for detection of new alleles. One reason is that mutations can be studied both in haploids and diploids, the other is the ease of propagation and growth rate assays. We screened for mutations that appeared in diploids and therefore were initially masked by wild-types but then, after tetrad dissection analysis, were revealed as growth defective haploids. We found that a deleterious or lethal mutation occurs spontaneously at a rate of about one per thousand of diploid genome replications. This seemingly slow pace of mutation rate in unicellular organisms with small genomes becomes substantial when extrapolated for species that have several times larger genomes and many cell replications per sexual generation. The collected mutations were subsequently assayed for their dominance and epistatic interactions (the latter only for non-lethals). Heterozygous effects of lethals and non-lethals were very small and equal on average which means that the former were much better masked. Non-lethal mutations in heterozygous loci interacted mostly multiplicatively suggesting that neither their synergism nor antagonism were strong.

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369 - Evolution of azole resistance in yeasts: Genetics and genomics

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We established twelve replicate experimental populations of *Candida albicans* to study the dynamics and mechanisms of adaptation to the azole antifungal agents. The experimental populations were founded from a single drug-sensitive cell; six populations were evolved with inhibitory concentrations of fluconazole and six were evolved without drug, over 330 generations. While all populations evolved with fluconazole adapted to the presence of drug, they followed strikingly different trajectories. The experimental populations also diverged in fitness. We measured changes in genome-wide gene expression that became entrenched during adaptation and persisted in the absence of drug using DNA microarrays. Cluster analysis of the 301 genes with significantly modulated expression identified three patterns of adaptation to drug. One pattern was unique to one population and included upregulation of the ABC transporter gene, *CDR2*. A second pattern occurred at a late stage of adaptation in three populations; for two of these populations profiled earlier in their evolution, a different pattern was observed at an early stage of adaptation. The succession of early- and late-stage patterns of gene expression, both of which include upregulation of the major facilitator gene, *MDR1*, must represent a common program of adaptation to this antifungal drug. We compare these results with the dynamics and mechanisms of evolved fluconazole resistance in experimental populations of *Saccharomyces cerevisiae*.

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370 - Predicting the evolutionary potential of plant pathogenic fungi: a model framework

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Plant pathogenic fungi are notorious for evolving rapidly in agroecosystems. Hence they offer good models for studies in experimental population genetics and evolution. Interactions among five population genetic forces (mutation, migration, selection, drift, recombination) ultimately affect the evolutionary potential of fungal

populations. We propose a model framework for predicting the evolutionary potential of a range of plant pathogenic fungi based on assessment of the life history traits underlying the individual population genetic forces. The proposed 'evolutionary risk' model is flexible and provides a quantitative prediction of the expected relative rate of fungal evolution in experimental studies. The components of the model can be tested individually and in combination. We believe this model will be useful for formulating specific hypotheses and designing experiments regarding evolution and population genetics for a wide range of fungi. A preliminary test of the model based on the observed rate of breakdown of resistance genes in 35 plant pathosystems showed a significant correlation with predicted rates of evolution. This suggests that the model may be useful to predict the relative rates at which pathogenic fungi will evolve virulence. We hypothesize that the model will also be useful for predicting the rate at which fungi develop resistance to fungicides.

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371 - Molecular mechanisms involved in the evolution of the modern yeast genomes, proteomes and metabolomes

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Saccharomyces cerevisiae is one of the most important industrial and laboratory model organisms. It can successfully grow in the almost complete absence of oxygen (anaerobic yeast), it utilises sugars via fermentation even at aerobic conditions (aerobic-fermenting yeast), it constantly produces respiration-deficient mitochondrial mutants (petite-positive yeast) and the genome shows large segmental duplications (post-genome-duplication yeast). Recently, several related yeasts have been studied to get an idea about the origin of the modern traits found in the contemporary *S. cerevisiae* species. It is likely, that the progenitor of the modern *Saccharomyces* yeasts, as well as other related genera, was an aerobic and petite-negative yeast, which was not very prone to aerobic alcoholic fermentation. In the next step, some of the yeast lineages progressively decreased their dependence on oxygen by reshaping several of their metabolic pathways. For example, as soon as the fermentation pathway could provide enough energy for growth, the mitochondrial genome became dispensable, and several biochemical pathways, as de novo pyrimidine biosynthesis, could become independent on the integrity of the respiratory chain. A progressive independence of these pathways from the presence of oxygen has promoted the anaerobic life style. The origin of these gross metabolic changes might have coincided with extensive chromosome reorganisation and the origin of the large segmental genome duplications.

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372 - Pathogenicity factors involved in fungus-fungus interactions and biotechnological applications for disease control

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Fungus-fungus interactions play an important role in biological processes that affect crop production and food quality, as well as animal and human health. They may serve as model systems useful in the understanding of various microbe-other organisms interactions. Further, the mechanisms involved represent sources of genes, promoters and compounds which have interesting biological properties. However, we are still quite far away from having a substantial understanding of these interactions. Our group, studies the *Trichoderma*-host system, which is based on several and complex mechanisms able to protect crops from pathogens and stimulate plant growth. By applying a variety of biochemical, genetics and molecular techniques, including the use of targeted gene disruption and transgenic expression of *Trichoderma* genes, we have identified pathogenicity factors required by the mycoparasite to attack the host and behave as a biocontrol agents. A few chitinases and glucanases, within the extensive and redundant lytic enzyme system of *Trichoderma* spp., have been found to contribute substantially to the antagonistic ability of these fungi. In vivo testing of knock-out mutants having one or two different ORFs interrupted, have indicated that *Trichoderma* uses different sets of genes depending on the strain and the host fungus. In addition, gene disruption affected expression of other biocontrol genes, and produced unexpected and potentially useful effects.

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373 - Pathogenicity factors in nematode-trapping fungi

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The nematode-trapping fungi comprise a rather large group of soil living fungi that can infect nematodes by forming special morphological structures (traps) like mycelial networks, adhesive knobs or constricting rings. Following the development of the traps, the fungi infect the nematodes through a sequence of events: attachment to the host surface, penetration, followed by invasion and digestion of the host tissue. Among the pathogenicity factors that have been studied in some detail in these fungi are extracellular serine-proteases and lectins. To identify additional enzymes, toxins etc. that are involve in the killing and paralyzing of infected nematodes, we have started an EST sequencing project in the fungus

Monacrosporium haptotylum which captures nematodes with the aid of adhesive knobs. In this fungus, functionally intact trap cells can be isolated from the mycelium. Three different cDNA libraries are analyzed representing transcripts of mycelium, knobs and knobs infecting *C. elegans*. We have so far sequenced and analyzed ca 5.000 transcripts. Annotation of the transcripts displaying high or moderate similarities to sequences in Genbank indicate major metabolic differences between the saprophytic mycelium, the infection structures (knob) and the fungus when infecting *C. elegans*.

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374 - Gene structure and evolution of fungal secondary metabolites involved in plant disease

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In filamentous fungi, the genes necessary for the biosynthesis of a particular secondary metabolite are typically clustered. Examples include penicillin, trichothecene, gibberellin, aflatoxin, lovastatin, and HC-toxin. Because known genetic mechanisms would tend to break up clusters, their prevalence and persistence suggests that clustering confers selective advantage either to the organism or to the cluster itself. Since housekeeping pathways tend not to be clustered in fungi, the selective advantage is probably related to the unique properties of secondary metabolite pathways, for example, their weak selective advantage. In the plant pathogenic fungus *Cochliobolus carbonum*, the genes for biosynthesis of the cyclic peptide HC-toxin are clustered within a 600-kb region of DNA called the 'TOX2' locus. TOX2 comprises at least seven genes, encoding a non-ribosomal peptide synthetase, a putative HC-toxin efflux carrier, at least three other structural biosynthetic genes, and a pathway-specific transcription factor. In all known HC-toxin producing isolates, these genes are all in multiple functional copies. All of the genes are completely absent from toxin non-producing isolates, suggesting that acquisition of the HC-toxin biosynthetic genes occurred after speciation of *C. carbonum*, perhaps by horizontal gene transfer. The organization of the TOX2 genes within and between isolates suggests that duplication and translocation occurred subsequent to the acquisition of the TOX2 genes.

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375 - Glycosylation of cell wall proteins: importance in pathogenicity of *Candida albicans*

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Candida albicans is the most common agent of serious human infection caused by a fungus. Cell wall mannoproteins act as the immediate point of contact between the fungus and host surface epithelia, endothelia and phagocytic cells. Therefore, glycosylation of cell wall proteins has a direct impact on the outcome of host-fungus interactions. We are exploring the biological roles of the O- and N-linked mannan in via the analysis of strains in which a range of genes encoding mannosyl transferases have been disrupted. Periodate oxidation of mannan on the *Candida* cell surface or deletion of either *MNT1* or *MNT2*, which are involved in O-glycosylation, results in strains with reduced adherence and attenuation of virulence. Mutations in N-glycosylation have multiple phenotypes including changes in drug sensitivity, adhesion and in some cases changes in morphogenesis. Mutants with deletions in the *MNN4* gene are almost devoid in phosphomannan, which has been implicated in recognition of *C. albicans* by macrophages. However, *mnn4* mutants were still able to be taken up by macrophages suggesting that this epitope is not a key mediator of macrophage recognition. Analysis of glycosylation mutants to date establishes that the carbohydrate epitopes of mannoproteins play key roles in aspects of *C. albicans* related to its pathogenesis including adherence, cell wall permeability, drug sensitivity, virulence, and yeast-hypha morphogenesis.

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376 - Genomic analysis of pathogenicity factors of the insect pathogen *Metarhizium anisopliae*

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The ascomycete *Metarhizium anisopliae* attacks many pest insects and is widely used as a mycoinsecticide. It has been studied extensively at the genetic and biochemical level and serves as a model insect pathogenic fungus. *M. anisopliae* secretes a large number of enzymes with potential targets in the insect host including proteases, chitinases, lipases, esterases as well as toxic secondary metabolites. ESTs and microarrays offer unprecedented opportunities for the identification and characterization of these complex fungal responses to host-related signals. ESTs of *M. anisopliae* were obtained under growth conditions that optimize production of secreted proteins. The EST sequences revealed many potential virulence factors and categories of biologically active molecules previously unreported in *Metarhizium* and sometimes other fungi. The largest and most diverse category were the proteases representing more than 30% of all transcripts. To analyze expression of all discovered genes and in particular potential pathogenicity determinants cDNA microarrys were made. Several clusters of genes had distinct expression patterns and were specifically up- or down-regulated during growth on insect cuticle. For example many proteases were differentially expressed in different media and at various time-points. These results demonstrate the suitability of such genomic approaches for understanding pathogenicity as a whole and identifying and characterizing pathogenicity factors in *M. anisopliae*.

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377 - Fungal biodiversity in Colombian Amazon forests: species composition, ecology and function

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This work constitutes an effort to establish the role of fungi during regeneration processes of tropical lowland forests, and was performed at the plots of Tropenbos Colombia in Araracuara and Peña Roja, in central Caquetá of Amazonian Colombia. The plots represent different regeneration stages and primary forests and have been investigated for a long time at Tropenbos Colombia. Our project generated new data on the occurrence of macro- and microfungi along a regeneration gradient in Colombian Amazon lowland forests. Functional inferences suggest a considerable role for the putative ectomycorrhizal fungi and litter decomposing fungi in the nutrient cycling of these forests. Decomposition of leaf litter can be relatively fast, but seems to be influenced by the environmental conditions. Shortly after trees are cut down, many wood-inhabiting fungi appear, which may occur as endophytes in healthy trees. The formation of fruit bodies may be a result of the stress imposed by the death of the hosts. After the felled trees are burnt, soil borne microfungal populations are dominated by heat resistant fungi. Many new taxa of macro- and microfungi have been found, and await formal description. New ideas about the role of microfungal populations in nutrient cycling led to a new research proposal to study the role of the microbial interface connecting the roots of the trees with the environment in secondary and primary forests.

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378 - Diversity and complexity in Ascomycota from Venezuela

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Since the beginning of the 20th century and up to 1950, there was a remarkable interest of the US towards Latin America. The US Department of Agriculture sent US-trained mycologists to conduct inventories on the natural resources of several Latin American countries, and as a result, neotropical mycological knowledge widened. That was the period when Hans Sydow, and Chardon and Toro published the results of their expeditions to Venezuela. More recently, there has been an emphasis in fungal systematics, an interest towards basic research, and more participation of local mycologists, all of which have helped improved knowledge on fungi in general. Programs such as Flora Neotropica and Flora Amazonica Project, have contributed toward an increased knowledge of fungi of the neotropics. R. W. G. Dennis, has been a major contributor towards neotropical fungal knowledge, specifically in regards to ascomycetes. In his 'Fungus Flora of Venezuela and Adjacent Countries', 1970, he treated approximately 1346 species of Ascomycetes. This talk will present ascomycete collectors in Venezuela over the last 50 years, as well as numbers of publications dealing with neotropical ascomycetes in major mycological journals and congress presentations. in this same area. Knowledge of the total numbers of Ascomycetes for some neotropical areas will be compared with other groups of fungi from the same geographical regions.

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379 - Ectomycorrhizal fungi associated with *Dicymbe* (Caesalpiniaceae) in the Pakaraima Mountains of Guyana

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Mycological explorations in the Pakaraima Mountains of western Guyana have unearthed unique forests dominated by ectomycorrhizal (EM) tree species of the genus *Dicymbe* Spruce ex Benth. (Caesalpiniaceae, tribe Amherstieae). This remote region of tropical South America is situated on the central Guiana Shield and is characterized by highly oligotrophic soils. While EM associations are still poorly known from South American rain forests, the discovery of EM *Dicymbe* forests extends the known range of EM associations substantially from previously documented sites in the central Brazilian Amazon and southern Venezuela. Species of *Dicymbe* reach extreme levels of dominance (> 90% of stand basal area) in a mosaic of primary forests in the Pakaraima Mountains. *Dicymbe* stands are sharply demarcated from surrounding mixed rain forests lacking in EM trees, and harbor a rich EM mycota. Collecting efforts have currently recorded > 150 species/morphospecies of EM fungi occurring in close association with *Dicymbe* species. These fungi are primarily basidiomycetes representing most of the commonly accepted EM families, including Boletaceae, Russulaceae, Amanitaceae, Cantharellaceae, Cortinariaceae, and Paxillaceae. Putative EM ascomycetes include *Elaphomyces* spp. and *Pseudotulostoma volvata* O.K. Mill. & T.W. Henkel, a new taxon in the Elaphomycetaceae. Ongoing systematic work on these fungi will be discussed, with a focus on new and unusual taxa.

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380 - Polypore diversity in Costa Rica

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The systematic study of polypore fungi in Costa Rica started with the collections by Dr. Josiah L. Lowe in 1963. Recent studies have contributed to the knowledge of this group in Costa Rica. Currently, 81 genera and 284 species of pore fungi have been reported for Costa Rica. It has been calculated that the total amount of species will be around 350-400. Most of the species reported are cosmopolitan, and are distributed through out the country. Nevertheless, the highest diversity is found from sea level to 900 m with less taxonomic diversity on high elevations (2000-3000 m).

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381 - Biodiversity of *Russula* in Costa Rica: a first account

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Information on *Russula* in Costa Rica is scarce and very fragmentary notwithstanding the fact that many hundreds of specimens have been collected, especially in the last ten years. Only very few new *Russula*'s have been described from Costa Rica so far, but it is clear that the genus is highly divers and is characterized by an important degree of endemism. As can be expected, the Costa Rican taxa - at least in the more temperate mountain areas - reveal strong similarities with the *Russula*'s from eastern North America. Unfortunately however, even in North America the knowledge of *Russula* remains rather superficial. With the exception of some subsections, there exists no complete, modern, taxonomic study of American *Russula* that can serve as an adequate basis for a revision of the genus in Costa Rica. To illustrate the above, some examples of biogeographically or otherwise interesting *Russula*'s will be presented in more detail. The relation between species diversity, host distribution and general ecology will be addressed and compared with observations from other continents.

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382 - Diversity of Costa Rican wood-inhabiting pyrenomycetes and loculoascomycetes

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Ascomycetes constitute the largest known group of fungi worldwide, occurring in all ecosystems and geographic areas as saprobes, pathogens and endophytes. Almost all historical reports of pyrenomycetes and loculoascomycetes for Costa Rica pertain to leaf-inhabiting fungi. Wood-inhabiting taxa in these groups are virtually unknown - one of the only reports listed seven species in three genera in one family. A total of 531 specimens were collected from 1996 to 2001. The specimens were distributed among 213 species in 45 families. Species in six families were frequently encountered: 26% of the fungi collected were in the Chaetosphaeriaceae, 20% in the Lasiosphaeriaceae, 12% in the Annulatascaceae s.l., 7% in the Xylariaceae, 7% in the Melanommataceae, 6% in the Hypocreaceae, 6% in the Platystomaceae and 6% in the Tubeufiaceae. In addition to our own collections, specimens have been examined from parataxonomists collecting in 5 conservation areas in 2001 and 2002. Parataxonomists collected 1133 specimens of ascomycetes on trunks and 455 specimens from plots. Field identifications reported specimens (% of trunk or plot collections) in the following groups: Xylariales (32% trunk; 35% plot), Lasiosphaeriaceae (13% trunk; 8% plot), Hypocreales (8% trunk; 7% plot), Melanommataceae (8% trunk; 4% plot), Platystomaceae (4% trunk; 2% plot) and others. Comparative data from collecting trips to lowland French Guiana and Puerto Rico show proportions of taxa similar to those for Costa Rica.

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383 - Diversity of neotropical smut fungi

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After ten years of field work in neotropical countries, morphological investigations, and study of the literature, a monograph on neotropical smut fungi (Ustilaginomycetes, Basidiomycota) is accepted for publication in Flora Neotropica. 227 species in 34 genera are described. Among these 17 species and 5 genera were described as new taxa and ca. 34 new combinations were necessary. Further interesting results were obtained concerning the diversity of soral morphology, germination of teliospores, host ranges, and geographical distribution of the different species of smut fungi. However, much work remains to be done before we might have a rather complete understanding of the existing diversity of smut fungi in the neotropics. An overview of the diversity of the known neotropical species of smut fungi will be presented.

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384 - The importance of fungi

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Why study fungi? Politicians and grant awarding bodies focus on the applied, the popular or the trendy. Fungi sadly are rarely seen to fit into these categories. It is however our duty as mycologists to demonstrate that fungi are one of the Worlds greatest living resources and estimates of 1.5 million species or more are becoming increasingly likely. Their track record for the benefit of man is without question. Where would we be without penicillins, cyclosporins cephalosporins and other important pharmaceutical products? At least 80 percent of the worlds plants have mycorrhizal associations without which they would grow poorly or not at all. We could be justified in saying no fungi no world! These of course are beneficial activities but their global significance as pathogens of economic plants and, more recently though HIV, man should not be underestimated. Imagine the political reaction if fungi were the perfect bio-weapons! This symposium follows on from Vancouver where the then President of IMA stated stand up and be proud to be a mycologist because if you dont there will be few left in ten years time. Eight years on is a good time to reflect and prepare for the future. Mycology is in our hands and it is our responsibility to make the subject exciting, emphasize its relevance and to woo the appropriate individuals and organizations.

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385 - Awareness to Public

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Promoting fungi at the grass roots is essential for the development of a healthy appreciation of the role fungi play and as mycologists its our duty to the community to inform. The paper will present case-studies in which pubic/mycological interface has been forged. It is necessary to inject interest in fungi at a range of levels from schools to adult education programmes. There are more people in Britain than ever before collecting and eating wild fungi and keen to know about their quarry and many natural historians who wish to know about their natural heritage but lack the knowledge and resources or where to look for such information. These are very receptive audiences. Children have strong influences on their adults and they are the adults of the future. The informed public is a useful ally in that it understands well-presented media coverage, lobby on behalf of mycology and in addition includes a volunteer army happy to spend long hours collecting, recording and monitoring. In contrast many others, including politicians, consider fungi of nuisance value or even deadly and rarely think (or know) of the benefits. Celebrities can help to disseminate the message. If mycology is to be supported by Government agencies then they and voters require to know how important fungi are to everyday life and well-being of our planet. Mycologists are the people who know about fungi so it is up to us; if all fails we cannot blame anyone else!.

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386 - Integration of mycology with other disciplines

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One vision of mycology is that our discipline is undervalued and underfunded. The accuracy of such a view is open to debate but reiteration of this negative view undermines the value and potential of mycology. Other disciplines also feel disadvantaged and have equal cause for concern about the future. Therefore, it may seem perverse or naïve to present on overtly positive vision of mycology in the current climate, but there are many opportunities for the modern era of mycology. The mycological remit is broad with highly studies model systems taking their place amongst the most important and fashionable and systems in which to explore basic biological processes. Genomics and post-genomic data bases and technologies are being developed by cell and molecular biologists, bacteriologists, parasitologists and botanists that will have tremendous utility in the analysis of fungi. The fungi are rich in organisms with critical roles to play in ecology, biotechnology, medicine, agriculture and other aspects of human endeavour, and remain one of the few relatively unexplored corners of global biodiversity. With the right tools, a multidisciplinary approach and an open mind, mycology can be seen to be at the beginning of an exciting and highly competitive era. This talk will accentuate this naively positive view with selected examples of how mycology is already evolving in this new era.

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387 - Educational aspects of promoting mycology

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From the standpoint of recent experiences, I will discuss the need for active promotion of mycology at all educational levels. However strongly, and however loudly, the case for the importance of fungi is put, we face an uphill struggle to have mycology included in general educational courses. Inclusion as a 'specialist study of a taxonomic group' is feasible in college and university courses, but there is little sympathy for the view that a

course on 'biology' (at any level) should naturally include a major input dealing with fungi. The traditional division of biology into animal biology and plant biology still rules - from the school curriculum to the university degree programme. Changing this blinkered (and essentially ignorant) view of the natural world is likely to take a long time. We have to create an understanding that fungi form an essential and normal part of the biology that surrounds us, and I believe that we need to start with school, and even pre-school, children if we are to achieve this change in attitude.

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388 - Mycology in Thailand

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Thailand traditionally has a strong base in plant pathology and this is continuing. However in the last ten years mycology has received increasing attention and support in both the university and government sectors. Mycology, however, is not seen by the younger generation as a commercial subject and considerable effort in the university sector is required to switch on perspective students. The British Council has recently approved a higher education link programme between our universities and Liverpool John Moores University linking with the British Mycological Society aimed at stimulating the teaching of mycology through workshops and teaching manuals. BIOTEC as a government funded organization is running a number of research programmes mainly targeted at applied aspects of mycology. This presentation will review the development of mycology in Thailand through to the present day and evaluate the current situation and future prospects.

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389 - Conservation of the fungal diversity in Cuba

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One of the main objectives of Darwin Initiative project, 'Fungi of the Caribbean' was to produce national conservation strategies for fungi in Cuba. The strategy is based on the analysis of the available information in the computerised databases of the project and in the Cuban mycological literature. This proposal constitutes the first document that in our country, in the Caribbean region - and possibly in Latin America - approaches the specific problem of the fungal diversity conservation and one of the few ones that exist in the world. The document is structured in two fundamental parts: in the first one we offer the most current state of knowledge of Cuban mycobiota; while in the second part we develop the strategy and action plan in correspondence with the 'holes' identified in this study. The goals of this proposition belong together with those traced in the Cuban National Strategy of Biodiversity. However, the objectives and actions answer the particularities that the rational use and conservation of this important group of organisms has. The strategy and action plan highlights those areas where basic information about fungi are lacking, and discusses the best way to prepare 'red data list' for the fungi. The question of what scientific society would be most suitable to represent mycology in Cuba is also addressed, as well as the importance that has the habitats preservation for the conservation of the fungal diversity.

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390 - Conservation of macrofungi in the Pacific Northwest United States

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The Northwest Forest Plan was developed in 1994 to conserve biodiversity and species viability by maintaining habitat in the forests of the Pacific Northwest United States. More than 400 species of concern are listed in the official Record of Decision, including mammals, birds, amphibians, mollusks, arthropods, plants, lichens, mosses, and fungi. Of the 400 species, the largest proportion (more than 50%) is fungi. The goal of the Forest Mycology Team of the USDA Forest Service, Pacific Northwest Research Station, Corvallis, Oregon, is to provide scientific knowledge on the biology and functional diversity of forest fungi and to apply this knowledge to fungal conservation as well as forest ecosystem health and sustained productivity. Information regarding taxonomy, species distribution and occurrences, population dynamics, and environmental variables are essential to improve strategies for species conservation. We are developing a number of tools to assist land managers and scientists in this endeavor, including: survey techniques, population analysis methods, and habitat-based models for predicting species presence and potential habitat. Using the 'most noble polypore' *Bridgeoporus nobilissimus* as a case study, we illustrate the concepts, issues, and management approaches relating to conservation of rare forest fungi on public lands in the United States.

391 - The IUCN threat categories as a challenge for mapping fungi

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IUCN criteria for Red lists (2000) demand mapping on an even base and on a fine scale for all taxa studied. To overcome the weakness of classical mapping projects, i.e. spatial, temporal, and taxonomical inconsistencies three new approaches were tested. The first approach includes sampling on transects at stratified randomly chosen forest plots of the Swiss National Forest inventory by trained mycologists. The second approach is directed towards volunteers with observations at randomly selected grid points in all habitat types. Modelling with spatially explicit models and expert models as the third approach are chosen to simulate the potential distribution and to define indicator values for each species. A comparison of the different approaches shows a similar abundance pattern for most agarics and boletes and contradicts objections towards classical mapping approaches. Species distribution in all approaches follow the same pattern, a Weibull distribution with few abundant and many rare species. To overcome problems of scale for the range estimation the link with habitat types and their simulation models is essential. Sub-datasets allow to test size-dependant effects on species richness and conservation value. For some habitat types such as bog vegetation area alone seems not to be a very important criterion for fungi.

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392 - Fungi as indicator organisms for endangered forest ecosystems

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Indicator fungi are species, easily identifiable by paramycologists, which co-exist with rare and Red Data Book organisms. A 'classical' inventory of the Scandinavian forests used only 5 rare species earlier, but 109 species and 16 large species groups of 'signal-species' were added later in Sweden. In the first case, many valuable habitats may be omitted; in the second case, good knowledge of fungi is needed by surveyors. In Finland, 33 species are used as indicators, in Estonia, 51 species and 5 species groups (genera). Selection of indicators should be based on a significant statistical correlation between their presence and general species richness. Such a study would be extremely time-consuming and has not yet been carried out anywhere. Lists of indicators have been compiled intuitively on the basis of observations by experienced mycologists. There are many examples of the existence of such a dependence. In an old forest of 19 ha protected since 1924 in Estonia, 23 indicator species but also half (97) of the Estonian polypores and 140 other wood-rotting fungi have been found. Use of indicators is an arbitrary tool but useful in demonstrating the natural value of sites to non-specialists. In Estonia, mycologists participated in the project 'Estonian Forest Conservation Area Network' (1999-2001; 51 new selected conservation areas); paramycologists have found indicator fungi at 175 sites studied during the inventory of Woodland Key Habitats (2000-2002).

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393 - The impact of environmental pollution on macrofungi

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An update of the literature on the impact of environmental pollution on the mycoflora will be presented. Much emphasis has been laid on nitrogen immissions, and the question is raised which other types of pollution may affect, positively or negatively, the quality of the mycoflora. Since the first reports on effects of air pollution on the mycoflora appeared, several types of air pollution have drastically been changed. The question will be addressed whether these changes have affected the mycoflora. Finally, an update will be presented on the mechanisms involved in pollution affecting the mycoflora.

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394 - Effects of forest management on wood-inhabiting fungi in Scandinavian boreal forests

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The species composition of wood-inhabiting fungi was investigated in 90 managed and 34 unmanaged spruce and pine forests in SE Norway. Altogether, we found 56 Polypore and 234 Corticoid species. Comparisons of logs with similar properties (standardized tree species, decay class and dimension class) showed a significant reduction in species number per log in managed spruce forests compared to unmanaged forests, but there was no such effect in pine forests. The species number per log in managed spruce forests was 90-45% of that in the unmanaged forests, and the reduction was highest on logs of large dimensions. A closer inspection revealed that species preferring medium and very decayed spruce logs were disfavored in managed stands, whereas species on early decay classes and decay generalists were unaffected. Similarly, species preferring large spruce logs were disfavored in managed forests. Forest management had strongest impact on low-frequent (rare) species in the spruce forests (more than 50% reduction), whereas common species were modestly affected. The Corticoid species seemed more adversely affected than the Polypores. These results indicate that many wood-inhabiting species in spruce forests are adversely affected by temporal (continuity) gaps in the supply of dead wood, whereas the species in pine forests seem more adapted to forest disturbances. This implies that forest management practices should secure sites with a continuous supply of dead wood in spruce forests.

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395 - Conceptual problems of Ecological Continuity and its bioindicators

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Old, undisturbed forest stands may be important for biodiversity through their content of microhabitats or for the long periods available for colonisation. The term Ecological Continuity (EC) has been used to ascribe value to old forest stands, but its use may lead to underestimation of the importance of forest dynamics and dispersal. If bio-indicators of EC are to be used, species with low dispersal capacity should be chosen. However, many lichens and other fungi seem to have a patch-tracking lifestyle, and to merely indicate specific microhabitats. This is exemplified with data from two papers on wood-inhabiting ascomycetes and basidiomycetes in temperate deciduous woodland. In the first paper, a negative correlation between species richness and stand age in hazel woodland was reported. The number of Red List species was not affected by stand age. In the second paper, fine woody debris (FWD; diam 1-10 cm) was found to be very important for species richness in oak dominated woodland. This finding is discussed in relation to the required time for microhabitat formation. 75% of the ascomycetes were found only on FWD, compared to 35% for basidiomycetes. The volume of CWD (coarse woody debris; diam > 10 cm) and FWD, and the number of Red List species on CWD and FWD were similar. Indicators of EC should be sought among microhabitats (logs), terrestrial molluscs, vascular forest plants, bryophytes and lichenized fungi which are 'perennial stayers', and perhaps ground-floor fungi.

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396 - *Cortinarius* subgenus *Myxacium*: phylogeny and distribution in North America

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Subgenus *Myxacium* is a relatively small group within *Cortinarius*, the largest genus of agarics. The subgenus in North America presently comprises 3-4 sections with 15-25 species. A review of names used for *Myxacium* taxa will be presented. Distribution maps will be shown for selected taxa within this region. To begin understanding relationships within *Myxacium* phylogenetic analyses were performed using nuclear DNA sequences from the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene on 23 *Cortinarius* taxa representing 7 subgenera, and later on 42 taxa from the Northern Hemisphere. Evidence from these studies suggests the subgenus *Myxacium* is polyphyletic as currently recognized. A summary of current knowledge of phylogenetic relationships within *Myxacium* will be discussed together with individual species and their probable taxonomic placement.

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397 - Multiple-gene phylogenies indicate a recent radiation of *Cortinarius*

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The main obstacle for a broadly acceptable classification of *Cortinarius* (Agaricales, Basidiomycetes) is the huge number of species, and the lack of good, non-transitional characters. In this study, we tested suitability and power of three protein coding genes in combination with nuclear rDNA to resolve deeper relationships within *Cortinarius*. We also compare the resolving power of increased taxon sampling versus increased character sampling. To address these questions, portions of ATPase subunit 6 (ATP6), elongation factor 1-alpha (EF1-alpha), NADH-ubiquinone oxidoreductase chain 5 (NADH5) and nuclear rDNA (ITS and LSU) were sequenced for 18-132 taxa, respectively. Sequences of 44 species were combined totalling 4955 bp of DNA. Datasets were analyzed separately and combined with Maximum Likelihood and Maximum Parsimony with different weighting schemes. Ribosomal DNA and protein-coding genes failed to provide adequate discriminatory power for deeper relationships. The combined gene genealogy yielded the best resolution and thus provides the most robust initial hypothesis of phylogenetic relationships within *Cortinarius*. We suggest that the difficulties to resolve the evolutionary relationships of *Cortinarius* are linked to two sequential rapid radiation events in the evolution of this ectomycorrhizal mushroom. Thus, unresolved branching orders during these time periods may represent an accurate representation of the evolutionary history of *Cortinarius*.

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398 - Taxonomy of genus *Cortinarius* based on phylogeny obtained from nuclear rDNA sequences

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ITS and LSU rDNA sequences of 48 species (ITS) and a subset of 29 species (LSU) of *Cortinarius* from the Northern Hemisphere were studied. *Gymnopilus sapineus* was the outgroup taxon. Phylogeny from the ITS data was studied by: maximum parsimony followed by successive weighting (MPsw), maximum likelihood analysis (ML), and neighbour joining (NJ). In all analyses, the subgenera *Telamonia* and *Dermocybe* were monophyletic. MPsw also rendered subgenus *Phlegmacium* as monophyletic if we accept *Rozites caperatus*, *C. limonius* (formerly subgenus *Leprocybe*), and subgenus *Myxacium* section *Colliniti* belonging to this subgenus. A tentative taxonomy of *Cortinarius* is: (1) subgenus *Dermocybe* with its 'classical' inventory. (2) subgenus *Telamonia* comprising the 'old' *Telamonia* species (minus *C. obtusus*) together with species formerly placed in *Sericeocybe* or *Leprocybe*. (3) subgenus *Phlegmacium*, also comprising some species from *Leprocybe* and *Myxacium*, and the genus *Rozites*. (4) subgenus *Cortinarius*, a paraphyletic group positioned at the bottom of the phylogenetic tree consisting of miscellaneous species from the 'old' subgenera. The LSU sequence data had few variable sites, and the phylogeny was not so obvious as from ITS. MPsw gave following indications: *Telamonia* is monophyletic, *Rozites* is inside *Cortinarius*, *C. cinnabarinus* belongs to *Telamonia*, *C. obtusus* falls outside *Telamonia*. The analysis could not support *Phlegmacium* as monophyletic. *Dermocybe* appeared paraphyletic on the LSU tree.

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399 - Evolution of subgenus *Phlegmacium* in light of its pigment chemistry - a case of 'trivialization' and loss of character diversity?

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Cortinarius subgenus *Phlegmacium* includes many spectacular, bright-coloured species, especially in sect. *Fulvi* ss. lat. (incl. sect. *Scauri* ss. Mos. p.p.). Section *Fulvi* has more than 60 different anthraquinonic pigments, mainly of the atrovirin, flavomannin and phlegmacin series. These pigment series are probably developed independently, from different precursors, and occur in different species. It is thus likely that sect. *Fulvi* is a polyphyletic group, even though it is morphologically homogeneous based on numerical character analyses. According to chemical pathways, the atrovirin-containing species (subsect. *Atrovirentes*), and the ones with the least methylated flavomannins and phlegmacins (subsect. *Splendentes* and *Rufoolivacei*) are the most primitive. These species are characterised by (i) high pigment concentrations, (ii) high diversity of pigments, and (iii) easy oxidized compounds. The supposed primitive pigment characters are co-occurring with extreme 'phlegmacioid' features such as (i) bright colours, (ii) strongly glutinous surfaces, (iii) marginate bulb, (iv) citriform, coarsely verrucose spores, and (v) lack of brown encrusting-parietal pigments. The supposed derived species possess more 'trivial', 'telamonioid' characters such as brown colours and a clavate stem. It seems thus that the ongoing evolutionary trend in *Phlegmacium* is a kind depauperation of pigment diversity, going from spectacular to more trivial looking organisms.

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400 - Molecular systematics in the genus *Inocybe* (Agaricales)

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Three genes are employed to infer the phylogeny of *Inocybe*, a genus of ectomycorrhizal agarics distributed world-wide but particularly diverse in temperate regions of the northern and southern hemispheres. Partial DNA sequences of RPB1, RPB2, and nuclear large subunit ribosomal RNA (nLSU) all indicate *Inocybe* is monophyletic and composed of several lineages. Combined RPB1 and RPB2 DNA sequences and amino acid data reveal the *I. calamistrata* group (sect. *Cervicolores*) occupies the most basal branch in the clade and that subgenus *Mallocybe*, which includes the *I. dulcamara* group, is monophyletic. However, these results are not supported by all data sets. Members of the *I. rimosa* group (sect. *Rimosae*) appear polyphyletic based on RPB1 and nLSU data. Strong support is shown in all instances for the monophyly and derived status of species with metuloid cystidia, but nodulose-spored taxa are polyphyletic. Examples of cystidiate clades include the *I. lanuginosa* group and *I. geophylla* group. Several novel relationships have also been detected: (1) a southern hemisphere mallocyboid clade including the tropical West African *I. aureoplumosa* and undescribed taxa from Australia, including a secotioid form; and (2) a monophyletic group of several species with nodulose spores from neotropical *Dicymbe*-dominated forests in Guyana. RPB1 and nLSU data suggest the saprophytic genus, *Phaeomarasmius*, is indeed sister to *Inocybe*, although these two data sets are incongruent for some taxa.

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401 - Distribution of *Cortinarius* in the world flora and their relations to phanerogams. An update

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The genus *Cortinarius* was for a long time regarded as being restricted to the temperate Northern and Southern Hemisphere associated with Pinaceae, Fagales, *Eucalyptus*, *Tilia* and *Dryas*. The work of Favre initiated the interest in

alpine and arctic mycology and numerous Cortinarii have been described from the Northern Hemisphere associated with *Salix* and *Dryas*. The increased exploration of tropical fungi have brought to light quite numerous ectomycorrhizal taxa but the number of Cortinarii remain low. Few species are known from Congo, the Amazon area and South India associated with leguminous and sapotaceous genera or Dipterocarpaceae. The distribution of some special groups like subgenus *Cortinarius*, subgenus *Leprocybe* sensu stricto, the *Cortinarius orellanus* group, subgenus *Dermocybe*, subgenus *Phlegmacium* subsection *Coleopodes*, the *Cortinarius salor* group and others are discussed.

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402 - Species and speciation in the *Hebeloma crustuliniforme* complex

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Within the genus *Hebeloma* species have traditionally been defined typologically by their morphological differences. In many cases this has not lead to uncontroversial species delimitations. An extreme example is the *Hebeloma crustuliniforme* complex, where a number of species have been described, but where the borderlines between these species have been disputed. Here we used sexual intercompatibility to define species within this species complex. We found a minimum of 20 intercompatibility groups (biological species). A phylogeny including species of the other main groups of the genus *Hebeloma* shows that the *Hebeloma crustuliniforme* complex consists of two well-supported clades that together possibly do not form a monophyletic group. Speciation is the process of becoming a species. Under the biological species concept, speciation can thus be defined as the origin of sexual incompatibility. Within a monophyletic group consisting of nine closely related ICGs belonging to the *Hebeloma crustuliniforme* complex, four ICGs had varying levels of partial incompatibility (within groups 100% compatibility, between 0.4, 8.3 and 15.0%). We estimated phylogenies of different individuals of these ICGs to reconstruct the evolution of intercompatibility. Our results indicate that generalizations between the level of intercompatibility and genetic divergence within this species complex can not be made.

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403 - Correlations between classical systematics and molecular phylogenetic hypotheses in the genus *Cortinarius*

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A wide spectrum of *Cortinarius* species from South America, Australia, Asia and Europa were studied by means of comparative morphology and anatomy, scanning electron microscopy of basidiospores, and molecular phylogenetic methods. Sequencing of both the ITS region and the D1/D2 region of the nuclear coded large ribosomal subunit (LSU) allowed estimation of phylogenetic relationships both within the genus *Cortinarius* and also between *Cortinarius* and closely related taxa. We present results of our molecular phylogenetic analyses and discuss them in relation to classical taxonomic concepts in *Cortinarius* and our own macro- and microscopical observations.

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404 - A molecular phylogeny of *Gymnopilus*, using ribosomal ITS DNA sequence data

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Gymnopilus P. Karsten is a genus of wood-rotting basidiomycetes with over 150 species distributed worldwide. *Gymnopilus* produces a rusty-brown spore print and has rough spore ornamentation, commonly without a plage. The genus has recently been revised in Australia and there are several detailed accounts of the genus for other regions including Norway, North America and Great Britain. In conjunction with an ongoing revision of New Zealand species, we are sequencing the internal transcribed spacer region of nuclear ribosomal DNA for phylogenetic analyses using parsimony and maximum likelihood. We will present a phylogeny of the genus incorporating both southern and northern hemisphere species. There is considerable similarity between the New Zealand and Australian *Gymnopilus* flora, and several species pairs exist that span the northern and southern hemispheres.

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405 - Myconet

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Myconet MYCONET is an international project intended for the development of a natural classification of the Ascomycota. Relevant literature is screened and all new data that may necessitate changes in the classification are presented in the series Notes on ascomycete systematics (at present c. 3500 Notes). An Outline of the Ascomycota is

continuously updated. Decision on changes is taken by eight Boards of experts, viz. for Ascomycota (higher taxa), Taphrinomycotina / Saccharomycotina, Arthoniomycetes / Lecanoromycetes, Chaetothyriomycetes / Dothideomycetes, Eurotiomycetes, Leotiomycetes, Pezizomycetes, and Sordariomycetes. Results are published in the electronic journal Myconet and in hard copies of the same journal.

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406 - FungalWeb

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The FungalWeb has been established to make a mycological web site that can provide the most current and accurate fungal taxonomy. The users it is designed for range from professional mycologists in academia and industry to advanced high school students. What has been done is to provide a classification for all fungal telemorph genera, based on a combination of phenotypic and genetic data. The FungalWeb as it is available today is the result of a joined effort of a number of leading experts, together covering all the fungal taxonomic groups. The planned next steps for improving of the web site are the following: Extend the web site to cover the genera *Aspergillus* and *Penicillium* to species level. This will be done summer 2002, building on contribution of data from Samson, Frisvad and Pitt. Extend the web site to as far as possible also include and accommodate implacement of the anamorphs. This will be done based on guidance and data from K. Seifert. Planned implemetation fall 2002. In a second generation FungalWeb it is planned to establish links to all major mycological web sites and databases, including pictures, secondary metabolites, pathogenesis, ecology, industrial use etc. Further, FungalWeb needs resources for scientific and technical maintenance and development. Attempts will be made to have this secured on coordination with the GBIF initiative.

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407 - LIAS - A global information system for lichenized and non-lichenized ascomycetes

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The LIAS project was initiated in 1995. It is a multi-authored information system for collecting and distributing descriptive and other biodiversity data on lichens and non-lichenized ascomycetes. Its goal is to provide a working space for cooperation and collaboration of experts (http://www.lias.net/). The data of the two core databases are stored in DiversityDescriptions (= DELTAAccess) and made available through several web interfaces. Using HTML data submission forms automatically generated from the LIAS databases, sets of descriptive data can be established and interactively revised over the internet. Taxon names are linked to various types of information (e. g. more than 700 partly multi-stated descriptive characters), allowing mycologists and lichenologists to co-operate online and add their data to a common system for multiple usage (identification keys, database generated natural language descriptions, revisions etc.; with public or restricted access). Currently more than 60 mycologists and lichenologists world-wide are contributing as authors or revisers. LIAS is co-operating with several flora and database projects (e. g. the Sonoran Lichen Flora Project, the Checklists of Lichens Project) and the Internet Portal 'Mycology.Net'. It will serve as Global Species Database for lichens in context with the Species2000 initiative and the EuroCat project.

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408 - Recent Literature on Lichens

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"Recent Literature on Lichens" (RLL) is a series published in The Bryologist since 1951. It aims at listing all recently published papers in lichenology, with a complete bibliographic reference, keywords, and abstract - including mention of all new scientific names and combinations. The authors are William L. Culberson (in the period 1951-1978), Robert S. Egan (1979-1991), and Theodore L. Esslinger (from 1991). The lists have been computerized by Egan and Esslinger, and the database is put on the Web by the present author (http://www.nhm.uio.no/botanisk/lav/RLL/RLL.HTM). Together with "Mattick's Literature Index", originally a card index in Berlin computerized and expanded by Harrie J.M. Sipman, the data set now goes back to 1532, and consists of more than 32,000 records. Members of Myconet's Lecanoromycetes Board may use RLL as a tool when scanning for taxonomic papers for review.

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409 - Saccharomycotina and Taphrinomycotina

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Gene sequence comparisons have shown that presently described, genera, families and orders in the Saccharomycotina and Taphrinomycotina are often not phylogenetically circumscribed. Single gene analyses are generally inadequate for genus circumscription, but for the Saccharomycetales genera seem to be strongly resolved in datasets comprised of around 5,000 nucleotides. Family groupings in such analyses are generally only weakly supported. Consequently robust molecular phylogenies of higher level classification may require 20-50 gene sequences.

410 - Lecanoromycetes

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In the current Myconet classification, lichenized discomycetes are mainly placed in the two classes Arthoniomycetes and Lecanoromycetes with 6 orders, about 64 families, 72 Arthoniomycetes and 494 Lecanoromycetes genera. The class Arthoniomycetes with one order is represented by nearly 400 known species, the class Lecanoromycetes might well comprize ca. 6.300 species. Following the Myconet classification, the order Lecanorales s. str. is segregated into four suborders, whereas some other units formerly treated at suborder rank, have been (re-)assigned the status of orders. In the last years there were main activities in molecular phylogenetic research concerning both classes. From 2930 (partial) 18S rDNA sequences actually existing for Pezizomycotina, ca. 1,6% refer to Arthoniomycetes and ca. 10% to Lecanoromycetes. For Arthoniomycetes additional 78 ITS sequences (ca. 1.2%) have been published aside from a negligible number of 26S and mitochondrial sequence data. For Lecanoromycetes, nearly 900 ITS sequences, 250 sequences of 26S and a small number of mitochondrial sequence data are available in GenBank. To estimate the support of existing classifications of the two ascomycete classes by published molecular data, topologies of 18S-trees of 20 publications are evaluated and compared with phylogenies calculated from 600 high quality sequences of 1600 bp minimum length. Supplementary information from ITS- and LSU-based phylogenies is respected in the considerations.

411 - Chaetothyriomycetes and Dothideomycetes

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Major results of recent studies, mainly based on molecular methods, concerning the phylogeny of these two classes of ascolocularous fungi are presented and discussed. This includes the inclusion of lichenized fungi (e.g. Verrucariales) in the Chaetothyriomycetes and their relationships to other ascomycetes. Recent studies on the Dothideomycetes mainly dealed with the subdivision of this large class of fungi into natural lineages. Results and prospects for further is discussed using some examples.

412 - Eurotiomycetes

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The morphological signature of the Eurotiomycetes embodies fungi that generally produce spherical, evanescent, eight-spored asci within more or less globose ascomata. Ascospores are minute, single-celled and hyaline or very pale in colour. DNA sequence analyses underline the convergent nature of this signature and have been the basis of arguments to align taxa with the typical eurotiomycete signature with other classes (e.g., *Cryptendoxyla*, *Myxotrichum*). Similarly, DNA analyses have been used to align taxa with quite anomalous morphology within the Eurotiomycetes (e.g., *Pseudotulostoma*). Understanding the nature of the ecological pressures creating these morphological variants will help us rationalize the taxonomic journeys of taxa among the classes of the Ascomycota.

413 - Leotiomycetes

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Molecular sequence data have suggested a more or less severe rearrangement of some genera or species within the Helotiales, or even their removal from the order to which they have previously been placed according to morphological characters. Whether these modern results can be accepted in every case remains to be seen. Morphological studies are based on a high number of species and specimens, while molecular studies often still concern only one or a few species per genus. Some morphologies are so complex in structure that a polyphyletic origin advocated by molecular data in some cases seems quite impossible to imagine. Furthermore, identification of species is still problematic in the Ascomycetes, considering the fact that a large number of taxa still remains to be described, followingly erroneous specific or even generic names may have been attached to specimens from which molecular data derive. Nobody has control over correct determination when using data from GenBank. The potential of descriptive light-microscopical morphology is far from being fully exhausted. This especially concerns the various characters of the living fungus (vital taxonomy) which offer a huge amount of stable information on both generic and specific levels, but also concerns exact studies of apical apparatus, croziers etc. Such carefully gained morphological results often coincide with those of the sequence data, especially if a combination of characters is used for the generic concepts.

414 - Pezizomycetes

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Molecular phylogenetic studies in the Pezizomycetes (with single order Pezizales) have over the past five years revealed new information on relationships between families and genera. Pezizales currently includes 14 families, and is composed of at least 3 distinct lineages: A) Ascobolaceae and Pezizaceae, B) a Discinaceae-Morchellaceae clade, a Helvellaceae-Tuberaceae-Karstenellaceae clade and a monotypic Rhizinaceae clade, C) Ascodesmidaceae, Glaziellaceae, Pyronemataceae, Sarcoscyphaceae and Sarcosomataceae. The placement of the monotypic family Carbomycetaceae is unknown. Lineage C is the most heterogeneous lineage and the delimitations of all included families are problematic. To resolve family level relationships within lineage C it will be necessary to evaluate the largest and presumed polyphyletic Pyronemataceae in the context of closely related taxa (Ascodesmidaceae, Glaziellaceae, Sarcoscyphaceae and Sarcosomataceae). The Pyronemataceae has been a 'default' family for pezizalean taxa with uninucleate spores and iodine negative asci, which lack unifying anatomical characters by which they could be segregated into putative natural families. The evolutionary relationships of *Ascodesmis* and *Glaziella*, and the justification of the families Ascodesmidaceae and Glaziellaceae, or their circumscription, is still uncertain. Lineages A and B appears to be well-separated evolutionary lineages and the delimitation of families within these has to a large extent been clarified.

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415 - Sordariomycetes

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Current classification of higher taxa in the class will be discussed. The genera currently accepted within the Xylariaceae will be given brief comments as to their current status and various problems will be raised. Several if not most of the so called core genera of the family can with the present knowledge not be considered monophyletic.

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416 - Laboulbeniomycetes, Spathulosporomycetes and Ascomycota inc. sed.

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The two classes Laboulbeniomycetes and Spathulosporomycetes are briefly presented and Ascomycota with uncertain affinities are discussed.

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417 - Integrating biological data within an evolutionary framework

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The rapidly expanding field of bioinformatics is providing researchers with many tools and techniques for building, updating, and accessing biological data. With the exponentially increasing amounts of data from SNPs, gene expression profiling, proteomics, clinical and epidemiological studies, the focus is now on developing the conceptual and practical tools for understanding and exploring biological data to its fullest potential. One of the biggest challenges facing researchers is the move away from studying one gene or one molecular data set at a time to looking at multiple genes or multiple genetic and phenotypic data sets simultaneously, in a holistic fashion. The integration of multiple molecular and phenotypic data sets is accelerating the pace of biological discoveries and our overall understanding of biological systems. Computational methods are being developed to allow researchers to make the critical link between genotype and phenotype and in the process distinguish between associated and causative genetic variation. Evolutionary theory is at the core of these computational algorithms and is providing the conceptual bridge between bioinformatics and biological inference.

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418 - The utility of haplotypes in evolutionary analysis

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Genetic variation in natural populations is organized on chromosomes in linked sets that often exhibit some correlated structure or linkage disequilibrium. When considering sets of adjacent polymorphic sites, it is often of use to note that only a subset of possible arrangements occur. These 'haplotypes' have proven highly useful for making a variety of inferences about population structure, migration, ages of polymorphisms, and departures from neutral evolution. An overview of the methods for inference of haplotypes from genotype data will be presented, along with illustrations of the applications of haplotype analysis in populations of *Drosophila* and other organisms.

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419 - Inferring ancestry from DNA sequences; modeling and inference with the coalescent process

R.C. Griffiths

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A unique gene tree describing the mutation history of a sample of DNA sequences can be constructed as a perfect phylogeny under an assumption of non-recurrent point mutations. There is much interest in thinking of the data as a tree, rather than just sequences. This talk will describe, with examples, how coalescent trees and gene trees are used to model and infer ancestry of a sample of DNA sequences.

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420 - Demographic inferences from molecular data using Bayesian and maximum likelihood methods

R. Nielsen

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A popular approach for inferring demographic parameters, such as levels of gene flow between populations, or rates of population growth, is to collect molecular data and to use this data to estimate demographic parameters based on specific population models. In the past there have been few attempts to test these models and to develop methods for choosing between alternative models. In this talk I will present some recent methods for hypothesis testing and model choice using molecular population genetic data. In particular I will investigate models for estimating levels of gene flow and population divergence times.

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421 - Progress and pitfalls in aflatoxin studies

D.M. Wilson

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Aflatoxin contamination of foods and feeds is a global problem which is most severe in the warmer portions of the globe. Aflatoxin research is difficult because the fungi that produce the aflatoxins are not aggressive pathogens and often occur as saprophytes in feedstuffs. Aflatoxin contamination can occur before harvest, during harvesting and drying and in storage. The environmental conditions favoring contamination before harvest generally includes late season drought and high temperatures. The relationships between damage and contamination begin in the field and progress throughout the marketing channels with moisture content being the most critical post harvest factor. There are different marketing regulations in the USA and European Union. The market for the product sometimes dictates the marketing and management strategies. Countries with few or unenforced regulations sometimes have highly contaminated products in the marketplace. Therefore, management strategies must be tailored to the climate and the country. There are at present no completely effective management tools to eliminate aflatoxin contamination from the human food chain.

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422 - Fumonisin exposure in different populations and reduction of fumonisin by the process of nixtamalization

M. Dombrink-Kurtzman

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In countries where a major part of the daily diet is maize, the presence of fumonisins in maize products represents an emerging health concern. The U.S. Food and Drug Administration has issued recommended guidance levels for maize. Although unequivocal harm to humans has not been demonstrated, fumonisins have been shown to cause diseases in horses and pigs and to produce tumors in laboratory rats. Exposure to fumonisins is highest in South Africa and China due to high intake of maize and the potential for environmental conditions favoring mycotoxin production. Fumonisins, mycotoxins produced by *Fusarium verticillioides* (synonym *F. moniliforme*) and *F. proliferatum*, can be present in normal-appearing maize. The alkaline-cooking process, nixtamalization, is used to produce many maize products in Mexico, Central America and the United States. Research had shown that masa and tortillas from Mexico and the United States contained fumonisin B_1. To determine the fate of fumonisins during processing, maize naturally contaminated with fumonisins underwent nixtamalization for production of tortillas. Material was analyzed at each step for fumonisins, weights and moisture contents so that mass balance determinations could be calculated. Less than twenty percent of the fumonisins remained in the tortillas. Nixtamalization appears to be a way to reduce significantly fumonisins occurring in maize products.

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423 - Human exposure to ochratoxin A: Analytical methods for determining ochratoxin A in wine and beer and as a biomarker in human urine

M. Pascale* & A. Visconti

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Ochratoxin A (OA), a mycotoxin widely distributed in various foodstuffs and beverages, has been shown to be nephrotoxic, hepatotoxic, teratogenic and immunotoxic to several animal species and to cause kidney and liver tumours in mice and rats. IARC has classified OA as a possible carcinogen to humans (Group 2B). The widespread occurrence of OA in human blood provides evidence of a continuous human exposure to this mycotoxin. Wine and beer are products widely consumed by adult individuals and, due to the high frequency of contamination with OA, they may represents a serious source of daily OA intake for human. A new analytical method for the determination of OA in wine and beer has been recently developed by the authors and validated by an inter-laboratory study. The method, based on the use of immunoaffinity columns and HPLC, has been recently adopted by the AOAC International, CEN (European Committee for Standardisation) and OIV (Office International de la Vigne et du Vin) as official method. In a recent study carried out in the UK, a good correlation between OA consumption through the diet and OA concentration in urine was observed suggesting that urine could provide a useful marker of OA intake. A rapid and accurate method, based on immunoaffinity columns and HPLC, to quantify OA at pg/ml levels in urine has been developed by the authors. The method can be used as a rapid and non-invasive tool to assess human and animal exposure to OA in epidemiological studies.

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424 - Changes in concentration of mycotoxins during storage of wheat

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Species of the genera *Fusarium*, *Penicillium* and *Aspergillus* are well-known for their occurrence on grains and their ability to produce mycotoxins. Whereas *Fusarium* invade and damage grains predominantly on the field, they are increasingly displaced by *Penicillium* and *Aspergillus* during storage. *Fusarium* species produce a variety of mycotoxins, of which deoxynivalenol (DON), a B-trichothecene, is detected most frequently and in highest concentrations. DON is mainly produced on the field by *F. graminearum* and *F. culmorum*. Ochratoxin A (OTA), which is one of the mycotoxins produced by *Aspergillus* and *Penicillium* species, does not normally occur before harvest and is therefore considered to be a storage toxin. Due to its carcinogenicity and nephrotoxicity it is regarded to be the toxicologically most important mycotoxin occuring during storage of wheat. It was the purpose of the studies presented to investigate the influence of different suboptimal storage conditions on the viability and mycotoxin production of *Fusarium* and *Penicillium*/*Aspergillus* species and to deduce information about their inter-relationship during suboptimal storage. For that reason, DON and OTA contents were quantified and set into relation to the frequency of fungal isolation and biomass.

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425 - *Penicillium* species in food leftovers intended for feed

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Swill feeding was widespread in Norway until feeding of unsterilized swill was prohibited due to the high risk for spreading of contagious animal diseases. However, there has been increasing interests for utilisation of food leftovers for animal feed, especially for pigs and fur animals. The authorities want to avoid food leftovers on waste disposal sites and at the same time utilize this resource in production of an economic feed. Plants producing liquid feed from food have been established several places in Norway. Food leftovers from large-scale households and food industry are actual resources. Since food leftovers have variable composition, a high number of mould species and mycotoxins may be expected. In a descriptive study of the fungal flora of food leftovers intended for feed, altogether 89 samples of food waste were subjected to mycological examinations. Genus *Penicillium* was demonstrated in 88% of the samples with mean count $5{,}5 \times 10^5$ KDE/g. Altogether 31 *Penicillium* species were demonstrated, of which 8 were demonstrated in ≥10% of the samples. The species most frequently demonstrated were *P. crustosum*, *P. roqueforti*, *P. viridicatum*, *P.brevicompactum*, *P. aurantiogriseum*, *P. chrysogenum*, *P. expansum* and *P. echinulatum*. Genus *Penicillium* does produce a high number of mycotoxins, which makes it difficult to choose indicator-toxins for feed based on food leftovers. Further studies on toxin production in food leftovers are in the process.

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426 - Mutualism and antagonism in bark beetle-fungus-mite interactions

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Bark beetles present an economically and ecologically significant model for the study of multilevel symbiotic interactions. The southern pine beetle - *Dendroctonus frontalis* - is associated with both mutualistic and antagonistic fungi. The bluestain fungus - *Ophiostoma minus* - may initially assist the beetle in killing its tree host. However, as beetle larvae begin developing, it competes with the beetle for host tissue. The beetle also carries two fungi in specialized mycangia. These fungi (*Ceratocystiopsis ranaculosus* and *Entomocorticium* sp. A) are nutritional mutualists of the beetle. These mycangial fungi are able to grow and fructify within the host tree, providing nitrogen rich tissue for the beetles to feed upon - unless they are interfered with and outcompeted by *O. minus*. All three fungi interact in both positive and negative fashion with phoretic mites, which are carried into the tree by the beetle. These mites in turn vector and feed upon two of the beetle associated fungi (*O. minus* and *C. ranaculosus*). Though *O. minus* usually outcompetes the mycangial fungi, abiotic factors (such as water potential) may alter these interactions. In addition, the fungi are not of equal benefit to the beetle, or the mites. We review recent research into the biology and ecology of this complex system, pose hypotheses as to the effects of these interactions on the population dynamics of this destructive beetle, and consider the implications to the field of fungal symbioses.

427 - Epidemiology of southern pine beetle-fungal interactions

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The effects of microbes on the dynamics of animal populations may be due to direct interaction between associates and hosts, or may be indirectly mediated by other organisms within the community. We studied a fungus (*Ophiostoma minus*) that is, at various times and stages, an antagonist and competitor of the southern pine beetle (*Dendroctonus frontalis*), a mutualist of several mites associated with the beetle, and a facultative pathogen of the pines that play host to this beetle. We hypothesized that the population dynamics of *D. frontalis* are influenced by negative feedback through community interactions involving other (mutualistic mycangial) fungi and *O. minus*, and mites that transport and feed upon the associated fungi. We found *O. minus* abundance within bark to be positively related to mite abundance but negatively related to *D. frontalis* survival. The abundance of *O. minus* gradually increased as *D. frontalis* infestations progressed through time. Changes in *O. minus* abundance were more correlated with mite abundance than with the prevalence of *O. minus* on attacking beetles. Factors that affect the population dynamics and behavior of these mites, and the growth of *O. minus* in pines, likely play an important role in the population dynamics of the southern pine beetle.

428 - The consequences of oligophily in bark beetle-fungus associations

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Fungi are ubiquitous associates of bark beetles (Coleoptera: Scolytidae). Investigations have revealed a diverse array of association types ranging from mutualism to antagonism. Monophilic (one symbiont) and polyphilic (many symbionts) associations appear to be rare, while oligiophilic (two or a few symbionts) associations are common. Oligophily is likely to have several important consequences for both the host beetle and the symbiotic fungi. For any given association, fungal associates vary greatly in their effects on host beetle fitness, and therefore, are likely to differ in their influence on host population dynamics. Additionally, interactions, including competition for hosts, among multiple fungal associates of a beetle species may determine the relative abundance of each in a population, which, in turn, may also influence host population dynamics. Oligophily may be a less desirable state than monophily in associations that involve at least one mutualistic fungus. In such associations, for at least some beetles in a population, the mutualist can be displaced by less beneficial associates or by detrimental fungi, resulting in lower overall fitness. Some associates may actually be cheaters in the system,conferring few to no benefits to the host. While cheaters are potentially detrimental, beetles may be unable to develop effective means of avoiding cheaters without also negatively impacting beneficial associates.

429 - Relationships among hosts, horntails, and fungi in Japan

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Wood discoloration of *Chamaecyparis obtusa* (hinoki) and *Cryptomeria japonica* (sugi) trees by the fungus associated with horntails, *Urocerus japonicus* and *U. antennatus* was studied in Japan. Wood discoloration in hinoki and sugi caused by the horntails and the fungus was found in several prefectures. The emergence periods of *U. japonicus* and *U. antennatus* were from the beginning of July to the middle of October and from the middle of May to the middle of August, respectively. Cultures isolated from the mycangia of adult females of horntails showed the same cultural characteristics. Basidiocarps found on felled logs of hinoki and sugi were identified as *Amylostereum laevigatum* based on morphological characteristics. The cultures from the basidiocarps had the same cultural characteristics as those from the mycangia of horntails. Two mycangial isolates produced on basidiocarps on the stem segments of sugi by

artificial inoculation and were identified as *A. laevigatum*. Isolates from the basidiocarps and the mycangia of horntails had similar ITS and peroxidase A sequences. The wood of all inoculated trees showed discoloration, with no difference in shape and pattern of discoloration among the two isolates from the basidiocarps of *A. laevigatum* and two from the mycangia of horntails. The inoculated fungi were reisolated from the areas of discoloration in the inoculated trees. This is the first report on wood discoloration of hinoki and sugi caused by the horntails and *A. laevigatum*.

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430 - Relationships amongst the fungal symbionts of Siricid woodwasps and their spread in the Southern Hemisphere

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The Basidiomycetes genus *Amylostereum* is best known for its symbiosis with siricid wood wasps. In this study, the phylogenetic relationships between *Amylostereum* spp. and other Basidiomycetes were investigated, using sequence data of the nuc-IGS and mt-SSU rDNA regions. *A. areolatum* was more distantly related to the three other species of *Amylostereum*, than they were to each other. *A. ferreum* and *A. laevigatum* were the most closely related species. These data support hypotheses relating to the mating behaviour and ecology of *Amylostereum* spp. Furthermore, contrary to previous suggestions, the *Amylostereum* spp. were more closely related to *Echinodontium tinctorium* (Echinodontiaceae) than to the Stereaceae. Apart from phylogenetic studies, vegetative compatibility and PCR-RFLP analyses were also used to study the spread of the introduced *S. noctilio-A. areolatum* complex in the Southern Hemisphere. Isolates of *A. areolatum* from South Africa and South America were found to represent a single VCG. PCR-RFLP profiles from nuc-IGS region showed that all isolates from the Southern Hemisphere shared the same profile, which differed from that of other populations. This genetically uniform population of *A. areolatum* has emerged from its obligate relationship with *S. noctilio*, which disperses only asexual fungal propagules. These results suggest a single or limited introduction of the *A. areolatum-S. noctilio* into the Southern Hemisphere.

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431 - Interspecific combative interactions - an overview

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Competition by fungi for nutrients in dead organic resources is effectively brought about by competition for space. Such competition can be divided into primary resource capture (obtaining uncolonized resources) and secondary resource capture (combat to obtain resources already colonized by other fungi). Combat occurs not only in organic resources but also when mycelia grow out of the resource, e.g.into soil, in search of new resources. Combative mechanisms include antagonism at a distance, mycoparasitism, hyphal interference and gross mycelial contact. Outcome of interactions can be deadlock, where neither species gains territory from the other, or replacement, where one mycelium partially or completely wrests territory from the other. A hierarchy of combative abitlity can be discerned amongst fungi that inhabit a particular resource, but within this hierarchy there is intransitivity and outcomes can vary depending on abioic variables and the presence of other organisms. Interactions can dramatically alter mycelial function even when the outcome is deadlock. The review will be illustrated largely with examples of wood decay fungi and of wood decay fungi interacting with ectomycorrhizal mycelium.

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432 - Interactions among wood-inhabiting fungi: implications for fungal succession and biocontrol

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Interactions among wood inhabiting fungi are important for succession and have also implications for biocontrol. Early establishment secures resources for the mycelium while late arrival to a substrate necessitates strategies for entering an already established decay community. Mycelial parasitism and replacement in wood has proven to be one important driving force in succession in wood. Replacement involves developmental changes in the mycelia and can often be facilitated by production of antimicrobial secondary substances. Other key factors for succession include differences in ability to handle recalcitrant compounds, differences in growth rate, differences in responses to gaseous regime and water potential etc. We will describe experiments indicating both species specific and general replacement patterns, metabolite production, and gene expression during interaction between various wood inhabiting fungi. Special reference will be given to the possibilities to find biocontrol against economically important root rot fungi such as *Heterobasidion annosum*.

433 - Primary decayers - a key to understanding biodiversity in decaying wood?

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Primary decayers, here defined as fungi initiating and causing extensive wood decay, have in several cases been found to be important determinants of subsequent decay development and species composition in decaying wood. Based on own research this concept of decay pathways is reviewed, and it is discussed if such pathways are well defined and whether they are shaped by interspecific interactions, by passive facilitation, or both. Further, the importance of decay pathways for biodiversity in decaying wood is considered, and it is discussed whether decay pathways are important to consider in the development of management guidelines aiming to preserve and restore biodiversity in forests.

434 - Tritrophic relationships in the phyllosphere: fungal parasites and hyperparasites in action

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Traditionally, the interactions between plant parasitic fungi and host plants are regarded as closed, two-species systems. However, both parasites and their hosts are, in fact, components of complex multitrophic interactions in which parasitic fungi are often attacked and killed by hyperparasites or other antagonists. Parasites, by definition, have a negative effect on host fitness, so hyperparasitism should be favourable for plants infected with parasites. However, studies on the possible role of hyperparasites in the natural control of plant parasites are missing from the literature. There are a few quantitative studies even on the natural occurrence of hyperparasitism that represents only the first step towards evaluating the impact of hyperparasites on host fungal and plant populations in nature. This paper synthesizes the current knowledge on structural, physiological and evolutionary aspects of natural host-parasite-hyperparasite relationships. A case study is also presented in which the effects of *Ampelomyces* hyperparasites on the fitness of powdery mildew infected *Lycium halimifolium* plants were studied by measuring the chlorophyll content of the healthy and infected leaves with and without hyperparasites in the field.

435 - Understanding the communicating mycelium - Translocation, past present and future

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A fungal mycelium with its cytoplasmic continuum is a single organism. The mycelium is thus a network of communicating daughter nuclei. Communication consists of both signalling and sharing of material resources, like nutrients and energy. The study of nutrient and organelle translocation has a long history in mycology and some classical works will be briefly presented. The difference between large mycelia and the unicellular organisms in need for resourse redistribution are mainly two. 1. Need for fast high capacity nutrient translocation: Four different mechanism has been suggested, simple diffusion, simple diffusion + active uptake, active translocation by cytoplasmic movements and pressure driven bulk flow through vessel hyphae. There appear to be large differences between fungal mycelia of different species in their ability to translocate nutrients which might reflect the mechanisms employed. 2. Need for nutrient storage: To be able to scavenge nutrients quickly from the environment for redistribution there has to be efficient uptake systems and somewhere to store what has been taken up. Nutrients are taken up as small molecules but their storage have to be in osmotically neutral form. Great advances have been made in understanding the mechanisms for nutrient reallocation in mycelia but there are still much to do. Long distance signalling as well as nutrient storage needs attention in future research to be able to understand the physiology of mycelial organisms.

436 - Bidirectional transport - an insurmountable obstacle to the use of tracer isotopes in quantitative translocation studies?

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Tracer isotopes have been used in a long range of studies to demonstrate translocation of various substances through fungal mycelia. In a microcosm experiment, translocation was studied in rhizomorphs of the wood rotting fungus *Hypholoma fasciculare*. Non-destructive electronic autoradiography was used in combination with the two radioactive phosphorus isotopes 32P and 33P to show that phosphorus was transported in two directions simultaneously. This experiment confirms earlier studies suggesting that phosphorus, and most likely a range of other substances, are transported by mechanisms based on circulation throughout the mycelium rather than direct unidirectional transport from sources to sinks. The net translocation between two parts of the mycelium cannot be quantified by adding a tracer isotope at the supposed source only, as the transport in the opposite direction must also be assessed. Even when tracer isotopes are added at both the source and the sink, conclusions about the net translocation are difficult to draw, as the tracer concentration in relation to the concentration of the studied analogue (the specific activity), also has to be known at both sites. Tracer isotopes may thus not be very useful as tools in quantitative translocation studies. The more straightforward method of destructive serial harvests followed by determination of total pool sizes is likely to be a better alternative.

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437 - In vivo imaging of nutrient dynamics in woodland fungi

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Nitrogen translocation by fungi, although a key ecosystem process, is poorly understood because current tools to study it have limited spatial and temporal resolution. In a novel technique to image transport of an amino-acid analogue in realistic microcosms, *P. velutina* was grown on a scintillation screen, and transport of ^{14}C-AIB was quantitatively imaged with a photon-counting camera. Fourier analysis of transport showed a rapidly propagated pulsatile component, with period ranging from 18.3h at 19 °C to 11.6h at 25.5 °C. Velocity of transport (23 mm h^{-1}) was significantly faster than diffusion. Pulses in the inoculum and the mycelium were asymmetric and complementary, with the mycelium photon signal mirroring that from the inoculum. This can be mimicked by a model that includes exchange of amino acids in outgrowing mycelium with a storage pool in the inoculum mycelium. The vacuoles may both store amino acids and act as a transport pathway. Confocal microscopy showed a dynamic vacuolar network in which bulk movement of vacuoles and transient contacts between them *via* tubules were seen. FRAP and FLIP techniques showed an exchange of content between the vacuoles. By combining amino acid tracking in mycelium with confocal imaging, observed global nitrogen movements in the mycelial system can be explained in terms of cellular and metabolic events.

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438 - The septal pore cap as a key structure in cell to cell transport

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The function of the septal pore cap (SPC) in basidiomycetes remains largely unknown. The connection of the SPC to the endoplasmic reticulum, and its position close to the orifice of the dolipore septum, may suggest several functions of the SPC. These may include a role in the transport of subcellular structures from one cell to another; a sieve function; and a barrier function during cell ageing, stress or cell lysis. While the application of tomography is a prerequisite for the fine analysis of the SPC, its use in combination with the conventional methods may complete the overall view. Interpreting the data obtained after the different preparation methods for electron microscopy may lead to a better understanding of the SPC structure. We suggest that the SPC in *Rhizoctonia solani* plays a key role in intercellular transport of for example mitochondria and ribosomes in the hyphal filaments. Moreover, the SPC may be involved in plugging the orifice of the septal pore channel. This plug-formation is not yet understood, but likely a signaling pathway exists to coordinate the assembly of plug-material at the orifice. Possibly, this pathway starts with an initial cytoplasmic signal that indicates that the orifice needs to be plugged, then the concerted action of the endoplasmic reticulum, the SPC and the filamentous network would finally result in the formation of a plug. Once plugged, no intercellular transport is possible. Most likely this plugging process is reversible.

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439 - *Armillaria* air pores and rhizomorphs really do conduct oxygen

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Armillaria luteobubalina Watling & Kile produced 'air pores' at the origin of rhizomorphs in cultures on agar. Air pores grew upwards into the air from the colony surface, attaining a height of about 7 mm. When mature they were pigmented like the surface of the colony. Rhizomorphs originated below the air pores, grew down into the agar and then turned horizontally beneath the agar surface, eventually growing submerged in the agar to near the edge of the Petri dish. Air pores consisted of an aggregation of hyphae intertwined to form a cylinder. The pigmented layer at the surface of the colony itself extended into the base of the air pores, where it was elevated into a mound inside the base of the air-pore. Beneath the whole of the pigmented layer of the colony there was a region of loose hyphae with extensive gas space between them. This gas space extended into the base of the air pores and was continuous with the central gas canal of the rhizomorphs. This gas space was also continuous with the internal spaces of the air pore (and atmosphere) through gaps in the pigmented layer in its basal region. Conductance to oxygen of agar blocks with air pores was measured with oxygen electrodes: a substantial oxygen conductance averaging about 7 x 10-10 m3 s-1 was observed. We conclude that the air pores conduct oxygen into the gas space below the pigmented mycelium of the colony, where the rhizomorphs - which can also conduct oxygen - originate.

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440 - Control of motility in tubular vacuole systems in fungi

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Tubular vacuole systems occur in mycorrhizal, saprotrophic and pathogenic fungi. In an Australian *Pisolithus* they consist of two distinct components: less mobile large compartments linked to the plasma membrane variously interconnected with motile tubular elements. These systems are implicated in endosomal processing, intra- and inter-cellular transport, storage, and enhanced interaction with the cytoplasm. In mycorrhizal associations they may facilitate transport of elements between nutrient absorbing hyphal tips and the interface with the plant partner. Recent evidence for and against this hypothesis will be evaluated. Tubule formation is a widespread phenomenon in organelle systems of eukaryote cells and offers an alternative to transport via vesicles. Vacuolar tubule formation is a regulated process that is modulated by both external and internal conditions and in *Pisolithus* is dependent on microtubules but apparently not on filamentous actin. Results with other inhibitors support a role for GTP-binding proteins in regulation of vacuolar tubule formation and the data are consistent with involvement of a dynamin-like GTPase.

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441 - The use of genomic repeated sequences to characterize arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal fungi (Glomales) form a root symbiosis with more than 80% of land plant families, improving plant mineral uptake and general plant health. The different arbuscular mycorrhizal fungal species which have been described do not generally show host-plant specificity, suggesting a great plasticity of the fungal genome. To date, knowledge concerning genome organization and structure in these fungi is poor, so limiting the understanding of their biology at a molecular level. It is however known that arbuscular mycorrhizal fungi have a large genome size, and evidence points to the presence of a high proportion of repeated sequences. Phylogeny analyses and identification of arbuscular mycorrhizal fungi has been based on ribosomal or non-ribosomal repeated sequences, and the former have been used to show genome variability between nuclei of a same fungal isolate. In order to improve knowledge about genome organization in arbuscular mycorrhizal fungi, different types of repeated sequences have been characterized and their distribution in the fungal genome analyzed. Sequences containing elements found in transposons have been isolated, and their role in the evolution of the genome of these fungi will be discussed. Part of this work is supported by the European Project GENOMYCA (QLK5-CT-2000-01319; http://www.dijon.inra.fr/bbceipm/genomyca/).

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442 - Vegetative incompatibility and genetic diversity among geographically different isolates of *Glomus mosseae*

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Population studies based on vegetative compatibility tests revealed the occurrence of genetically different isolates and of vegetative compatibility groups within the same fungal species in pathogenic, saprophytic and ectomycorrhizal fungi. Successful anastomoses occur widely between hyphae belonging to different individually germinated spores of the same isolate of arbuscular mycorrhizal (AM) fungi, though nothing is known about mycelial compatibility between conspecific isolates. We studied mycelial compatibility between geographically different isolates of the worldwide distributed AM fungal species *Glomus mosseae*. Vegetative compatibility tests performed on germlings belonging to the same isolate showed that all isolates were capable of self-anastomosing, whereas no anastomoses were detected in pairings between germlings of different isolates. Microscopic examinations detected hyphal incompatibility responses, consisting in apical wall thickening, followed by protoplasm withdrawal and septa formation, in hyphal swellings produced by the approaching hypha on the surface of the contacted one. The intraspecific genetic diversity of *G. mosseae* revealed by vegetative compatibility tests was confirmed by total protein profiles and ITS-RFLP profiles. Since AM fungi lack a tractable genetic system, vegetative compatibility tests may represent a powerful tool for the detection of genetically different mycelia and for investigating population structure of these obligate symbionts.

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443 - Investigating the propagation pathway of endobacteria in *Gigaspora margarita*

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Intracellular bacteria live in the cytoplasm of many isolates of arbuscular mycorrhizal fungi belonging to Gigasporaceae. On the basis of 16S rDNA analysis these endobacteria were assigned to a new taxon closely related to the genera *Ralstonia*, *Pandorea* and *Burkholderia*. To understand their propagation pathways through the life cycle of *Gigaspora margarita* (BEG 34 isolate), in vitro mycorrhizal cultures of transformed roots were produced. The presence of bacteria in spores from filial generations have been monitored by using confocal microscopy observations together with molecular analysis. We demonstrate for the first time the vertical transmission of endobacteria from a single 'parental' spore (F0) to filial spores through germinating and symbiotic mycelia. A quantification of the endobacteria population was carried out using 3D volume reconstruction. To verify whether the presence of the whole plant could determine differences in the transmission of bacteria, a F1 generation of spores was produced on clover pots. In contrast with the in vitro conditions, all the F1 spores from monosporal inocula were free of bacteria, suggesting that bacterial migration is controlled by multiple factors. Endobacterial activity was investigated by using Acridine Orange. The bacterial distribution pattern and activity, closely related to the fungal life cycle, reinforces the hypothesis of a strong symbiotic association between the two organisms. Research supported by the EU Project GENOMYCA.

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444 - Molecular bases of appressorium formation in AM fungi

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The appressorium development is the first morphogenetic change which precedes the formation of the symbiotic association between arbuscular mycorrhizal (AM) fungi and their host roots. This event takes place after recognition of yet unknown plant signals which trigger the developmental decision of abandoning the so-called asymbiotic life stage. Upon recognition of those signals the fungus changes its straight pattern of hyphal tip growth to form a swollen structure that hooks over a rhizodermis cell and later develops the penetration peg among two adjacent epidermal cells. The physical contact with the host root is essential for the appressorium development. To investigate the molecular bases for this morphogenesis we took a comparative molecular approach and studied the changes on gene expression that occurred to the fungus upon induction with a host root. We induced appressorium development on water agar medium by bringing into contact parsley seedlings with germinated spores or sporocarps of AM fungi. A time course of appressorium development showed that first induction takes place around 120 h, while at 168 h a plateau in the number of appressoria is achieved. We investigated both, early gene expression and later gene expression in order to selectively search for genes involved in signaling and recognition or for genes responsible for structural changes. Results concerning our progress in this topic will be presented.

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445 - Extensive tubular vacuoles in arbuscular mycorrhizal fungi

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Hyphae of *Gigaspora margarita* were stained with Oregon Green 488 carboxy-DFFDA that is known to accumulate in the lumen of fungal vacuoles, and were observed by laser scanning confocal microscopy. Germ tubes had vacuoles with one of the following types: A, longitudinally oriented bundles of long tubules; B, both tubular and various sizes of spherical vacuoles in various proportions; C, a mass of spherical vacuoles. Stained germ tubes with cytoplasmic streaming always showed A or B types of vacuoles, but never C type. Tubular vacuoles were extremely fragile when exposed to laser irradiation; many small spheres were formed. Bundles of tubular vacuoles were also observed in extraradical hyphae and intercellular hyphae of *G. margarita* from co-cultures with onion seedlings. Tubular vacuoles were observed also in the germ tubes of *G. rosea*, *Glomus leptotichum*, *Gl. intraradices*, *Scutellospora cerradensis* and in hyphae of other members of Zygomycota, *Rhizopus stolonier*, *Absidia repens*, *Mucor meguroence*, *Choanephora infundibulifera*, *Mortierella chlamydospora*, *Syncephalastrum racemosum*, *Linderia bicolumnata*. These results suggest that tubular vacuoles are universal in Zygomycota. Ultrastructure of rapid freeze-freeze substituted germ tubes of *G. margarita* showed longitudinally oriented profiles of vacuoles occupying most of the cell volume.

446 - Biolistic transformation of AM fungi: Advances and perspectives

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Arbuscular mycorrhizal (AM) fungi of the unique phylum Glomeromycetes are ubiquitous in nature and constitute an integral component of terrestrial ecosystems, forming symbiotic associations with plant root systems of over 80% of all terrestrial plant species. Understanding the root interactions of AM fungi is one of the most challenging and exciting areas of research. The development of transformation strategies for AM fungi has provided a great opportunity for the enhancement of our understanding of the biology of AM fungi but also for the interactions between plant roots and the surrounding soil environment. In this presentation we discuss the development of transformation strategies for AM fungi by biolistics and highlight the areas of this technology which are being further developed for the stable transformation of these elusive organisms. These include the use of genetic reporters, vector construction and transgene persistence.

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451 - Study the effect of different techniques on diversity of freshwater hyphomycetes in the river Nile (Upper Egypt)

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Four different techniques were applied, leaf mapping of *Eucalptus rostrata*; randomly leaf sampling; Millipore filtration; spores in foam, for aquatic hyphomycets communities study in the river Nile. *Triscelophorus monosporus*, *Anguillospora longissima*, *Flagellospora penicilloides* and *Tetracladium marchalianum* were the dominant species in the all techniques used. Aquatic hyphomycetes diversity was high by using leaf mapping (23 species) and randomly leaf sampling (31 species) but it was low by using Millipore filtration (11 species) and spores in foam (8 species) techniques.

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452 - Do marine fungi in Egypt form a distinct group ?

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Diversity of subtropical marine fungi is comparable to that recorded from temperate and tropical ones. Clear line can be drawn between marine mycota in temperate and tropics, however that is not the case when we compare marine mycota of tropical and subtropical sea shores. Mangroves in tropical and subtropical locations harbor similar marine taxa, though specific groups of fungi are found more frequently in tropical (e.g. *Aigialus* spp., *Lophiostoma* spp., *Pyrenographa xylographoides*, *Acrocordiopsis patlii* and *Quintaria lignatilis*) or subtropical (e.g. *Lignincola laevis*, *Lulworthia grandispora* and *Periconia prolifica*) mangroves. Mangroves in tropics have a higher fungal diversity than that in subtropics but several factors may account for this. Marine mycota diversity in subtropical habitats varies from one location to another. Mangroves and sea shores in Egypt are harsh environments (i.e. high salinity and temperature accompanied with low humidity) surrounded by desert. Frequent species recorded in samples collected from the Red Sea coast were different from other subtropical habitats (e.g. *Swampomyces* spp. and *Kirschsteiniothelia maritima*), while frequently recorded species from other subtropical locations were completely absent (e.g. *Lignincola laevis*, *Halorosellinia oceanica* and *Massarina* spp.). Mycota of *Avicennia marina* and *Rhizophora* sp. in Egypt will be compared with a discussion for several ecological parameters.

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453 - Impact of harvest of tree tops and branches of Norway spruce on the biodiversity of fungi

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There has been an increased use of logging waste, tree tops and branches, in Swedish forestry for biofuel purposes. The aims of this PhD-project are 1) to identify and describe the fungal communities associated with fine woody debris (FWD), 2) to analyse to what degree fungal communities overlap in composition of species between FWD, coarse wood debris (CWD) and the litter layer, 3) to analyse impacts of removal of logging waste on the diversity and community structure of these fungi and 4) from a fungal perspective, model the carbon availability in a spruce forest generation by considering a) the amount and quality influx of litter from CWD, FWD, cones, needles and the field layer and b) the decomposition rate of these fractions. I will present results from the first study, where we have identified and compared the fungal species composition in branches, tree tops and stumps, seven years after thinning. The identification was conducted by analyses of ITS sequences both directly from wood samples and from pure cultures. As a comparison, sporocarps were monitored on the same samples.

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454 - *Septoria* diversity, biogeography and life history

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The fungal genus *Septoria* Sacc. contains about 1000 names and is a paraphyletic group considered as coelomycetous anamorphs of the Dothideales genus *Mycosphaerella* Johanson. Sexual holomorphic species with *Septoria* anamorph phenotypes in the life cycle number about 14%. Most *Septoria* species are strictly asexual and very probably had independent evolution. Cryptic sexuality for some anamorphic species (*S. clematidis-rectae*, *S. convolvuli*, *S. cruciatae*, etc.) may be supposed because spermatial synanamorphs are formed in pure culture. A morphological continuum among *Septoria* anamorphs with related holomorphs and anaholomorphs confirms the aggregative character of the group. Observed homology of conidiogenous cells, conidia and conidiomata leads to reassessment of these fungi, as has already happened with the *Cercospora* complex of genera. *Septoria* species are with wide geographical distribution. Most common are fungi with a broad Eurasia or Europe and Middle Asia, Eurasia and North America geographical distribution. Sporadic dispersion within the limits of temperate-subhumid, temperate-semiarid, and submeridional-semiarid natural habitats is inherent to

anaholomorphs. Continuum dispersion is more typical for holomorphs with *Septoria* anamorphs spread over the Eurasian-American region, while constituent teleomorphs sometimes are geographically limited with disjunctions in arid areas.

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455 - New species of *Marasmius* (Basidiomycetes, Tricholomataceae) from tropical Africa - I

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Descriptions, drawings of microscopic features and photographs of 12 new taxa (11 species and 1 variety) of the genus *Marasmius* s. str. (Basidiomycetes, Tricholomataceae) of the sect. *Epiphylli*, *Fusicystides*, *Globulares*, *Hygrometrici* and *Neosessiles* from tropical Africa are presented. Six species (*M. albidocremeus*, *M. camerunensis*, *M. lacteoides*, *M. muramwyanensis*, *M. kigwenensis*, *M. tshopoensis*) belong to the sect. *Globulares*, two species and one variety (*M. minutoides*, *M. minutoides* var. *angustisporus*, *M. nyika*) to the sect. *Hygrometrici*, one species to the sect. *Epiphylli* (*M. foliiphilus*), sect. *Fusicystides* (*M. longicystidiatus*) and sect. *Neosessiles* (*M. bururiensis*). Species presented here represent a part of results of the monographic studies of the genus *Marasmius* in tropical Africa.

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456 - Macrofungal species proposed for a Tuscan red-List (Italy)

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Due to the close relationship between fungi and environment, conservation of fungal species often depends on environmental protection. This is particularly true for entities that grow exclusively in certain habitats; if their habitat is threatened, so are they. Areas of particular phytogeographical interest and/or limited size were listed for Tuscany under the 'Bioitaly' project which implements the Habitats Directive 92/43; many of these areas were surveyed from the mycological point of view in the Tuscan mapping project. The mycoflora of some of these areas was not systematically monitored until recently. The results for the relict mountain mires and autochthonous spruce forest in the National Natural Reserve of Campolino in the upper Sestaione Valley (pSIC IT5130001), the coastal dunes of Burano Lake (pSIC IT5190032) and Diaccia Botrona (pSIC IT5190011) are reported here. Information on environmental features is given for each of the areas listed, together with a list of epigeous macromycetes to be included in a future Tuscan Red-List. The IUCN category of each fungal species is indicated. The exsiccata of taxa proposed for the regional Red-List are preserved in the Herbarium Universitatis Senensis (SIENA).

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457 - The new project of mapping macrofungi in Tuscany (Italy)

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The Association of Tuscan Mycology Groups (AGMT) was formed in 1993. It currently coordinates 22 mycology groups, is active in scientific research and in the field of education. In collaboration with the Tuscan Agency for Development and Innovation in Agriculture and Forestry (ARSIA), the Tuscan Regional Administration, the botanical gardens and the universities, AGMT is beginning a new programme involving census, mapping and conservation of epigeous fungi in Tuscany. The first census (1995-98) led to publication of a check-list of about 1000 taxa. Besides adding to the check-list, the new convention will draw up a regional red-list based on IUCN criteria (2000). The first part of the programme involves bibliographic research to create a Tuscan mycological database. The data of the mapping projects presented by the Italian Botanical Society (SBI), the Confederatio Europeae Micologiae Mediterraneensis (CEMM) and the European Council for the Conservation of Fungi (ECCF) will then be extrapolated. The database will include data on vegetation and substrate, frequency, and chart coordinates for each taxon. The exsiccata will be kept in the Herbarium Universitatis Senensis (SIENA). Details of the project can be viewed on the ARSIA website.

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458 - *Fusarium* species newly recognized from Japan since 1990

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Taxonomy of *Fusarium* in Japan has long been based on the Snyder & Hansen's taxonomic system, especially among workers in the phytopathology. The taxonomic system was really epoch-making and recognized only nine species within the giant anamorphic genus based on the present sense of *Fusarium* mycology. Application of taxonomic systems contradictory to it, such as Wollenweber & Reinking's or Booth's, was also tried in Japan, but recognition or description of a new taxon was

scarce. Most of study efforts have, therefore, been concentrated to find infraspecific groupings such as formae speciales or phytopathogenic races. A substantial reinvestigation of *Fusarium* species has been conducted in the last decade, according to the species concept based on recent molecular-phylogenetic analyses and precise comparative morphological studies. Presence of more than 40 species of *Fusarium* in Japan has been until now recognized. Among them, *F. fractiflexum*, *F. kyushuense* and *F. nisikadoi* were new species and *F. circinatum*, *F. concentricum*, *F. fujikuroi* (= *F. moniliforme* MP-C), *F. globosum*, *F. incarnatum* (= *F. semitectum* var. *majus*), *F. proliferatum* (= *F. moniliforme* MP-D), *F. phyllophylum*, *F. verticillioides* (= *F. moniliforme* s. str. = MP-A) and so on were new records from Japan, including renaming.

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459 - Physical and chemical effects of fungi and lichens in rock weathering

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The epilithic lichen thallus frequently contain microdivided minerals and biominerals, meanwhile mycobiont hyphae invade the rock surface. Within the rocks the protolichens and hyphae constitutes an important part of the endolithic biomass. The studies of organo-mineral phase constituted by minerals and hyphae, algae, cyanobacteria and bacteria should be perform by microscopy techniques that do not disturb the biofilm structure. These techniques should allow investigation of the internal ultrastructure of the biotic components, leading to their identification. Such techniques should also permit the observation of mineral physical changes induced by microorganisms. The equipment which allows the carrying out of this work is the SEM operating in Backscattered mode (SEM-BSE). In situ SEM-BSE (EDS) observation of the organo-mineral phase allows detection of the biomobilisation processes and minerals biotransformation. In Antarctic extreme environment, biologically transformed minerals have been observed, and as a result, diagenetic iron hydroxide nanocrystals and clays have been found around chasmoendolithic hyphae in Beacon sandstone. Also in granitic rocks the biominerals, such as calcium oxalate and silica deposit have been found close to the fungal cells. In the biomobilisation processes as well as in the biomineral formation, the fungi are involved leaving a distinct traces of their microbial activity.

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460 - Mycorrhizal fungi of seven *Paphiopedilum* species in Thailand

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Healthy roots from mature plants of seven *Paphiopedilum* species, namely *Paphiopedilum concolor*, *P. concolor* var. *striatum*, *P. exul*, *P. godefroyae*, *P. niveum*, *P. parashii* and *P. villosum*, were collected from various locations in Thailand. Mycorrhizal fungi were isolated using a modification of the Masuhara and Katsuya method. Root pieces were washed with tap water and surface sterilized for 5 min in 5% NaOHCl and then rinsed twice in sterile distilled water. The root segments were then cut into longitudinal sections and the epidermal layer was removed. Cortical cells containing pelotons were macerated and placed on 1/6 NDY medium and incubated for 3-7 days. After the incubation period, hyphal tips were transferred to PDA slants and kept as pure cultures. Identification was based on morphological characters as observed under a light microscope. Nuclei were stained with safranin O using the Bandoni method. Three genera and six species of mycorrhizal fungi were found as follow: *Rhizoctonia repens* (*P. concolor* and *P.exul*), *R. ramicola* (*P. exul*), *Ceratorhiza goodyerae-repentis* (*P. godefroyae*), *Epulorhiza calendulina* (*P. concolor* var. *striatum* and *P. exul*), *Rhizoctonia* sp.1 (*P. parashii*) and *Rhizoctonia* sp.2. Nuclear staining revealed that all strains were binucleate except *Rhizoctonia* sp.2 (*P. niveum*) which was multinucleate. Pure cultures were maintained on slant PDA, tissue paper, soil and liquid paraffin for further studies on molecular phylogeny and symbiotic germination.

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461 - Canadian Collection of Fungal Cultures (CCFC/DAOM

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The culture collection maintains ˜ 11,000 plant pathogenic and wood rotting fungal cultures representing ˜ 2,900 species. The catalogue can be viewed on the World Wide WEB: http://sis.agr.gc.ca/brd/ccc. The CCFC is supported by the taxonomic expertise of one of the largest groups of systematic mycologist in North America, with internationally recognized expertise in most major taxonomic groups. Research is concentrated on solving agricultural problems. The collection originated as an amalgamation of individual working collections and now serves as a primary Canadian repository for research strains. It functions as a gene bank for this microbial genetic resource and provides pure cultures to researchers in agriculture, forestry, medicine, private industry and biotechnology. Authenticates strains have been provided for research programs involved in: crop resistance to fungal diseases, biological control, diagnostic probe development and identification, screening for new metabolites and

enzyme pathways, post harvest diseases, and mushroom spawn. The methods of long term preservation of cultures by cryostorage (vapour phase) and lyophilization are aimed at longevity and the stabilization of genetic properties. An extensive oil collection is also maintained. Isolates are available to researchers at a fee. The CCFC is a member of the WFCC and abides by all international regulations concerning the distribution and transport of biological organisms.

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462 - Identification of genetic diversity in the ascochyta blight pathogen of chickpea [*Ascochyta rabiei* (Pass.) Lab.] by used RAPD markers

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The poor information about genetic diversity in the ascochyta blight fungus is one of the basic difficulties for breeding resistance cultivars of chickpea to blight disease in Iran. In this study we have employed RAPD marker for distinguish genetic variation in DNA of this fungus isolates. We have used 12 random primers for comparison of 26 selected isolated isolates from 16 Iranian provinces. Ten primers have showed polymorphism among isolates. The OPK-01 primer defined the highest number of polymorphism (11) and the OPK-09 primer defined only 2 polymorphism bands. On the base of all primers, isolates classified into 22 genotypes. The genetic diversity index was estimated - and revealed high variation in Iranian population of this pathogen. The pair-wise genetic distance has estimated for all isolates. This experiment demonstrated that RAPD markers have high ability for genetic analysis of this pathogen population. The comparison of the results of RAPD marker with virulence test showed the very low correlation coefficient (0.03) between the virulence and genetic matrixes.

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463 - Survey of *Phytophthora* species in oak forests in Austria and Turkey

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Oak trees are occupying a wide range of environments and are of enormous ecological and economical importance. Since the early 20th century oak ecosystems have been suffering from decline and mortality. Species of *Phytophthora* cause serious disease problems in natural oak stands and plantations. During the course of a survey in declining and healthy oak stands in Austria and Turkey between 1999-2001 ten *Phytophthora* species were isolated from soil samples using the oak leaflet baiting method. In total, 86 sites (35 in Austria and 51 in Turkey) were investigated. *Phytophthora* spp. were recovered from 17 and 38 stand in Austria and Turkey, respectively. The assemblage of species included *P. quercina*, *P. citricola*, *P. cinnamomi* (A2), *P. gonapodyides*, *P. syringae*, *P. europaea*, *P. cryptogea*, *Phytophthora* sp.1 and *Phytophthora* sp.2. In addition, *P. cambivora* (A2) was isolated from beech (*Fagus sylvatica*) trees admixed in oak forests in Turkey. The associated oak species were *Quercus robur*, *Q. petraea*, *Q. cerris*, *Q. pubescens*, *Q. frainetto*, *Q. hartwissiana* and *Q. vulcanica*. Among the recovered species *P. quercina* showed the widest distribution and *P. citricola* was also common. Isolates resembling *P. citricola* could be separated into four distinct groups due to morphological, physiological and cultural characteristics. The results of this study suggest that *P. quercina*, *P. cinnamomi* and *P. cambivora* are a potential threat to oak ecosystems in Austria and Turkey.

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464 - A new species of *Ceratocystis* discovered on *Eucalyptus nitens* in Australia

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Ceratocystis fimbriata and *C. eucalypti* are the only two species of *Ceratocystis* reported to occur on *Eucalyptus*. *C. fimbriata* is recognized as a serious pathogen, especially in Uganda, Congo and Brazil, where it causes rapid wilting and death of these trees. *C. eucalypti* is considered native to Australia and is not pathogenic. It does, however, colonize fresh wounds on *Eucalyptus*. This study was conducted to determine whether *Ceratocystis* spp., including *C. eucalypti*, would infect artificially induced stem wounds on *Eucalyptus nitens* (Canberra) and *Eucalyptus globulus* (Cann River), Australia. Ten trees were wounded in October 2000 at each site and wounds were examined a month later. Perithecia, characteristic of *Ceratocystis* spp., were found covering the wounds and also in isolations using carrots as bait. Pure cultures were obtained by transferring ascospores masses from perithecial necks onto 2% MEA plates. The *Ceratocystis* sp. collected from *Eucalyptus nitens* has hat-shaped ascospores, different to those of *C. eucalypti* but similar to those of *C. fimbriata*. It differs, however, from *C. fimbriata* and all other *Ceratocystis* spp. in its unique pear-shaped perithecial bases. DNA sequence data from the ITS and 5.8S rRNA operon confirmed that the fungus from *Eucalyptus nitens* in Australia is unique, with *C. fimbriata* and *C. albofundus* as its closest relatives. The *Ceratocystis* sp. discovered in this study is in the process of being described as a new taxon.

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465 - Mycorrhizal fungal richness in ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) plantations in Patagonia (Argentina)

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In Argentina, there are about 2,000,000 ha of grasslands suitable for afforestation with fast growing non-native conifers along the piedmont of the Patagonian Andes, and ponderosa pine is the most widely planted species. Many of this planting sites are under water stress conditions. The aim of this study was to survey mycorrhizal species richness and their distribution along the precipitation gradient in ponderosa pine plantations in Patagonia. Eleven plantations, four under a precipitation regime of 1000 mm (humid plantations) and six under 400-600 mm (dry plantations), were surveyed for two springs and two autumns for hypogeous and epigeous fungi. *Amphinema byssoides* was the most widely distributed species, found in all plantations. The other species differed in their presence according to the humidity gradient. Humid plantations presented 9 different taxa, each plantation displaying a species richness between 4 to 6. *Hebeloma mesophaeum* was present in all of them, followed by *Suillus luteus* and *Rhizopogon rubescens*. Dry plantations presented 5 different taxa, and each plantations displayed a species richness between 1 to 4. *S. luteus*, *H. mesophaeum* and *R. rubescens* were the most widely distributed. Data showed that species richness was strongly dependent on precipitation. The principal species, though, were the same, but their relative occurrence varied in different conditions.

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466 - Macromycetes diversity of Russia and its conservation *ex situ* in the collection LE (BIN)

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Modern social and economic conditions in Russia are characterized by numerous negative factors and processes which influence on biodiversity. Loss and destruction of natural locations are the main danger for mycobiota diversity. For long-term purposes of macromycetes conservation *ex situ* specialized culture collections serve. During the past ten years the main trend of development of the Komarov Botanical Institute Basidiomycetes Culture Collection - LE(BIN) has been considering as conservation and maintaining of macromycetes cultures with emphasis on preservation of macromycetes from reserved territories. About 250 new macromycetes cultures were isolated during field works in Russian reserves such as 'Nizhne-Svirsky' (Leningrad region), 'Les na Vorskle' (Belgorod region), Island Valaam (Karelia) and others. LE(BIN) species representation was increased by new cultures from genera *Clavicorona*, *Collybia*, *Marasmiellus*, *Marasmius*, *Mycena* and *Lentinellus*. Strain diversity of *Collybia*, *Marasmiellus*, *Marasmius* and *Mycena* was also increased essentially. Considerable representation of biological species of the genus *Lentinellus* is maintained in the Collection. The genus *Clavicorona* is presented by six strains of the typical species *Clavicorona pyxidata*. Cultures of ectomycorrhizal fungi, rare species and ones that were recommended for conservation in Russia are also preserved in the Collection LE(BIN).

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467 - Ecological, management, policy and community research in British Columbia relevant to the commercial harvest of wild forest mushrooms

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Tricholoma magnivelare (Pine Mushroom) and *Cantharellus formosus* (Chanterelle) are commercially important wild forest mushrooms in British Columbia. Recently, research to integrate these mycological resources into forest planning has been carried out. Productive Pine Mushroom habitat throughout BC was characterized and the common factor found was dry and nutrient poor soil. It was then determined in north western and south central BC that it is possible to protect and manage Pine Mushroom habitat without an unacceptable impact on timber harvesting because of the relatively small area of highly productive Pine Mushroom habitat and low tree productivity of this habitat. On Haida Gwaii, a study determined that Chanterelle productivity on sites burned prior to regeneration was greater than on similar sites with no fire history. In British Columbia over 95% of the forestland is publicly owned which creates challenging policy and regulatory issues. A study of social and economic potential of non-timber forest products (NTFPs) on Haida Gwaii discussed many of the issues resulting from the annual influx of mushroom pickers. A subsequent study assessed how property rights could be used to overcome concerns and enhance stewardship. Integrating many of these findings and concerns, the Northern Vancouver Island NTFP Demonstration Project is researching how best to manage non-timber forest resources in a way that is environmentally sustainable, economically viable and socially equitable.

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468 - The BIOTA Southern Africa project: concepts and first results

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The BIOTA (Biodiversity Monitoring Transect Analysis in Africa) Southern Africa project is a long term, multidisciplinary project that aims at monitoring biodiversity along the rainfall gradient from the Cape of Good Hope in South Africa to the Angolan border in Namibia. The Cape of Good Hope receives 500 mm rain/year. The area at the Namibian/South African border is extremely arid with 50 mm rain/year, a transition between winter and summer rainfall occurs here. Annual rainfall in summer increases towards northern Namibia, which receives 450 mm/year. In addition to rainfall regimes, different land management systems are compared. This poster presents first results from a study of Namibian fungi, focussing on soil fungi, arbuscular mycorrhizae, and rust fungi. Soil fungi were isolated by soil washing; arbuscular mycorrhizae were studied with morphological and molecular tools. This revealed differences in species composition along the Namibian part of the transect. Rust fungi are monitored to assess their biodiversity under different climatic and management conditions. In Namibia rust fungi are collected countrywide to compile a national checklist.

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469 - Some Aphyllophorales in mediterranean ecosystems

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The knowledge on distribution of Aphyllophorales in Mediterranean area has a pre-eminent position both from an ecological and taxonomic point of view. This interest is essentially due to the peculiarity of the Mediterranean vegetation patterns and, consequently, of the wood-inhabiting fungi. The drop in rainfall during the last years, with the consequent increase in aridity, determined considerable changes in the vegetation with a short-lived fruiting period as first effect. In this context, we report the results of researches concerning the Mediterranean Aphyllophorales that, in spite of the studies carried out during the last years are till now poorly known. Between the collected species are worthwhile: *Neolentiporus squamosellus* (Bernicchia & Ryvarden) Bernicchia & Ryvarden, growing on burnt *Juniperus oxycedrus*, in Supramonte di Orgosolo (NU) Sardinia. It is the only species of *Neolentiporus* present in the northern hemisphere while *N. maculatissimus* Rajchenb. is growing in the southern hemisphere. *Echinodontium ryvardenii* Bernicchia & Piga is the first European collection of *Echinodontium* while all previous collections are North American or Asiatic. It must looked upon as a relict of a previously larger distribution. *Piloporia sajanensis* (Parmasto) Niemelä is a rare species with a boreal distribution, collected in Sardinia on *J. oxycedrus*. It is suggested that the isolated locality could be an Ice Age relict from a time when Italy was covered with coniferous forests.

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470 - Fungal biodiversity in a regeneration series in Colombia Amazonia

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Fungal biodiversity has been investigated in a series of secondary forests and two types of primary lowland forest in Colombian Amazonia. Functional inferences suggest a role for ectomycorrhizal fungi and litter decomposing fungi in the nutrient cycling of these forests. Decomposition of leaf litter can be relatively fast, but seems to be influenced by the environmental conditions. Shortly after trees are cut down, many fruitbodies of wood-inhabiting fungi occurred. This raises the question whether these fungi may have occurred as endophytes in the healthy trees. The formation of fruit bodies by these fungi may be a result of the lack of stress imposed by the death of the hosts, and the availability of a suitable substrate. After the felled trees are burnt, soil-borne microfungal populations are dominated by heat resistant fungi. Many new taxa of macro- and microfungi have been found, and await formal description. Analysis of secondary metabolites in soil borne Penicillia revealed some clues to explain the observed uptake of iron during decomposition of leaf litter. Initial observations suggest the presence of c. 15 undescribed species of *Penicillium*. A novel antagonistic interaction between bacteria and certain soil borne basidiomyceteous yeasts has been observed. Associated research has been initiated to establish potential use of fungi by the indigenous populations.

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471 - Yeasts of the world, an interactive CD-ROM

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This CD-ROM presents a complete taxonomic data set of all currently accepted yeast species, including morphological and physiological data, and ribosomal DNA sequences. The interactive software contains modules for the comparison and integrated use of physiological, sequence and morphological information, facilitating the identification of yeasts using complementary data sets. Many species are illustrated by microscopic and macroscopic images. This product will be useful in a wide range of yeast studies throughout the agro-industrial and medical sciences.

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472 - Biodiversity of discomycetes associated with coniferous trees

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Ligniphylous discomycetes are developing on the various woods. Ones of they have special ferments for destroying of a broad-leaves wood. Other has special ferments for destroying of conifers. They to some extent show a species specialization according to plants. Discomycetes are not aggressive wood-destroying fungi. But some species of discomycetes produce diseases, death or a depression of a developing of plant. These fungi settle on a bark of robust trees and provide a saprotrophic cycle of life. It is not get up 1,5 meter on a stem of tree. If a resistance power of tree slacken, fungus change self cycle of life to parasitic. Some species of Discomycetes develop fruit-bodies only on coniferous trees. At first they invade the bark of healthy trees and live as saprotrophic fungi. If a resistance of trees is weakened, these fungi change their way of life and become parasitic. When the tree dies, these fungi come back to the saprotrophic way of life. In accordance with literature data, 43 species of Discomycetes were reported on *Pinus* spp., 37 - on *Abies*, 18 - on *Picea*, and 12 - on *Larix*. In coniferous forests, Discomycetes develop on attached and fallen needles and branches in the litter. In the latter case their number is smaller in comparison with wood-destroying species.

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473 - The Biodiversity of Basidiomycota in Thailand

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The biodiversity of Thailand's fungi is poorly known. Until 1990 less than 500 spp. (of all fungi) had been reliably reported. Since 1990 increased effort has been made to survey this rich biodiversity. Thailand is entirely tropical offering a range of habitats from rain forest in the south to dry deciduous forest in the north. Elevations are from sea level to over 2500 m (Doi Inthanon). In the last ten years ca. 3100 specimens, from 40 orders, 96 families, 326 genera and 677 spp. have been added to the BIOTEC Herbarium. Most accessions were from 1992 to 2001 from various parts of Thailand. Within the herbarium there are significant deposits from many researchers having either short-term or long term associations with young Thai researchers. These activities continue to the present. A major effort is being made with the marasmioid and mycenoid fungi of Thailand but in the course of that work other agarics have also been collected. Notable records of these for Thailand include: *Cintractia amazonia*, *Fistulina hepatica*, *Crinipellis zonata*, *Amanita hemibapha*, *Strobilomyces velutipes*, *Dacryopinax spathularia*, *Clavulinopsis miniata*, *Irpex flavus*, *Steccherinum rawakense*, *Craterellus verrucosus*, *Anthracophyllum nigritum* and *Mutinus bambusinus*.

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474 - Marasmioid and mycenoid fungi of Thailand

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Marasmius (1600+ spp.) and *Mycena* (1200+ spp.) are the most diverse genera of saprobic basidiomycetes. The greatest species diversity is in the tropics, although hundreds of taxa are found in temperate regions of both southern and northern hemispheres. Nearly all species are saprotrobic, having important roles in litter decomposition, nutrient recycling/retention, pedogenesis etc. A few are parasitic on important food crops. Importantly, some form antibiotics such as strobilurin. Many species are host- or substrate-specific accounting for some of the high diversity seen in tropical forest. Over 460 samples were collected from various parts of Thailand. Of these, 93% were identified to species or morphotaxa while 7% remain unidentified. To date, 100 genera and 51 species are known. Samples were classified into three major groups of fungi; namely marasmioid, mycenoid and other macrofungi. Marasmioid fungi were 25% of the collection,

mycenoid 40% and other agarics 35%. Of the marasmioid fungi, 78% were the genus *Marasmius*. Two new species of marasmioid fungi have been named: *Incrustocalyptella orientalis* and *Clitopilus chalybescens*. New records of *Marasmius* include *M. conicopapilatas*, *M. papuraceus*, *M. purpureostriatus* and *M. micraster*. Of the mycenoid fungi, 57% were *Mycena*. Notable new species include: *M. dermatogloea*, *M. khonkhem*, *M. palmicola*, *M. pseudoseta* and *M. minicoseta*.

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475 - The diversity of Ingoldian fungi in Thailand

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While much work on Ingoldian fungi is from temperate regions knowledge of tropical species is poor. Surveys were made in the last 10 yr, especially for Khao Yai National Park. Foam samples were collected from 8 sites in Thailand. Rivers were ca. 5-10 m with overhanging forest. Foam samples were fixed in the field before spotting to microscope slides for examination. From all sites, 49 spp. were found. A sample from Khao Yai had only 2 spp. while one from Bala Hala had 25. This river (Khlong I-Gading) supported 28 spp. including 11 not reported elsewhere. By contrast, the similar Lumtakhlong (Khao Yai) had 11 spp. in total: none were exclusive to this river. In the north of Thailand Nam Nao National Park had 10 spp. recorded but half were exclusive to this site. *Triscelophorus* spp. were present in all streams. Other commonly recorded genera included *Lunulospora* spp. and *Anguillospora* spp. *Clavatospora tentacula* and *Brachiosphaera tropicalis* were also regularly found. Of the 49 spp. reported, 26 were known from a single site. Most species were present as single spores in foam samples. But species of *Anguillospora*, *Campylospora*, *Flabellospora* and *Triscelophorus* were represented by many spores. While these reports were mostly of mitosporic forms of Ascomycota a significant find was the discovery of *Erynia conica* and *Erynia rhizospora*. These insect pathogenic Zygomycetes are mostly known from temperate regions and poorly surveyed in the tropics.

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476 - Novel characters of New Zealand polypore fungi: septate basidiospores and spore print colour

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About 170 species of polypore fungi in 60 genera are currently recognised in New Zealand, with over 80% of accepted names differing from those used in the most recent monograph published in 1965. Half of the genera contain a single New Zealand species, and only one genus, *Australoporus*, is considered to be endemic to the region. Many collections of polypore fungi in the New Zealand Fungal Herbarium (PDD) bear misapplied Northern Hemisphere names. Among these collections we recently discriminated two new species that have septate basidiospores: *Polyporus septosporus* and *Dichomitus newhookii*. These species are characterised by occasionally uni- to multiseptate basidiospores, a feature newly recorded among Aphyllophorales, and apparently also unknown among agarics. Septation appears to be confined to older spores, especially those dispersed from the tubes. Spore print colour, considered taxonomically useful for agarics, also has application for polypore fungi and provides the only useful field character to differentiate two common New Zealand species of *Ganoderma*.

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477 - Endophytic and plant pathogenic fungi from terrestrial orchids in Thailand

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Healty leaves and roots of two terrestrial orchids, *Ludisia discolor* and *Spathoglottis plicata* collected from Lopburi and Chaing Mai provinces were used to isolated endophytic fungi. A modification of Okane's method was employed. The samples were washed with tap water and the leaves were cut into small pieces of 5x5 mm. Leaf pieces and the whole root were surfaced sterilized for 1 min in 70% EtOH and 2 min in 10% sodium hypochlorite and rinsed 2-3 times with sterile distilled water. Leaf and root segments were placed on sterile filter paper and transferred on 2% malt extract agar and incubated for 2-3 days. Endophytic fungi found on leaves included *Gliocladium penicilloides*, *Colletotrichum coccides*, *Nodulisporium gregarium*, *Pestalotiopsis guepinii*, *Pestalotiopsis* sp. and *Xylaria* spp., whereas 2 isolates of binucleate *Rhizoctonia* and 1 multinucleate species were found from the healthy roots. In a second investigation, disease leaves of three terrestrial orchids: *Spathoglottis affinis*, *Spathoglottis plicata* and *Phaius tankervilleae* showing spot symptoms, were collected from Bangkok, Lopburi and Trang Provinces. The tissue transplanting method with 10% clorox and potato dextrose agar was used. Plant pathogenic fungi found in this study were *Cladosporium cladosporioides*, *Colletotrichum gloeosporioides*, *Curvularia eragrostidis*, *C. lunata*, *Curvularia* sp., *Cylindrocladium* sp., *Drechslera hawaiiensis*, *Fusarium oxysporum*, *Pestalotiopsis guepinii*, *Phomopsis* sp. and *Phyllosticta* sp.

478 - A preliminary study of Polyporales and poroid Hymenochaetales from Equatorial Guinea

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Within the scope of the project 'Catalogación y estudio de la diversidad vegetal y fúngica del bosque virgen continental de Guinea Ecuatorial' the basic aim of this work is to elaborate a catalogue of Polyporales and poroid Hymenochaetales from the area. We start with the study of samplings collected from 1998 to 2000 at different places of the territory, in most cases at the 'Monte Alen National Park ' (ECOFAC). Up to the date, the material corresponding to the genus *Amauroderma*, *Favolus*, *Ganoderma*, *Lenzites*, *Loweporus*, *Microporus*, *Oxyporus*, *Polyporus*, *Phellinus*, *Pycnoporus*, *Rigidoporus*, *Schizopora* and *Trametes*, has been examinated.

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479 - Additions to the discomycetes of the Dominican Republic and the Caribbean Region

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The discomycetes of the Dominican Republic and the Caribbean region are poorly known, especially the inoperculate. In a previous presentation made by S. A. Cantrell she reported for the Dominican Republic 9 and 12 new records in the Pezizales and Helotiales, respectively. Some of the species were new records for the Greater Antilles and the Caribbean. In a recent collecting trip made in January 2002, 111 discomycetes were collected divided in 22 Pezizales, 81 Helotiales, and 8 Rhytismatales. Some new additions within the Pezizales are: *Helvella macropus*, *Plectania* cf. *platensis*, *Pachyella babingtonii*, a beautiful black-green *Peziza*, a burnt-orange *Kompsoscypha* and an orange-yellow *Pulvinula*. Within the Helotiales, some new additions are: *Dicephalospora rufo-carnea*, *Podophacidium xanthomelum*, *Rhizodiscina lignota*, *Hyaloscypha aureliella*, *Polydesmia* cf. *dumontii* and *Hyalorbilia inflatula*. So far, we know that there are 75 species of discomycetes in the Dominican Republic, divided in 28 Pezizales and 47 Helotiales. Within the Pezizales, 24 are new reports for the Domincan Republic, 10 for the Caribbean region and 5 might represent new species. For the Helotiales, all are new reports for the Dominican Republic, 10 for the Caribbean region and 15 might represent new species.

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480 - Mycogeographic affinities of Tabasco (México) polypores

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A quantitative mycogeographic analysis was made to asses the mycological affinities of the southern tropical Tabasco State (Mexico) based on the inventory of 61 wood polypore species (Cappello, 2001). The Multi-Variate Statistical package was used to perform UPGMA groupings. In the first part of the analysis Tabasco's polypores were compared with polypore listings from the mexican phytogeographic regions as defined by Rzedowski (1978) and in the second part they were compared with selected regions of the world such as Africa Asia, Cuba, Europe, Northamerica (divided in regions after Gilbertson & Ryvarden, 1986), Central and Southamerica. Interesting Tabasco appears somewhat separated when compared with the mexican regions and the remaining areas form two distinctive groups. Furthermore Tabasco forms a distinctive group with SE northamerica, Cuba and southamerica when compared with the chosen world areas which in part agrees with Guzmán (1973), Ryvarden (1998) and Welden & Lemke (1961) findings.

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481 - Epiphytic lichens on different fractions of Norway spruce *Picea abies*

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The use of logging residues as fuel in Sweden has increased during the last decades but still only a small part of the total wood fuel supply is today being harvested. Since there is a potential for a further increase in the harvest of logging residues efforts are now being made to investigate the effects this may have on the forest flora and fauna. My research is focused on fruticose, foliose and crustose lichens, both epiphytic and epixylic. As a first step it is of great interest to examine the structure of the lichen community on a whole-tree basis. Several lichen studies have been made on basal trunks and basal branches but tree tops and branches higher up in the canopy have been poorly investigated. The aim of this study is to describe the species composition of epiphytic lichens on different fractions of Norway spruce *Picea abies*, a common tree in northern Europe. The study area, Kilsbergen, is located in the boreonemoral zone of Sweden northwest of the city of Örebro (59°17'N, 15°13'E). In this area I selected ten forest stands dominated by Norway spruce and with similar stand characteristics. In each stand I then made a whole-tree inventory of epiphytic lichens on two felled individuals of Norway spruce.

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482 - Filamentous fungi isolated from soil of mangrove at the Itamaracá island, state Pernambuco, Brazil

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Filamentous fungi were isolated from mangrove soils collected at the Itamaracá Island, State of Pernambuco, Northeast Brazil, using the serial dilution technique. Samples (1ml) from the final dilution (1:1000) were tranferred to Petri dishes with Sabouraud plus chloramphenicol and maintained at room temperature (28°C ±1°C), with the growth of colonies followed for 72 hours. The following taxa were identified: *Aspergillus niger, A. sydowi, A. terreus, A. ustus, Cladosporium sphaerospermum, Eupenicillium brefeldianum, Eurotium chevalieri, Penicillium corylophilum, P. lividum, P. restrictum, Scopulariopsis sphaerospora, Spegazzinia tessarthra, Talaromyces bacillosporus, T. trachyspermus, T. flavus* and *Stilbella sebacea*. *Scopulariopsis sphaerospora* and *Talaromyces bacillosporus* are being referred for the first time to Brazil.

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483 - The mycoflora of hot spring soil of northern Taiwan

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An investigation on the mycoflora, particularly of thermophilic and thermotolerant fungi inhabiting on sulfurous hot spring soils of northern Taiwan resulted 12 taxa viz. *Aspergillus fumigatus* var. *fumigatus* (66.85%), *A. fumigatus* var. 1 (green colony) (7.86%), *A. fumigatus* var. 2 (brown colony) (4.81%), *A. niger* (1.14%), unidentified *Aspergillus* sp.(0.045%), *Papulospora thermophila* (2.72%), *Chrysosporium* sp. (0.18%), *Scytalidium thermophilum* (0.045%), *Sporotrichum* sp. (0.045%), Mycelia Sterilia sp.1 (white colony) (6.63%), M. Sterilia sp. 2 (yellow colony) (5.27%) and M. Sterilia sp.3 (gray colony) (4.405%). 2202 colonies were isolated from three sampling sites, i.e. site 1 (hot springhead), site 2 (2 m from site 1) and site 3 (4 m from site 1). Fungal colonies isolated as well as species richness in three sites were as follows: 32.92% in 9 taxa from site 1, 37.87% in 11 taxa from site 2 and 29.21% in 8 taxa from site 3, respectively. The dominant species was *A. fumigatus* var. *fumigatus* that was isolated year around from three sampling sites. *A. fumigatus* var. 1 appeared from Feb. to Jun. (2000). In contrast, *A. fumigatus* var. 2 was isolated only in Aug. and Oct.(1999). Within the sampling range of present hot spring niche, there is an evidence of presence of ecotypes in *A. fumigatus* complex. *Chrysosporium* and *Sporotrichum* were isolated only from the soils without hot water treatment but *Aspergillus* sp. and *S. thermophilum* were isolated only from the soils pre-treated with hot water for 30 min at 60°C.

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484 - The fluctuation of marine Mastigomycota in estuary tidewater of Tsengwen River

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The occurrence and seasonal fluctuation of marine zoosporic fungi were investigated at four stations in the estuary tidewater of Tsengwen River, southwestern Taiwan. Thirteen species of Thraustochytrids and seven species of Chytrids were isolated. The highest species abundance (S) recorded in summer was 15. The highest species similarity (CC) recorded between the spring and winter was 77.78. According to their frequency at individual stations, *T. aggregatum*, *T. proliferum*, *U. minuta* were ubiquitous, while the rest were common to scare. Chytrids was only found at tidewater of stations 1L and 1M in summer and autumn. The presence of Thraustochytrids and Chytrids revealed fluctuation when the saltility descend to zero.

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485 - Two new species of *Amanita* from Korea

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Two new species of *Amanita* were collected in central areas of Korea from 1997 to 2000. They were identified, described and illustrated. One of them, *Amanita longstipeta* has turtle-shaped warts of crust and its base is longer under the soil than is above the soil. The other *A. aureofarinosa* is covered with golden yellow farina and annulus is absent. Both are solitary in soil with sand.

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486 - Database of Korean mushrooms

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In Korea about 2000 species of mushrooms (higher fungi) have been studied from basdiomyctes to ascomycetes. Among them, about 1500 species are constructed in database. This database contains several items; taxonomical system, the application, the ecological resources, the geographical distributions. Each of mushrooms is illustrated with descriptions, photographies and spores. Also, information retrieval system is available by using KRISTAL II for query searches on the Web in URL http://ruby.kisti.re.kr/^mushroom.

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487 - Macrofungi distribution in a region of the Sierra Madre Oriental (México): an approximation of area of endemism methods

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Several protocols of areas of endemism methods (Morrone, 1994, Jardine, 1972, Linder 2001) were applied to a mapped list of 220 species of selected groups of macrofungi (Stipitate hydnoids, polypores, *Amanita*, *Laccaria*, *Psilocybe* and *Scleroderma*. Taxa were chosen both for practical (better known and collected groups) and ecological(to represent both saprotrophs and biotrophs) reasons. The best result, lesser fragmented areas, was obtained with Linder's method of inverse weighting. Though three well defined groups were observed they were not supported enough to be considered robust. Nevertheless when these groupings were contrasted with vegetation and altitudinal levels maps it was found an acceptable non exact concordance with the last one; one subgroup related with lower altitudes is accordingly distributed. Contrary to our findings based on the application of parsimony methods, previous ecological studies in Mexico had shown a relation of macrofungi distribution and vegetation (Guzmán and Guzmán-Dávalos 1979, Varela and Cifuentes 1979, Heredia 1989) but they were based solely on similarity list comparisons. Tan & Wu (1986) in China rather found that macrofungi distribution was highly correlated with temperature which agrees better with our results. In spite of limitations to the use of herbaria data to apply areas of endemism methods, our results support to certain extent their applicability.

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488 - The 609 red listed fungi in Sweden: an analysis of their ecological characteristics, distribution and threats

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Red lists are extremely important instrument in both national and international conservation work. The recent Swedish Red List is based on an evaluation of factors that affect risk of extinction, a system to classifying species into threat categories developed by the World Conservation Union (IUCN), which is successively replacing the old systems around the world. The Red List comprises 609 species, of which 254 are assessed as threatened, selected from an assessment of the 4620 known Swedish macromycetes (http://www.artdata.slu.se/home.htm). In principle, all available knowledge of each of these fungi have been compiled and analysed; life forms, preferences of habitats and substrates, distribution within and outside Sweden, and threats. The poster will summarize their ecological characteristics and identify the major threats. The red list can be looked upon as a deficiency analysis of habitats and substrates. Among the most severe problem is the large scale on going fragmentation of valuable biotopes like primeval forest with a high amount of dead wood of appropriate quality and unfertilised grassland. Today, an alarming number of fungal populations survive only in small number of isolated populations. Reference: Gärdenfors U (ed) 2000. Red List of Swedish Species. ArtDatabanken, SLU Uppsala.

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489 - Polypores on *Pinus koraiensis* in NE China

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Pinus koraiensis is the main coniferous tree in NE China. The species of polypores on wood of the tree were investigated, and 79 species were recognized. Among them 23 species are considered as rare species, 31 are occasional and 25 are common. 16 species were found only in forest reserves, 12 in unprotected forests, and 49 in both forest reserves and unprotected forests. 6 species were found on dead trees, 28 on fallen trunks, 41 on rotten wood and 4 on charred wood. 19 species occur on *Pinus koraiensis* only, and 60 on other substrates as well.

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490 - 'Guide des champignons comestibles du Bénin', a tool for valorisation, preservation and sustainable use of West-african savanna woodlands

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Wild edible mushrooms are highly appreciated by local people in Bénin (West Africa). The majority of edible species in Bénin, however, are not cultivatable because they are ectomycorrhizal or associated with termites. In Bénin, as well as in the neighbouring countries, many ecosystems suffer from the influence of human activities. This guide is a tool for valorisation, preservation and sustainable use of West-african savanna woodlands and helps the reader to recognise the place of the fungi, their identity and function within the natural world. Aspects concerning morphology, ecology and distribution of fungi in general, as well as aspects on poisoning, cultivation, recording local knowledge, ethnomycology and methods for assessing natural productions and valuation of wild edible fungi. The guide presents over 70 colour pictures and drawings, accompanied by morphological descriptions, local names and information on distributions and edibility of more than fifty wild species. Everyone interested in collecting or studying West-African fungi will find this book a useful reference. Those dealing with the sustainable use and management of natural resources in tropical Africa, will find a multitude of techniques and tools for a better management and conservation of natural ecosystems in a participative way, i.e. with the participation and for the benefit of local people. This guide is to be released by the end of June 2002.

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491 - Soil microfungi in a banana (*Musa paradisiaca*) plantation in Tabasco (México)

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Soil fungi have been extensively studied all over the world (Viaud et al., 2000; Stchigel, et al., 2000) but poorly and rather recently in Mexico (Mena et al., 1998; Bills et al., 2001). Since Mexico has been recognized as one of the megadiverse countries (Guzmán, 1998) it is a priority to undertake studies increasing our mycobiota knowledge. Furthermore micromycetes are increasenly important as a source of new bioactive metabolites and enzymes for biotechnology (Pointing & Hyde, 2001). Soil samples were taken in a banana plantation at the Teapa county, Tabasco State (17°32'N, 92°57'E) in a three months intervals during August 2001 to May 2002. To isolate fungi plate dilution and direct soil techniques on PDA/streptomycin and bengala rose added were used. So far a listing of 30 species and 15 genera of mostly mitosporic fungi has been obtained, which agrees in general with other banana plantation soil fungi listings Goos (1960, 1963). Abundance and frequency data are provided to discern phenological and successional patterns.

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492 - Flora of the macrofungi of South Cameroon

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The macrofungi of South Cameroon have been surveyed for the first time including detailed illustrations of the micromorphology. In the megatherm forests with high precipitation of South Cameroon the Aphyllophorales are the most abundand fungal group with 60% of all collected material followed by agaricoid fungi (32, 7%). Fungi from the Auriculariales make 3% of all collections. Some important species are presented including illustrations of their macro- and micromorphology.

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493 - Mycorama - an international center of mycology in Switzerland

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In 1998 the Swiss Association of the Mycorama (ASM) - representing 400 members and 50 mycological societies - started with the planification of this project, examining its feasibility and finding the necessary funding. The start of the construction of the mushroom-shaped building is planned for the end of this year, in the Jura region near Neuchatel in Western Switzerland. The Mycorama will be an international museum presenting the many aspects of mycology, between myth and magic, medicine and gastronomy, ecology and economy, addressing itself as much to the public as to the mycologist. It will include facilities for the cultivation of edible species, a demo laboratory and a restaurant with typical mushroom dishes.

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494 - Mycorrhizal plants of traditionally managed boreal grasslands in Norway

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This poster reports on the mycorrhizal status of 82 plant species growing in traditionally managed grasslands in three different locations in the boreal and boreo-nemoral vegetation zone in the eastern part of Norway. Seventy-four species were found to have arbuscular mycorrhiza (AM). To our knowledge, we report AM for the first time in *Achillea ptarmica*, *Ajuga pyramidalis*, *Alchemilla glaucescens*, *Carex brunnescens*, *Carex pallescens*, *Crepis praemorsa*, *Hieracium lactucella*, *Rumex longifolius*, *Scorzonera humilis*, *Trifolium aureum* and *Trifolium spadiceum*. The rare and threatened species *Arnica montana*, *S. humilis*, *C. praemorsa*, *Gentianella* *campestris*, *Parnassia palustris*, *T. aureum* and *T. spadiceum*, all confined to grasslands, were found to possess AM fungi.

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495 - Fungi from the highlands of Eastern Iceland, an area at risk because of the proposed Karahnjukar hydropower project

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This study of the macromycota of the highlands (450-650 m a.s.l.) in Eastern Iceland was a part of research aimed at assessing the environmental impact of the proposed Karahnjukar hydropower project. Fungi were collected at the proposed site of a 57 km^2, 24 km long reservoir Halslon, to be formed by building dams in the river Jökulsa a Bru and flooding its channel and surrounding area. Collection took place in middle of August 2000, at 24 transects for vegetation analyses. The fungi were photographed and dried in the field and later identified. A total of 35 species of macromycetes and four plant parasitic micromycetes were identified. Most were typical highland fungi, e.g. *Omphalina pyxidata*, *Lactarius pseudouvidus*, *Russula nana*, *Laccaria laccata*, *Hebeloma mesophaeum*, *H. kuehneri*, *Inocybe calamistrata*, *Cortinarius subtorvus*, *C. alpinus*, *Lactarius pubescens*, *Puccinia septentrionalis*, *Exobasidium vaccinii-uliginosi* and *Rhytisma salicinum*, found in four or more transects. Those found in one transect only were: *Anthracoidea elynae*, *Bovista nigrescens*, *Entoloma sericeum*, *Galerina clavata*, *Galerina pseudomycenopsis*, *Geopora arenicola*, *Helvella albella*, *Hygrocybe turunda*, *Inocybe geophylla*, *I. hirtella*, *I. lacera*, *Laccaria pumila*, *Lyophyllum decastes* and *Scutellinia patagonica*, some of which are more common at lower elevation or confined to specific habitats. The known mycota of the highlands of Eastern Iceland is compared to that of Central Iceland.

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496 - Rapid approach to evaluate persistence of field-introduced AMF by nested PCR

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In China 74% of agricultural soils (more than 7 million hectares) are phosphorus-deficient. Arbuscular mycorrhizal fungi (AMF), which form symbiotic associations with plant roots, are involved in the maintenance of sustainable soil fertility. Colonization of plant roots by such fungi improves the availability and uptake of soil phosphate resulting in increased plant growth. Within the framework of a European Project (1) focussing on the use of AMF technology to improve staple food crop production in small-scale sustainable agriculture in China, one of our objectives is the use of molecular tools to monitor persistence, within host roots, of selected AMF inoculated onto three target food crops: maize, sweet potato and cassava. We have studied different techniques of conservation of mycorrhizal root systems and genomic DNA (DNAg) extraction. A method of grinding in a Tris EDTA buffer with the addition of activated charcoal was retained for its simplicity, rapidity and efficiency. The large ribosomal subunit of DNAg was amplified by nested PCR using eukaryotic universal primers for the first PCR, and primers specific for selected AMF(2) inoculated onto target crops, for the second. (1) INCO-DEV: MYCHINTEC No ICA4-CT-2000-30014. (2) van Tuinen, D., Jacquot, E., Zhao, B., Gollotte, A., Gianinazzi-Pearson, V. (1998) Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. Molecular Ecology 7: 879-887.

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497 - Influence of resource size on wood-inhabiting fungal communities in a tropical forest

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The diversity of ascomycetes and basidiomycetes wood-inhabiting communities were examined for different resource sizes of the host tree *Prioria copaifera* in five low-diversity and high-diversity forests in central and eastern Panama. A positive correlation between size of the resource and basidiomycetes diversity was found, large dead branches and trees had a large number of basidiomycetes than small branches or small diameter trees. The same significant relationship was found for ascomycetes in three of these five sites. A relationship between stage of decay and number of fungal species was also tested, and more basidiomycetes species were found at early stages of decay in three sites. However, this relationship was weak and explained less than 10% of the deviance in species diversity. Stage of decay did not have any effect on the number of ascomycetes species. A field experiment was conducted to determine the effect of the size of the resource on fungal colonization. Resource size was manipulated using different sized wood blocks of *Prioria*, placed in two sites and censured every 3 months for 15 months. The size of the blocks did had a positive, significant effect on the fungal species number, and the most common species were able to fruit in both small and large wood blocks. These results suggest that resource size, and therefore forest structure, is an important determinant of fungal community structure.

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498 - Description of *Lactarius indigo* and *L. deliciosus* mycorrhizas on nine pine species

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The main macro and microscopical features of mycorrhizas of *Lactarius indigo* Schw. ex Fr. and *Lactarius deliciosus* Fr. with five species of Mesoamerican pines (*Pinus ayacahuite, Pinus hartwegii, P. oocarpa, P. pseudostrobus and P. rudis*) are presented. Also description with microscopical and SEM pictures of mycorrhizas of *L. indigo* with *Pinus halepensis*, *P. nigra*, *P. pinaster* and *P. sylvestris* are shown. The work was made in a growth chamber and using plastic containers with peat moss-vermiculite substrate. Inocula of selected strains of *Lactarius deliciosus* and *L. indigo* were applied directly to the radicular systems of the seedlings. The results seems to be the first published for *Lactarius indigo*, a known edible species in America, especially in Mexico and Guatemala, and also the mycorrhizas of *L. deliciosus* with Mesoamerican pines. These results are very important to the production of mycorrhized plants in Central America, especially with these edible mushrooms, because they could be of use in potentiating reforestation and for providing extra income and food to farm people, specially in Guatemala, Honduras, El Salvador and Nicaragua.

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499 - Russian species of the genus *Alternaria* Nees

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Alternaria is a very widespread genus of dematiaceous fungi. Many species are serious pathogens of crops. Despite on long history of its investigation, there are some different opinions about genus taxonomy. Formerly closed species were distributed between two genera - *Alternaria* and *Macrosporium* Fr. In 1969 the name *Macrosporium* was abolished, and the name *Alternaria* became the only (Levkina L.Ì. 1984. Mycol. and Phyt. 18, 1). The genus *Alternaria* was revised several times. There are more than 100 described species now, but not all descriptions are admitted. The paper of Egorova about fungi on Russian Far East contains the most complete list of *Alternaria* species in Russia (Egorova L.N. 1999. Mycol. and Phyt. 33, 1). There are 43 well-known and 7 insufficiently investigated species in this region. They infect 72 plants. *A. citri* Ell. et N. Pierce, *A. mali* Roberts, *A. longipes* (Ell. et Everh.) listed by Egorova are considered *A. alternata* (Fr.) Kiessl. now (Rotem J. 1994). There are a few papers, which add some other species in total list. For instance, there is *A. saponariae* (Peck) Neerg. in the roll of Russian arctic fungi (Rus. Arctic Fungi. 1999). Our collections include some of these *Alternaria* species. In two main Russian mycological herbariums we reveal at least 5 species, which are absent in the list of *Alternaria* species from Far East. Using prior analysis of literature and herbarium material, we can conclude that there are about 50 *Alternaria* species in Russia.

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500 - Diversity of ectomycorrhiza in *Quercus rubra* L. stands of different age on reafforested mining sites

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Red oaks are planted on 15% of reforested mining sites in Lower Lusatia and thus belongs to the main tree species used in reclamation. The mining substrates are characterized by poor nutrient supply e.g. N and P. Under such adverse conditions mycorrhizal fungi may play an important role for the vitality and the survive of these trees. One aim of this investigation is to charakterize the morphotypes of mycorrhizae of *Quercus rubra*. We want to know if the fungi are above all generalists or if the fungi are species adapted to these extrem conditions. An other aim is to compare the ectomycorrhizal fungi which are common with the neophyt *Quercus rubra* and indigenous oak species like *Quercus petrea*. Soil samples are taken in oak stands of different age within an area of 720 m&^{2};. The mycorrhiza are separated by morphological and genetic methods. To characterize the extrem soil conditions, the samples are analysed of pH, phosphate, nitrate, ammonium, C, N, S, water retention and bulk density. First results show, that 19 morphotypes of mycorrhizal fungi can be separated by their mycorrhizae. Some fungi can be identified by their fruitbody, for example *Boletus edulis* or *Amanita muscaria*. *Scleroderma citrinum* and *Paxillus involutus* can be found as fruitbody and mycorrhiza. The generalists and 'early stage fungi' *Cenococcum geophilum* and *Paxillus involutus* have an important part in the fungi coenoses.

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501 - Aphyllophorales (Basidiomycotina) of the Atlantic Rain Forest in Northeast Brazil I. States of Sergipe and Alagoas

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Eight collections of Aphyllophorales were undertaken between October 2000 and July 2001 in 3 Atlantic Rain Forest remnants in Northeast Brazil. In the State of Sergipe 4 species of Polyporaceae (*Hexagona hydnoides*, *H.*

papyracea, *Megasporoporia cavernulosa*, *Trametes caperata*); 2 species of Podoscyphaceae (*Caripia montagnei*, *Cymatoderma dendriticum*) and 1 species of Hymenochaetaceae (*Phellinus gilvus*) were identified. In the State of Alagoas 19 species of Polyporaceae (*Coriolopsis rigida*, *Daedalea sprucei*, *Earliella scabrosa*, #*Echinochaete brachyporus*, *Hexagona hydnoides*, *H. papyracea*, #*Junghuhnia luteoalba*, *Lentinus crinitus*, *Lenzites stereoides*, *Megasporoporia cavernulosa*, *Nigrofomes melanoporus*, *Physisporinus vitreus*, *Rigidoporus linteus*, *Pycnoporus sanguineus*, *Trametes caperata*, #*T. lactinea*, *T. versicolor*, *T. villosa*, *Trichaptum abietinum*); 10 species of Hymenochaetaceae (#*Hymenochaete fulva*, *H. luteo-badia*, *Phellinus discipes*, *P. gilvus*, *P. fastuosus*, #*P. inermis*, *P. merrillii*, #*P. portoricensis*, *P. punctatus*, *P. rimosus*); 2 species of Ganodermataceae (*Ganoderma applanatum*, *G. lucidum*); 2 species of Podoscyphaceae (*Caripia montagnei*, *Cymatoderma dendriticum*); 2 species of Stereaceae (*Lopharia cinerascens*, *Stereum lobatum*); 1 species of Corticiaceae (*Trechispora farinacea*) and 1 species of Schizophyllaceae (*Schizophyllum commune*) were identified. All the species are new occurrences to the States and those marked with a # represent new occurrences to Brazil.

502 - Aphyllophorales (Basidiomycotina) of Atlantic Rain Forest in the Northeast Brazil II. State of Pernambuco

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Twelve collections of Aphyllophorales were undertaken between September 2000 and July 2001 in 4 Atlantic Rain Forest remnants in Northeast Brazil. In the State of Pernambuco 23 species of Polyporaceae (#*Coriolopsis asper*, *Fomes fasciatus*, *Fomitella supina*, *Hexagona apiaria*, *H. hydnoides*, *H. papyracea*, *Lentinus crinitus*, *Lenzites stereoides*, *Megasporoporia cavernulosa*, *Microporellus obovatus*, *Nigrofomes melanoporus*, **Nigroporus vinosus*, **Perenniporia martius*, *Polyporus dictyopus*, **P. guianensis*, *P. leprieuri*, *P. tenuiculus*, *Pycnoporus sanguineus*, *Rigidoporus microporus*, **R. ulmarius*, *Trametes caperata*, *T. membranacea*, *Trichaptum abietinum*); 11 species of Hymenochaetaceae (*Hymenochaete aspera*, *H. luteo-badia*, #*H. pinnatifida*, **Phellinus calcitratus*, *P. gilvus*, *P. fastuosus*, *P. linteus*, *P. rimosus*, *P. senex*, **P. tropicalis*, *P. umbrinellus*); 5 species of Ganodermataceae (*Amauroderma omphalodes*, **A. sprucei*, *Ganoderma applanatum*, *G. lucidum*, *G. resinaceum*); 3 species of Podoscyphaceae (*Caripia montagnei*, *Cymatoderma dendriticum*, **Podoscypha ravenelii*); 3 species of Stereaceae (*Lopharia cinerascens*, *Stereum australe*, *S. lobatum*); 1 species of Corticiaceae (**Trechispora farinacea*); 1 species of Hericiaceae (#*Steccherinum fistulatum*) and 1 species of Schizophyllaceae (*Schizophyllum commune*) were identified. The species marked with a # are new occurrences to Brazil and those marked with an * represent new occurrences to the State of Pernambuco.

503 - Aphyllophorales (Basidiomycotina) of Atlantic Rain Forest in the Northeast Brazil III. States of Paraíba and Rio Grande do Norte

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Seven collections of Aphyllophorales were undertaken between October 2000 and May 2001 in 3 Atlantic Rain Forest remnants in Northeast Brazil. In the State of Paraíba 11 species of Polyporaceae (*Antrodia albida*, *Fomes fasciatus*, *Fomitella supina*, *Hexagona hydnoides*, *Lentinus crinitus*, *Megasporoporia cavernulosa*, *Nigrofomes melanoporus*, *Polyporus dictyopus*, *Pycnoporus sanguineus*, *Trametes caperata*, *Trichaptum abietinum*); 3 species of Hymenochaetaceae (#*Hymenochaete dura*, *Phellinus gilvus*, *P. merrillii*); 2 species of Podoscyphaceae (*Caripia montagnei*, *Cymatoderma dendriticum*); 2 species of Stereaceae (*Stereum hirsutum*, *S. lobatum*); 1 species of Ganodermataceae (**Ganoderma applanatum*) and 1 species of Schizophyllaceae (*Schizophyllum commune*) were identified. All the species represent new occurrences to State of Paraíba, except the one marked with an *. In the State of Rio Grande do Norte 7 species of Polyporaceae (*Fomes fasciatus*, *Hexagona hydnoides*, *Lentinus crinitus*, *L. velutinus*, *Megasporoporia cavernulosa*, #*Perenniporia martius*, *Trametes caperata*); 7 species of Hymenochaetaceae (#*Hymenochaete fulva*, *Phellinus fastuosus*, *P. gilvus*, #*P. maxonii*, #*P. meleoporus*, *P. rimosus*, *P. umbrinellus*); 1 species of Corticiaceae (*Trechispora farinacea*) and 1 species of Schizophyllaceae (*Schizophyllum commune*) were identified. All the species are new occurrences to the State of Rio Grande do Norte. The species marked with a # in both States are new occurrences to Brazil.

504 - Floristics of Burroughs Mountain, Mount Rainier National Park, Washington State, USA

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Washington State houses three national parks with alpine zones, yet limited baseline documentation has been undertaken regarding species inventories or descriptive works of lichen communities. These areas are subject to disturbance from both hikers and climbers. Vascular plant surveys have been carried out in limited areas of the parks, but do not include lichens. In the summer of 2001 a study was conducted on Burroughs Mountain in Mount Rainier National Park to focus on an inventory of lichen species and to study these sensitive communities. At 14,410 ft (4392m) elevation, Mount Rainier reflects alpine communities rarely seen in other areas of the state or the Pacific Northwest. Burroughs Mountain is made up of

three plateaus ranging from 7000 to 7500 ft. Although each plateau is subject to the same climatic conditions and at very similar elevations, the vegetation of each plateau is drastically different. Snowmelt conditions and substrate appear to be major factors affecting the species of lichens found at each location. Analyses include lichen species richness, abundance, frequency, and the association of macrolichens with plant communities. Data was analyzed using PC-ORD and CANOCO. An inventory of lichens was also conducted at Camp Muir (10,000ft) to determine rare and sensitive lichen species found at this elevation. Highly subject to disturbance, this is the base camp for climbers hiking to the summit of Mount Rainier. A list of rare lichens collected will be presented.

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505 - Contribution to the knowledge of diversity and distribution of ascomycetes from Mexico

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Diversity surveys of Mexican Ascomycetes from terrestrial or aquatic environments are limited mostly by a lack of necessary resources for mycological biodiversity studies. However, currently in Mexico there is interest in biodiversity issues, especially in relation to the conservation of our natural resources. The importance of biodiversity in this country is well recognised, and there is a long history of Botanical, Mycological and Zoological research which still continues to the present day. The current study aims to assess the diversity of Ascomycetes from Mexico. The numbers of Mexican Ascomycetes recorded from the literature in relation to global estimates of fungal diversity are also addressed in this work. The Pezizales and Xylariales are the best studied groups, probably because they are among the largest Ascomycetes and therefore more easily collected. The most explored areas in the country are the State of Veracruz and the State of Mexico, both located in the central-southeast region of the country. The least studied areas are the State of Nayarit, the State of Aguascalientes, and the Peninsula of Baja California.

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506 - Biodiversity of keratinophyles in Indian hills (Shimla, H.P.) and Plain (Agra, U.P) and perforator nature of dermatophytes in relation to taxonomy

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A comparative biodiversity of keratinophyles reveals that hilly area was rich in both species content and relative diversity as showing 100% prevalence of keratinophyles where as plains shows 80% prevalence as these areas differ markedly in prevailing environmental conditions. The percentage prevalence of *Chrysosporium indicum* (26%) and *Microsporum fulvum* (26%) was maximum indicating its dominance in hilly area. In plains *Trichophyton equinum* (20%) and *T. rubrum* (20%) was dominant. Out of seven genera of keratinophyles three isolated genera of dermatophytes were subjected for their ability to perforate hair in vitro considering only their superficial pigmented characteristics. Fourteen isolates belonging to three genera of *Microsporum*, *Trichophyton* and *Epidermophyton* were found to have wide range of flexibility in pathogenecity evaluating that they can be distinguished on the basis of their perforating nature without identification. *M. fulvum* was found to be best perforator releasing maximum protein (5.98 µg/ml). Among the six strains of *M. baullardii* strain 6 was found to be non-perforator indicating their non-pathogenic nature although similar in microscopic and macroscopic morphology with other strains. So it can be considered as atypical strain and can achieve a nascent taxonomic status. The taxonomic status of all the fourteen isolates of three genera and seven species was studied with respect to their morphology.

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507 - Screening of keratinophyles isolated from Indian hill (Shimla, H.P.): A biotechnological approach

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Eighteen keratinophilic species was isolated from Indian hills (Shimla, H.P.) belonging to seven genera *Chrysosporium*, *Microsporum*, *Trichophyton*, *Epidermophyton*, *Emmonsia*, *Geomyces* and *Zymonema* were screened for their ability to degrade keratin (feather) in vitro. All test fungi was found positive for producing keratinase enzyme, total protein and sulphur containing amino acids on keratin degradation. *G. pannorum* and *M. fulvum* produced maximum keratinase (20.5 Ku/ml and 14.2 Ku/ml respectively, protein (6.42 µg/ml and 5.84 µg/ml respectively) indicating the biotechnological importance of their pathogenic nature.

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508 - Above and below ground community of ectomycorrhizal fungi on beech from four small gaps in mature natural and managed beech forests

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The mycelium of ectomycorrrhizal (ECM) fungi represents a crucial link between forest ground vegetation and biotic and abiotic sources of nutrients. Mapping of fruitbodies has been shown as unadequate for studies of the distribution of the fungal community. We have applied a combined method of mapping of fruitbodies, identification of their PCR-ITS-RFLP patterns and identification of types of ECM after anatomical characteristics. The abundance of types of ECM have been studied in four natural or man-made canopy gaps in beech forest, two in Denmark and two in Slovenia. The fruitbodies were mapped and collected in two growing seasons on the comparable research plots and the ECM root tips were analysed twice in a transect line through each of the plots. In soil samples (V=274 ml) all roots were cleaned, nonmycorrhizal, old mycorrhizal root tips and morphotypes were separated and counted. On each plot from 14 to 33 different species of ECM fungi were determined. In the soil samples from all four plots 37 different types of ECM were separated, from these 31 on beech. 21 of them have been determined to the species level. Only ca one half of the ECM species determined from mycorrhizal root tips corresponded to the fruitbodies from the same plot. Some of the fruitbodies were not yet found in ECM. For identification of unknown ECM root tips the PCR-ITS-RFLP patterns have been compared with the database of RFLP patterns, while sequencing of the ITS region in rDNA has also been started.

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509 - Biodiversity patterns: kinds of rarity of the corticioid fungi (Basidiomycetes) in Tierra del Fuego (Argentina)

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Usually fungi do not appear in the lists of endangered species. This is probably due to our limited knowledge of some ecological features of the species such as the vulnerability. The form of rarity, based on the geographic distribution, local abundance and habitat specificity, helps us identify the species that should be protected and the way this protection should be carried out in a certain area. During a floristic study of Corticiaceae s.l. of Tierra del Fuego the kind of rarity of the species was evaluated. Six extensive collecting were made along 3 years, visiting 18 localities selected in 5 areas. The kind of rarity of each species was estimated based on: 1. Frequency measured as the number of collecting where the species was recorded. 2. Geographic distribution range in Tierra del Fuego measured as the number of localities visited where the species was recorded. 3. Substrate specificity measured as the number of woody substrates where the species was recorded. A total of 104 were distributed in kinds of rarity as follows: Common (class 0): 15 sp.; slightly rare (classes 1-2): 26 sp.; rare (classes 3-4): 8 sp.; highly rare (classes 5-7): 55 sp. Of 26 endemic species of the area 13 were highly rare, 10 were slightly rare and 3 were common. Of 4 species with an austral distribution 3 are extremely rare and 1 slightly rare. This estimation of rarity considers the situation of the species only in Tierra del Fuego and did not aim to be generalized for other areas.

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510 - A comparative account of poisonous mushroom biodiversity of Turkey and northeast North America

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Mushroom poisonings are reported every year in Turkey. The primary reason for this is that local persons may be unfamiliar with poisonous mushrooms and are unable to distinguish between edible and poisonous mushroom species. Ninety two taxa of poisonous mushrooms occur in Turkey. These are listed here together with their locations as well as poisoning syndromes such as: Phalloides syndrome (ten species, one variety), Muscarine syndrome (nine species), Pantherina syndrome (three species), Gyromitra syndrome (seven species), Coprinus syndrome (one species), Psilocybin syndrome (four species), Paxillus syndrome (two species), Gastrointestinal syndrome (32 species). Furthermore, there are cases of mushrooms that may be poisonous if consumed raw (15 species) and mushrooms with suspected constituents (five species), as well as those reported to be poisonous if consumed with alcoholic drinks (three species). Similar poisoning accidents have been reported from Northeastern parts of North America, but are less common. This study demonstrates a comparison of these findings.

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511 - Saprotrophic mycelium mats in the southern rain forest of Mexico: effects on fine roots and soil arthropod diversity

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Mycelium mats are patches with a high rate of leaf litter decomposition and at the same time represent a high density of hyphae available for soil fungivores. We investigated the spatial and temporal distribution of mycelium mats on the soil of a rain forest in Mexico and whether fine roots and soil arthropods were influenced by mats. The abundance and size of mats varied seasonally. There was a low number of mats after the rainy season and in general they were small. By contrast in the dry and early rains seasons mats were abundant and on average they were larger than those found after the rainy season. Also there was an effect of mats on the distribution of fine roots. In the dry season the amount of observed roots in non-mat soil was 4-fold than that observed in mat soil and by early rains there was more roots in mat soil than in non-mat soil. Also, mats affected the chemical content of fine roots. Roots in mat soil had lower concentration of Ca y K over the year whereas effects on Na concentration varied among seasons. Soil arthropods were also effected by mycelium mats. Arthropod diversity was higher in mycelium mat soil than in non-mycelium mat soil. This study shows that the guild of mycelium mat forming fungi is an important component of the tropical rain forest in southern Mexico beyond its potential species richness. Mats effect the distribution and chemical content fine roots (plants) and diversity of soil arthropods two key components of ecosystem diversity.

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512 - A database of Italian keratinophilic fungi

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The term 'keratinophilic' is used both for fungi capable of decomposing keratin, including those that cause superficial mycoses in humans and animals (dermatophyte), and for fungi only able to use products of keratin's hydrolysis, or material which naturally associates to it (1). Since the beginning the 19th century many studies were carried out in Italy about this group of fungi, isolated from soil, humans or animals. An on-line database is being created which could be useful for mycologists, physicians and veterinaries needing information on keratinophilic fungi isolated in Italy. This database presently includes 118 infrageneric taxa and will be available on the web before the end of 2002. A list of Italian papers in which a species is cited is provided as well. This is the first step towards a larger nomenclatural database covering all of the Italian microfungi. Two other databases are being implemented, which will contain morphological and physiological information, and will be connected to the first one to generate a complex information system. This database is part of larger co-operative effort, involving several other Italian research centres, the MIUR project 'A network of databases on the diversity of terrestrial cryptogams in Italy'.

References 1. Kirk, P.M., Cannon, P.F., David, J.C. & Stalpers, J.A., 2001. Ainsworth & Bisby's Dictionary of the fungi. 9th Edition. CAB International, Wallingford UK.

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513 - Biodiversity informatics and the development of the GLOPP information system

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The GLOPP project (Global Information System for the Biodiversity of Plant Pathogenic Fungi) is creating an integrated information system on the biodiversity of plant pathogenic fungi and their hosts by gathering descriptive and collection data, literature references, resource information and taxonomic names. The IT project within GLOPP provides data entry applications and develops the public access system on the Internet. This task requires the development of new concepts and information models as well as substantial efforts in application development. To simplify analysis and improve collaboration with other developers, a modular and component based approach has been choosen. This 'DiversityWorkbench' application suite ultimately will contain major application components like DiversityDescriptions (derived from DeltaAccess version 1.7), DiversityReferences (with SpecialIndexing for organism interactions and distribution), DiversityCollection, DiversityTaxonomy and DiversityIdentify as well as support modules like DiversityUsers, the DiversityGazetteer, DiversityResources, or DiversityExsiccatae. Developers interested in collaboration are invited to discuss how the total development cost and effort can be shared.

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514 - Digitization of important European host-pathogen indices for fungi

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The GLOPP-LIT project focuses on capturing published or unpublished scientific information on plant pathogenic fungi. In the framework of the collaborative GLOPP project (Global Information System for the Biodiversity of Plant Pathogenic Fungi) the literature project provides a base layer of information for the identification of plant pathogenic fungi that is supplemented with more detailed

information from the projects working on specific groups. Scientific experts will guarantee the high quality standard of the database. Eventually, scientists will not only be able to search for specific parasite-host-interactions, also a literature reference and geographic relations are given. Major achievements of the GLOPP-LIT project are a digitization of the major published host-pathogen lists for Europe. In addition, highly detailed literature reviews of the distribution of Erysiphales, Peronosporales, and Uredinales in Germany are currently developed. These will lead to an improved understanding of the prevalence and distribution of important pathogen groups. It is hoped that the tools and processes currently developed for this purpose can be used to develop similar data sets in other countries as well.

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515 - Identification of basidiomycetes using image analysis of pigments and colony morphology

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It has been shown that image-analysis can be used for the identification of terverticillate species of *Penicillium* based on colour-calibrated images obtained from the VideometerLab system. The capability of this newly developed method to distinguish between fungal cultures that appear identical but are known to be different species is very convincing and implies its usefulness in recognizing fungal cultures. The establishment of a public database of images captured under standardized conditions will allow any scientist using the system to compare their data to reference information in the database. The species tested so far, however, represent only a minor part of the fungal diversity. Furthermore, these species often produce strongly pigmented cultures. In this study, we have challenged the system's ability to recognize and group species and strains of two genera of the Basidiomycota; three species each of *Polyporus* (Polyporales) which produce whitish/lightly coloured mycelia, and of *Pholiota*, Agaricales, which form mottled cultures. A total of 21 isolates were cultivated in triplicates on three different media in 9 cm Petri dishes, and images were captured of each plate on day 8 and 15. The results of the image-analyses, including groupings of strains and their growth on the various media were compared to the metabolite profile and taxonomy of the groups. The system facilitates comparison of subtle visual differences in an accurate and reproducible way.

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516 - Multispectral macroscopy for mycology

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Imaging technology has proved very useful for classification of fungi which are difficult to separate by other means without performing a labor demanding chemical analysis. Studies have been carried out using traditional trichromatic camera technology, producing three images corresponding approximately to the colors red, green, and blue, which are sufficient for many purposes where information on subtle color differences in the visible region is required. A new and innovative technology based on light emitting diodes (LEDs) that adds to the advantages of the trichromatic technology, is presented. By combining LEDs with a black-and-white digital camera, multiple advantages are obtained, of which an increased spatial resolution (in the megapixel range) and the possibility of using wavelengths outside the visible range, such as ultraviolet and near-infrared light, are the most notable. At present, up to ten relevant wavebands may be combined into the same unit producing multispectral images incorporating information not visible to the human eye, such as information on metabolites or chemical composition. In order to reduce the wealth of information produced by a multispectral unit to simple properties perceivable to the human brain, multivariate statistical methods are applied to the image data. By supervised or unsupervised learning these methods are used for building mathematical models relating image data to properties of interest, e.g. species, strain, or clone.

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517 - Host preferences of wood-decaying basidiomycetes in a cool-temperate area of Japan

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I examined host ranges of wood-decaying basidiomycetes in a cool-temperate forest in Japan. *Fagus* spp. and *Quercus* spp. are the main tree species within the forest. I marked fallen trees (more than 20 cm diam and 2 m long) within a 300 x 200 m plot, then listed polypores, stereoid, and hydnoid fungi on each tree. Diameter, tree species were also recorded for each tree. In total, 250 trees were marked, then 51 species of fungi (44 polypores) were recorded. Following species did not show any preference: *Bjerkandera adusta*, *Ganoderma applanatum*, *Rigidoporus cinereus*, *Stereum ostrea*, *Trametes versicolor*, etc. All collections of *Daedalea dickinsii* (26/26) were on *Quercus* spp. (including *Castanea crenata*). Other species as follows were also recurrent on *Quercus* spp.: *Hymenochaete rubiginosa* (39/41; 2 on undetermined trees), *Melanoporia castanea* (18/18), *Piptoporus soloniensis* (8/8), *Xylobolus* *frustulosus* (22/22), etc. The following species were frequent on *Fagus* spp.: *Fomes fomentarius* L type (7/7), *Fomes fomentarius* S type (19/21; 1 on *Styrax obassia*, 1 on an undetermined tree), *Protodaedalea hispida* (13/14; 1 on *Quercus* sp.), etc. *Fomitopsis* cf. *cajanderi* was always on *Prunus* spp. (4/4). *Melanoporia castanea* was usually on huge trees with more than 50 cm diam or on branches of huge trees. It is suggested that loss of old growth forests may reduce diversity of wood-decaying basidiomycetes such as those restricted to *Fagus* spp. and huge *Quercus* spp.

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518 - The discovery of *Cryphonectria cubensis* on native *Syzigium* spp. from South Africa

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Cryphonectria cubensis is a pathogen on *Eucalyptus* species and *Syzigium aromaticum* in tropical and subtropical regions worldwide. The pathogen has also been reported from *Tibouchina* species (Melastomataceae) in Colombia and South Africa. A previous hypothesis has been that *C. cubensis* was introduced into South Africa from South America. However, sequence data derived from β-tubulin, histone H3 genes and disease symptoms have been used to show that South African isolates of *C. cubensis* are different to those from South America, Central Africa and South East Asia. During disease surveys in indigenous forests of South Africa, fruiting structures resembling the anamorph of *C. cubensis* were found on native *S. cordatum* and *S. guanennsi*. Teleomorph structures were found on samples from the Northern and KwaZulu Natal provinces. The fungus from these native *Syzigium* spp. was broadly identified as *C. cubensis* based on morphology. Comparisons of β-tubulin sequence data showed that collections from the native *Syzigium* spp. group together with South African isolates from *Eucalyptus*. Results of this study indicate that the *Eucalyptus* pathogen in South Africa, currently known as *C. cubensis*, occurs on native *Syzigium* spp. in this country. Furthermore, they add convincing evidence to the view that *C. cubensis* in South Africa, is a species different to that occurring elsewhere in the world and that it is native to South Africa, where it has infected exotic *Eucalyptus*.

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519 - What does rare wood-associated fungi really want?

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Based on studies in Danish deciduous forests, we examine the importance of environmental conditions and wood quality for macro-fungal diversity (as sporocarps) in decaying wood, in order to provide practical conservation guidelines for this group. Although we found species richness to increase with wood volume, threatened species did not appear in general to prefer large logs. Rather, we found small logs to host more threatened species per volume unit than larger logs. Similarly, branches (diam. >10 cm) appeared to be richer in species than logs. Logs in intermediate stages of decay were found to be most species rich, especially with respect to threatened species. By analysing modified data sets we found that even a short gap in the supply of dead wood might significantly decrease the number of threatened fungi at forest level. Tree species diversity was found to be partly reflected in the diversity of wood associated fungi. However, some tree species appear to have rather similar fungal species assemblages and does not independently add to overall species richness at local scale. Local variation in microclimate was found to have limited importance for fungal diversity. Among localities, however, we found huge variation in the frequency of threatened species, which seem to be partly reflected by variation in microclimatic stress levels, partly by differences in forest history. Selecting the right sites for forests reserves is crucial for an efficient protection strategy.

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520 - Molecular analyses of arbuscular mycorrhizal fungal species communities

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Species communities of arbuscular mycorrhizal fungi (AMF) were studied using a set of primers specifically targeted at the ribosomal RNA genes. Small subunit and Internal Transcribed Spacer sequences were amplified from colonized roots and analyzed phylogenetically. Soil and roots were sampled from field sites under organic or conventional agricultural management widely differing in input intensity as well as from semi-natural grassland. Trap cultures set up from the respective field soils were also characterized. All sites are situated in the three-country-corner France/Switzerland/Germany, most of them on Loess soils. A wide range of fungi was detected in the trap cultures, comprising *Glomus*, *Acaulospora* and *Archaeospora*. The occurrence of generalist AMF and specialists for specific field sites or types of agricultural management was analyzed, as well as the spatial structure of AMF communities.

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521 - Lobariaceae (Lecanorales, lichenised Ascomycota) of Mauritius and La Réunion in the Indian Ocean

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The lichen family Lobariaceae is a prominent part of the lichen vegetation in the more humid areas of the tropical islands Mauritius and La Reunion situated in the Indian Ocean. During several excursions to the islands, the authors and also Hildur Krog, Oslo collected about 400 specimens of *Lobaria*, *Pseudocyphellaria* and *Sticta* from numerous sites. The collections include six species of *Lobaria*, five species of *Pseudocyphellaria* and 10-12 species of *Sticta*. Very limited information on the Lobariaceae of the islands has been published, and the closest comprehensive treatment is the macrolichen flora of East Africa by Swinscow and Krog (1988). *Lobaria holstiana* is not previously reported from the islands and *Sticta* cf. *cinereoglauca* appears to be new to Africa and adjacent islands. All of the species are pantropical, except *Lobaria holstiana* and *Sticta dichotoma*. *L. holstiana* is known from Tanzania, Mauritius and La Réunion, whereas *S. dichotoma* is found in Eastern Africa, the Mascarenes and Panama. However, *S. dichotoma* may be conspecific with the pantropical species *S. canariensis*. The photosymbiodemes of *Sticta dichotoma* and *S. cinereoglauca* are described.

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522 - The merosporagiferous fungi in Taiwan

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During an investigation of merosporangiferous Zygomycetes in Taiwan, eight species of the family Piptocephalidaceae (Zoopagales) were isolated. Two of these species belong to the genus *Piptocephalis*. They are *Piptocephalis cylindrospora* and *P. sphaerica*. Six species belong to the genus *Syncephalis*. They are *Syncephalis cornu*, *S. depressa*, *S. obconica*, *S. parvula*, *S. sphaerica* and *S. ventricosa*. All species represent new records to Taiwan. Previously none of the family Piptocephalidaceae was described from Taiwan. These eight species were all isolated from soil or dung in Taiwan, mainly mountain area. The morphological characters together with their hosts are reported.

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523 - Rehabilitation of nutrient-poor grasslands (waxcap grasslands) in dunes in the Netherlands: the effect of active nature-management on the mycoflora

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The effect of active nature-management in grasslands in an area in the dunes in the western part of the Netherlands is evaluated. The mycoflora in 17 semi natural wet grasslands with various management has been followed since 1985, two during all years, others irregularly. All 17 grasslands are situated in the Amsterdam Waterworks Dunes, a protected area of 34 km^2 southwest of Haarlem. They are mown, grazed or the topsoil has been removed. The dune area has served as a drinking water source for the city of Amsterdam which caused desiccation. Therefore infiltration started some 30 years ago with unpurified river water from the river Rhine. This caused an enormous increase of e.g. *Calamagrostis*, the direct motive to start active nature management in the former wet dune slacks. Evaluation after 15 years shows positive results for plants as well as macrofungi. There is a spectacular increase in number of species and fruitbodies of the macrofungi representative for Waxcap grasslands (*Hygrocybe*, grassland *Entoloma*, *Geoglossum*, *Trichoglossum*, *Clavulinopsis*, *Clavaria*). This type of grassland and its representative species have been under serious threat in the Netherlands since 1900. Of the investigated sites 5 have now 10 or more species of *Hygrocybe*, and two of the investigated sites belong now to the best Waxcap-grasslands in the Netherlands. There seems to be little difference in effect between grazing or mowing.

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524 - Plant pathogenic and saprophytic fungi from graminicolous hosts in Thailand

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In the present study, corn, rice and grass leaves showing leaf blight and leaf spot symptoms were collected from various locations in the North, North-East and Central Thailand. They were used for isolation of plant pathogenic and saprophytic fungi. Tissue transplanting method with 10% clorox and potato dextrose agar was used. The results indicated that dematiaceous hyphomycetes found on *Zea mays* included *Bipolaris bicolor*, *B. maydis*, *B. zeicola*, *Curvularia akaiiensis*, *C. eragrostidis*. *C. lunata*, *Nigrospora sphaerica* and *Nodulisporium gregarium*, whereas *Alternaria padwickii*, *Bipolaris hawaiiensis*, *B.*

oryzae, *Curvularia lunata*, *Drechslera halodes*, *Exserohilum rostratum* and *Nigrospora oryzae* were found on rice kernel and diseased leaves. Plant pathogenic and saprophytic fungi found on grass leaves included *Alternaria longipes* (on *Brachiaria mutica*), *Beltrania rhombica* (on *Digitaria cialiaris* and *Pennesitum pedicellatum*), *Curvularia akaii* (on *D. cialiaris*), *C. borreriae* (on *Echinochloa crusgalli*), *C. intermedia* (on *P. polystachyon*), *C. ovoidea* and *C. penniseti* (on *P. pedicellatum*), *C. sorghina* (on *P. setosum*), *Drechslera* spp. (on *Rhychelytrum repens*, *B. ruziziensis*, *Eleusine indica*, *Penicum maxinum*), *Exserohilum* sp. (on *P. padicellatum*), *Nigrospora oryzae* (on *Imperata cylindrica*, *Dichanthium annulatum* and *R. repens*), *N. sphaerica* (on *D. digitaria*, *R. repens* and *D. annulatum*) and *Pyricularia grisea* (on *B. mutica*, *P. padicellatum*, *Penicum repens* and *Cenchrus echinatus*).

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525 - Diversity of coprophilous fungi from wild and domestic animals in Central and North-East Thailand

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Coprophilous fungi are a large group of saprobic fungi that can survive thermal and chemical conditions in herbivore digestive tracts. They are adapted to extreme conditions and many species show distinctive morphological features. This group of fungi have been shown to be a source of antibiotics, organic acids, enzymes, biological control agents and other secondary metabolites of economic importance. In the present studies, 8 dung samples of domestic animals and wildlife, including buffalo, cow, deer, elephant, goat, rabbit, rat and toad, were collected from nine locations in Central and Northeast Thailand. The moist chamber, soil plate, alcohol and heat treatment methods were used to isolate these fungi. Identification was based on morphological characters observed with light and scanning electron microscopy.The results revealed that 27 genera and 14 species were found, including 3 Zygomycetes: *Absidia corymbifera*, *Cunninghamella* and *Rhizopus*; 18 Ascomycetes: *Ascobolus*, *Ascodesmis*, *Cercophora*, *Chaetomium*, *C. cupreum*, *C. globosum*, *Echinopodospora*, *Eupenicillium osmophilum*, *Eurotium amstelodami*, *Microascus*, *Neosartorya fumigata* var. *glager*, *Podospora curvicolla*, *Preussia*, *Saccobolus*, *Sporormiella*, *Sordaria fimicola*, *Talaromyces bacillisporus* and *T. rotundus*; 13 Hyphomycetes: *Acremonium*, *Arthrinium*, *Arthrobotrys*, *Aspergillus candidus* gr., *A. fumigatus* gr., *A. niger* gr., *Penicillium* spp., *Scopulariopsis*, *Stachybotrys* and *Trichoderma harzianum* and 1 Basidiomycete: *Coprinus*.

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526 - The habitat ecology and distribution of lichen *Lobaria pulmonaria* in Estonia

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Lobaria pulmonaria grows in biotopes of long ecological continuity and favours forests of natural conditions. The atmospheric pollution and destructive forest management has caused the decline of distribution in many parts of its distribution area, especially in Europe. The aim of this study was to review the substrate types, habitat demands and distribution of *L. pulmonaria* in Estonia. All the available data were accounted. The distribution analysis within suitable habitats was performed using Corine landcover types database in GIS. *L. pulmonaria* is totally epiphytic lichen in Estonia, it has been found on 15 tree species, almost exclusively on deciduous trees. One-third of registered records of *L. pulmonaria* are known on *Populus tremula*; it is common also on *Acer platanoides*, *Fraxinus excelsior* and *Quercus robur*. The most of the registered specimens (91%) grow in eutrophic boreal-nemoral, eutrophic paludifying and mesotrophic boreal forests, 6% are in semi-natural biotopes - wooded meadows, and 3% of records are known from parks (churchyards, former manor parks). *L. pulmonaria* is the most common in over 100 years old mixed forest, on average with 4-5 tree species or spruce dominated forests. At present *L. pulmonaria* is recorded in a few hundred localities in Estonia.

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527 - Waxcap grasslands in Slovakia - History, present state and perspectives

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Slovakia is rich in seminatural grassland habitats, because a small scale farming was the most common way of utilizing not very rich nature sources. Small fields, meadows and pastures were grazed and/or mowed and sometimes dung-fertilized. On these grasslands ecosystems very rich in biodiversity developed. In the second half of the last century many of these small plots were put together and managed industrialy, which ment absolute loss of their former character. Only at the most inaccessible places some of them were preserved, but many of their owners lost interest in utilizing them. Research of fungal diversity of these ecosystems has just started, but results are promising. In 1998 only 28 species of *Hygrocybe* were published and since then 11 new species were recorded and also the most endangered species as *H. calyptraeformis*, *H. spadicea* and *H. ovina* can be found. The best known localities: Landscape protected area (LPA) Biele Karpaty - meadows with more than 10 species of *Hygrocybe* recorded at single visit (*H. reidii*, *H. marchii*, *H. subpapillata*, together with some gasteromycetes and rare Clavariaceae. LPA Horná Orava - wet meadows and peatbogs with species as *H. calyptraeformis*, *H. substrangulata*, *H. coccineocrenata*, *H. turunda* a.o. National Park (NP) Slovenský Raj - seminatural grasslands of European importance with very high biodiversity. Mycological research has just started, but species as *H. nitrata*, *H. marchii*, *H. perzistens* and *H. substrangulata* were recorded.

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528 - Building of the central database of biological collections in the museums of the Slovak Republic

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In 2001 the project of building national database of biological collections, in which 31 Slovak museum take part, has started. Guarantee is the Nature Historical Museum (NHM) of the Slovak National Museum and the project is aimed to the identification and documentation of chosen components of the Slovak flora and fauna, with the accent on the rare and endangered species. At present the structure of database program is prepared and in 2003 first data will be filled in. Central node of the database will be at the NHM and in 2005 first outputs will be presented on the web. Herbary collections preserved in 25 museums contain 721 405 specimens. Fungi (including lichens) are 157 936 specimens. Main mycological collections are at: Slovak National Museum-Nature Historical Museum, Bratislava (BRA) - 146 877, Museum of the Tatra National Park (TNP) - 5 348, Forestry and Timber Museum, Zvolen - 1 910, Museum Nitra (NIM) - 1139, Central-Slovakian Museum, Banská Bystrica (SMBB) - 491, Povazie Museum, Zilina - 463, Orava Museum of P.O.Hviezdoslav, Dolný Kubín - 415, Slovak Museum of Nature Protection and Speleology, Liptovský Mikulás (MOP) - 334, Saris Museum, Bardejov (SMB) - 200 specimens.

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529 - Role of mobile introns in diversity of mtDNAs of imperfect black Aspergilli

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A high level of intraspecific mtDNA variability was reported earlier among black Aspergilli, indicated by various RFLP profiles of isolates of *Aspergillus niger*, *A. tubingensis*, *A. carbonarius* and *A. japonicus*. To study the reason for the mtDNA polymorphism physical- and functional maps of mtDNAs representing different RFLPtypes were constructed than the variable regions cloned and sequenced. These revealed that apart from minor nucleotide changes, the presence of introns at different positions and their appearance in various numbers in the mtDNAs are responsible for the mitochondrial genome diversity and size differences. The reasons of variability between two mtDNA types of *A. carbonarius* can be attributed to the presence or absence of a 1.1 kb group I intron in their *cox*2 subunit. Sequence comparison of two mtDNA RFLP types of *A. japonicus* revealed that they differed from each other in at least two group I introns in *cox*1 subunit and one group I intron in *cob* gene. Organisation of four *A. niger* mtDNA types proved to be very similar, but one of the three observed variable regions included a group I intron in the *cox*1 subunit was present in three different forms. The mobile introns (bearing ORFs) play an important role with their homing process generating recombinant mtDNAs in the interaction among mitochondrial genomes after mitochondrial transmission. The homing endonucleases activate a double strand break that is finally repaired by a repaire mechanism.

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530 - Arbuscular mycorrhizal status of plant species from Western Ghats of Goa, India

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Western Ghats are one of the hot spots of biodiversity in the world and the state of Goa lies in the heart of Western Ghats. In the present paper, AM fungal status of 62 plant species, *viz.* pteridophytes, herbs, shrubs, trees and plants with under storage ground organs from Western Ghats of Goa have been discussed. All the plant species selected for study, exhibited the presence of AM fungal association. The average root colonization ranged from 30% (pteridophytes) to 70% (shrubs). Whereas the average spore density of AM fungi ranged from 52 spores 100^{-1}g soil (pteridophytes) to 528 spores 100^{-1}g soil (plants with under ground storage organs). Species composition of AM fungi revealed the presence of five genera *viz.*, *Acaulospora* (9), *Glomus* (19), *Gigiaspora* (3), *Scutellospora* (5) and *Sclerocystis* (5) with species number given in parenthesis. A total of 41 species of AM fungi were recovered during the study. *Scutellospora gregaria*, *Glomus fasiculatum* and *Sclerocystis taiwanensis* were the most frequently occurring AM fungal species. The present paper is first report on AM fungal association in plant species from Western Ghats of Goa and contributes data necessary for further studies on AM fungi from this region of which very little has been explored so far.

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531 - Field identification of marne yeasts using DNA hybridization macroarrays

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Yeasts are a natural component of near shore and oceanic environments, however the extensive tests required for species identifications have hampered studies of their ecological roles. Current use of molecular techniques has resolved many of these issues. With electronic availability of extensive gene sequences in Genbank, species can be identified by sequence analysis and by design of species-specific PCR primers and hybridization probes. In this poster, we describe a macroarray hybridization assay, which can be used to identify yeasts directly from environmental samples or from cultured organisms. The technique is in a microtiter format in which species-specific probes are bound to the wells of the microtiter plates; genomic DNA from target species are amplified and labeled with biotin labeled universal primers. The probes are hybridized with labeled target DNA and a positive identification is indicated via a colorimetric assay. The results are unambiguous: a color change indicates a positive identification. The method is inexpensive, rapid, accurate and amenable to shipboard as well as laboratory use.

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532 - Fungal diversity in set-aide agricultural soil investigated using terminal-restriction fragment length polymorphism (T-RFLP)

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In a newly abandoned agricultural land the fungal community response to manipulation of the above ground vegetation was investigated. The field site consisted of 20 plots where the plant diversity was managed by either sowing 15 plant species or natural colonization was allowed to occur. The plant mixture contained grasses, legumes and forbs that all were expected to occur on the site. A subset of the plots (5 from each treatment) was inoculated with soil cores from a late successional stage. Five years after abandonment soil cores were taken from the plots and DNA was extracted using Bio101 soil extraction kit. The ITS region of the rDNA gene was amplified using fluorescently labelled fungal specific primers (ITS 1F/ITS 4). The PCR products were digested using HinfI and TaqI and analysed on an ABI 377 sequencer. Results from both restriction enzymes were combined and a principal component analysis performed. Also the species richness expressed as the number of restriction fragments in each sample were analysed. These results were compared with field observations of the development in plant species composition.

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533 - New and widespread genera of hypocrealean *Geosmithia* on a hidden place

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The genera of the polyphyletic genus *Geosmithia* (formerly *Penicillium*) are recently placed to Eurotiales and Hypocreales (*G. putterilii* and *G. lavendula*). The genetic relationships among 90 isolates of *Geosmithia* spp. collected from galleries of phloem boring insects and from soil were investigated using RAPD analysis, rDNA, microscopy and colony morphology. We found 14 groups that were distinguished by both RAPD patterns and morphological characters. Based on ITS1-5.8SrDNA-ITS2 sequences, phylogram relating representatives of RAPD groups to the described species was constructed. Two highly supported clades were found. First clade consisted of type strain of *P. pallidum* and 3 new groups, second clade contained 9 groups, with affinities to *G. putterilii* and *G. lavendula*. Some groups are very charakteristical by its morphology, fingerprint pattern and ITS region and represent new genera as well as undescribed subpopulations of *G. putterilii*. The only preference of RAPD groups was found for associated insect (*S. rugulosus* × *S. intricatus*), but not for host tree species or locality. This implies that *Geosmithia* fungi are mostly vectored by insect. The critical character of hypocrealean geosmithias called 'peg foot' is described by its morphology and ontogenesis. The newly found *Geosmithia* spp. (from *G. putterillii* complex) are very abundant in early fungal communities (next to yeast) of feedings of bark beetles.

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534 - Eastern Carpathian lichens: National and geomorphologic peculiarities

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The first Catalogue of the lichen-forming, lichenicolous and allied fungi of Eastern Carpathians Mts. including 1307 species (Kondratyuk, Lackovicova, Pisut & Popova 2002) allows to carry out the first attempt of comparison of lichen florae of their national portions and geomorphologic regions. The highest species diversity of fungi mentioned (1095 species) is hitherto known from Ukrainian part of Eastern Carpathian Mts., while the biggest Romanian part (only 529 lichen species known) is still in urgent need of special study. Polish and Slovak portions of Eastern Carpathian Mts. are represented consequently by 508 and 411 species. Among geomorphologic units of the Eastern Carpathian Mts. (accepted mainly after Kondraczky 1978), Eastern Beskydy region (within Ukrainian part) is the most well studied at the moment from lichenological point of view. 659 species of lichen-forming and lichenicolous

fungi are hitherto recorded from this region. More than 400 lichen species (namely 410) is known from Ukrainian Volcan Carpathians. From 300 to 400 lichen species is recorded from the following areas: Low Bieszczady, High Bieszczady, Chornohora, Chyvchyn-Grynyavsky Mts. and Marmarosh Mts. More than 200 lichen species (and less of 300) is known from Bukovsky Vrchy Mts., Gorgany and Svidovetz Mts. Similarity and differences of the species content of lichens of national and geomorphologic regions of the Eastern Carpathian Mts. region are discussed and illustrated.

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535 - Epiphytic lichens in Dushanbe

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Dushanbe, the capital of the Tajik Republik, is located in the Hissar Valley at a height of 800-900 meters above sea level. Dushanbe's climate is sharp continental. The average temperature in January is +1 °C and in July +28 °C. Epiphytic lichens in Dushanbe and its vicinity were investigated in 1997-1998 to learn study their role as air pollution indicators. Species composition of the epiphytic lichens on *Platanus orientalis*, *Acer negundo*, *Pinus eldaricus* was studied and the distribution of certain lichen examined. A list of lichens contains 15 species. On the ground of received data three zones of air pollution were delineated in Dushanbe. Most polluted zone (1) follows the distribution of *Lecanora hagenii*, *Phaeophyscia orbicularis* and *Anaptychia ulotricoides* etc. This (1) zone includes the separate sites along the main motor roads of the city. They are the sites with permanent intensive pollution. The zone of intermediate pollution (11) was delineated according to distribution of *Lecanora hagenii*, *Phaeophyscia orbicularis*, *Anaptychia ulotricoides*, *Candelariella aurella*. The third zone is located in the north-eastern outskirts of Dushanbe embraces almost the whole hilly massif. Therefore the number of epiphytic lichens species shows a tendency of decreasing of species number along the gradient of air pollution.

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536 - The influence of forestry on wood-inhabiting corticioid basidiomycetes in Switzerland

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Intensively managed forests show significantly less fruit bodies and a lower species richness of corticioid basiodiomycetes than forest reserves. Species richness does not only seem to be determined by the availability of woody debris, since also some strongly managed forests host a remarkably high species richness. Even rather rare species, e.g. *Tubulicrinis sororius* or *Brevicellicium olivascens*, could be found in managed forests, whereas they were expected to occur more likely in forest reserves. Forests, richer in different tree species, harbour a higher number of fungal species too, independent of their managing history. Nevertheless, the importance of forest reserves for the maintenance of a rich species community of corticioid basidomycetes is clearly confirmed. In order to address the question of diversity, abundance, host age, decomposing process, regional factors and the influence of modern forestry, 82 plots of 50 m^2 have been selected in the main forest types in Switzerland. A total of 3216 branches with fruit bodies of corticioid basidiomycetes have been harvested and thereon 233 species identified. Among the most frequent species are *Amphinema byssoides*, an important mycorrhizal partner of conifers and *Phlebiella vaga* or *Hyphodontia sambuci*, two wood-decomposing species. Preliminary results show a few species dominating, the majority of the species is found only rarily: species rank distribution is best explained with a steep power function.

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537 - Nitrogen impact on epiphytic lichens in an ancient oak forest in Denmark

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The lichen vegetation was studied in Kås Skov, an ancient oak forest in Northern Jutland, Denmark that is under protection of EEC habitat directive because of its high biodiversity. The very rich lichen vegetation is threatened by ammonia and ammonium deposition from animal farms in the region. In a floristic survey of epiphytes on tree trunks, several old forest indicator lichens previously recorded were not refound, however *Zamenhofia hibernica* and *Ramonia chrysophaea* were found as new to Denmark. Lichen vegetation from north and south side of trunks, as well as that of north- and southfacing branches of trees standing at the forest edge and interior, were compared. Multivariate analysis (CCA) showed that the lichen occurrence is more dependent on the position on the branch, than on where the branch is situated in the forest. Indicator Species Analysis (ISA) revealed that species generally regarded as nitrophilous, prefer branch parts 10 to 50 cm from the tip (3-8 years), at the edge of the forest. Contrary, thalli of *Hypogymnia physodes* contain 21% more nitrogen in the sheltered part of the forest compared to the forest edge. The high nitrogen content in *Hypogymnia physodes* from Kås Skov (2.1%) is compared at regional scale and showing that >15 kg N /ha/year is deposited. This dose is expected to cause changes in the epiphytic lichen flora, and the nearby lichen heathland.

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538 - Wood-inhabiting fungi in primary and secondary seasonally dry tropical forest, Costa Rica

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The relation between management regime, quality of coarse woody debris, and presence of some groups of wood-inhabiting fungi was explored in a tropical dry forest. A patch of primary forest and a nearby old secondary forest were surveyed in Santa Rosa National Park, Guanacaste, Costa Rica. A total of 81 species of poroid and stereoid fungi were found. The primary forest had 62 species, while 54 species were recorded in the secondary forest. Thirty-five species were common between the stands. More species were exclusively found in the primary forest than in the secondary one (27 vs. 20). The curve for cumulative number of species on increasing number of logs was slightly steeper for the primary forest than for the secondary. At least 84 logs had to be examined before a significant difference in species richness between the two forests could be detected. The results indicate that species richness of poroid and stereoid fungi is returning to the level of the primary forest in a regenerating dry forest habitat when the woody debris is allowed to stay in the forest and there is a patch of original forest in the vicinity. Relations between substrate quality and the occurrence of fungi in the different forest types were explored and reasons for the observed patterns are discussed.

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539 - Growth of mycorrhizal *Leucaena leucocephala* (Lam.) de Wit seedlings in copper contaminated soils

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The ability of the mycorrhizal symbiosis in favouring nutrient absoption in agricultural systems, specially in arid and semiarid areas, as well as the toxicity of heavy metals has been well documented. Due to the low infectivy potential of the AMF at the study area (Mineradora Caraíbas, Bahia State, Brazil), we evaluated the effect of the mycorrhizal association in seedlings of *Leucaena leucocephala* in soils with increasing proportions of copper contamination. Soils from an 'caatinga' area, not impacted (control) and impacted by copper mining (site that receives the waste product) were used. Seedlings were inoculated with *Glomus etunicatum* Becker & Gerd. and cultivated in substrate constituted by soil from the impacted area diluted in soil from the control area, in the following proportions: 0, 25, 50, 75, and 100%. In general, increasing the proportion of copper contaminated soil had a negative effect on plant growth. Mycorrhizal plants presented higher height, foliar number, and shoot and root biomass than those non inoculated, when maintained in soil with up to 50% contamination. The highest number of AMF spores (3.2 g^{-1} soil) was found in substrate with 25% contamination. The arbuscular colonization was higher than 40% in the treatments with up to 50% of copper contamination. The plant-fungi association promoted higher copper tolerance of the seedlings, but the mycorrhizal benefit was lost when soil contamination was higher than 50%. Financial support: CAPES and CNPq.

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540 - A survey of macrofungi at Pakua mountain range in central Taiwan

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The fungal flora, with special focus on macrofungi of Pakua mountain range in the vicinity of Chang-Hua city (24 degree 10' N, 120 degree 50' E) was surveyed. The elevation is between 20-80 m, the average temperature ranges from 18-27 degrees centigrade and the annual precipitation is about 1732 mm. Mostly specimens' collection was made primarily during rainy days from August 2001 to April 2002. Seventy specimens were collected and identified. Among the identified collection 60 specimens belong to basidiomycetes and 4 in ascomycetes. Tricholomataceae, Pluteaceae, Cortinariaceae, and Amanitaceae are among the dominant families in the flora. An estimated 24,000 fungal species inhabit in Taiwan, an island located at the northern end of pacific archipelago. However, only one-sixth of them have been reported. With the increasing pressure of economic development poses an urgent need for the investigation of the island's fungal resources. Pakua mountain range is among a unique spot that has never been systematically investigated.

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541 - Fungi (Basidiomycetes) in the Kielce town (Central Poland)

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The Kielce town (112 km^2, population over 220 000 inhabitants) has a dense ca 25% forest cover. Comparing to the other Polish or European towns, it is characterized by the presence of highly differentiated natural vegetation and variety of habitats. Macromycetes were mapped using carthogram method. The area of town was divided into the net of 1 km^2 squares. One square was considered as one locality. For each species the type of habitat, time of fruiting and distribution are given. The studies were intensely carried out from 1986 till 1994 and sporadicly until now and yielded the collection of over 450 species. Among them 6 species were new for Poland (*Hypholoma capitatum*, *Phlebia cremeoochracea*, *Phlebiella allantospora*, *Inocybe cryptocystis*, *Marasmiellus tremule*, *Pluteus pouzarianus*) as well many others rare in Poland and in Europe. In Kielce 65 species were listed in the red list for Poland (Wojewoda, Lawrynowicz 1992), for example: *Albatrellus confluens*, *Artomyces pyxidatus*, *Boletus queletii*, *Dacrymyces ovisporus*, *Ganoderma carnosum*, *Geastrum minimum*, *G. quadrifidum*, *Gleoporus taxicola*, *Hydropus marginellus*, *Hygrophorus glyocyclus*, *Phellinus hartigii*, *Pleurotus calyptratus*, *Ramaria flava*, *Stropharia albonitens*, *Tulostoma brumale*, *Volvariella pusilla* and 4 species are protected by law: *Langermannia gigantea*, *Meripilus giganteus*, *Phallus impudicus* and *Sparassis crispa*. Existing enormous biodiversity of fungi within town area is in my consideration a uniqe phenomenon.

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542 - Diversity of microfungi from soil and debris at termite mounds in Thailand

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Termitomyces species, a delicious mushroom, are found in the tropics. However, cultivation of this mushroom has not been successful for many reasons. Most species of microfungi found in termite fungus gardens belong to *Xylaria* and *Trichoderma*. These fungi may play an important role in stimulating the occurrence of *Termitomyces*. It is thus important to study microfungi from soil and debris at termite mounds, because some of these fungi can produce novel active compounds. For the present study, soil samples and debris from termite mounds were used to isolate microfungi. Samples were collected from various locations in Thailand. The following methods were used for isolation: alcohol and heat treatment, soil plate and dilution plate. Identification was based on macro- and microscopic characters when cultured on different media after observation under light - and SEM microscopes and making camera lucida drawings. Microfungi found in this study included *Ascodesmis*, *Aspergillus clavatus*, *Bartalinia*, *Beltrania querna*, *Beltraniella humicola*, *B. odinae*, *Beltraniopsis esenbeckiae*, *Bionectria*, *Chaetomium*, *Eupenicillium parvum*, *Eupenicillium* spp., *Eurotium* spp., *Fusarium*, *Gilmaniella humicola*, *Hamigera avellanea*, *Humicola fuscoatra*, *H. grisea*, *Mammaria*, *Neocosmospora*, *Neosartorya* spp., *Nigrospora oryzae*, *Nodulisporium*, *Paecilomyces* spp., *Pestalotiopsis*, *Phoma*, *Rhizoctonia*, *Scytalidium*, *Talaromyces* spp., *Trichoderma hamatum*, *T. harzianum*, *T. koningii*, *T virens*, *T. viride* and *Xylaria*.

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543 - Structural characteristics of naturally occurring ericoid mycorrhizas on five host species from Eastern Canada

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In spite of numerous published studies, many questions remain as to the detailed cellular interactions between the symbionts in ericoid mycorrhizas, especially in hosts growing in natural habitats. Plant species with ericoid mycorrhizas produce many fine roots (termed hair roots) that become colonized by few fungal species. This colonization is restricted to the enlarged epidermal cells of hair roots. A combination of light microscopy (including differential interference contrast) and laser scanning confocal microscopy was used to document the colonization patterns of epidermal cells and to detail the hyphal complexes of five native ericaceous hosts originating from Ontario and Quebec: *Vaccinium oxycoccus* L. (bog cranberry), *Ledum groenlandicum* Oeder. (Labrador tea), *Vaccinium myrtilloides* Michx., (velvet-leaf blueberry), *Kalmia angustifolia* L. (sheep laurel), *Gaultheria procumbens* L. (wintergreen). There was considerable diversity in colonization patterns among hosts, in the morphology of hyphal complexes, and in the nature of thick-walled epidermal cells. Of particular interest was the frequency of hyphal connections between adjacent epidermal cells, indicating that there is not always one entry point for each colonized epidermal cell. Further observations of other species in the large family Ericaceae will help to determine the full range in structural details of ericoid mycorrhizas.

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544 - Tatra Mts as the area for mycological investigations - presentation of a new project

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Tatra Mts., the highest part of Carpathian range, are localized at Polish/Slovak border. Although mountains are not very high (Gerlach, 2655 m asl), all vegetation belts are well developed in this area, including subalpine and alpine zones. Tatra Mts. offer then all kinds of mountain habitats and represent excellent model region for complex studies on mountain fungi in the Carpathians. Very little is known about fungi from this massif. Several papers were published (e. g. Kubicka 1963, Frejlak 1973, Fellner, Landa 1993), but, in total only ca. 550 and ca. 900 species were reported from respectively Polish (Wojewoda 1996) and Slovak (Kuthan et al. 1999) part of the massif, and

systematic observations are completely lacking. The aim of this contribution is to present a new project, which may be important for considerations on distribution and diversity of fungi in European mountains. The systematic research, carried out throughout two growing seasons so far, has been focused mainly on lower mountain belts, but observations in dwarf pine communities and alpine meadows have also been done. After only two years of study, the number of fungi recorded from this area increased considerably and some very interesting and rare taxa were found. The most interesting recent findings will be presented e.g. *Mycena oregonensis*, *Entoloma farinogustus* and other.

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545 - Macroscopic basidiomycetes of Sevan National Park (Armenia)

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Sevan National Park was established in 1978 to protect largest in Armenia and one of the largest alpine lake in the world, lake Sevan and the surrounding areas. Overall 150.100 ha are protected, including 24.800 ha of dry land. It is located between many of mountain ranges, at the 1897-2100 m above sea level. The aim of this investigation is to study the composition of the macroscopic fungies communities of the Sevan National Park. Special attention is paid to the biodiversity of edible, poisonous and medicinal mushrooms. As a material for this investigation was the collection of fungi gathered in different part of Sevan National Park, as well as critically treated collections of the Republic's herbariums and all other literature data. As a result of the preliminary investigation and special studies of macrofungi's biota in Sevan National Park, 186 species and subspecific taxa from 10 orders, 30 families and 84 genera of Gasteromycetes, Aphyllophoroid and Agaricoid mushrooms are identified. The biota is characterized by the domination of xylotrophs, humus saprotrophs and mycorrhizal fungi. There are also very interesting and specific species for this region, some of which are rare or endangered and have to be including in Armenian Red List. New approaches to the investigation and further theoretical study in this field are required. New approaches to the investigation and further theoretical study in this field are required.

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546 - Mushrooms in Mongolia

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Mongolia is an nation located between China and Siberia. The climate is cold and dry. The steppe and the forest-steppe occupy approx. 60% of the land. And the forest occupy approx. 10% of the land. One hundred and eighty eight mushroom specimens were collected and thirty three species were identified. Mushrooms in steppe decompose humus, dung and litter. *Tricholoma monogolicum* Imai is the most famous species in Mongolian steppe. This species grows on humus and forms fairy ring. Several species of *Agaricus* are common in the steppe. The forest vegetation consists *Pinus*, *Larix*, *Betula*, *Populus* and other trees. There are many species of mushroom (*Lentinus*, *Amanita*, *Suillus*, *Russula*, *Polyporus*, etc.) in the forest. Most of them are similar to European and northern Japanese species.

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547 - The Douglas-fir epigeous ectomycorrhizal basidiomycete community in the western North American Northern Spotted Owl zone

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A joint private/USA government research team is tracking fungal species richness in montane Oregon Douglas-fir forests in two different 1998-2003 studies. Target species include all 'epi-ecto' basidiomycetes + 40 other non-ecto species flagged in the US federal Northwest Forest Plan. A chronosequence study incorporates replicate 200 m^2 permanent strip transects in 25-, 55-, and 150-year old stands sampled every 2 weeks during fall & spring fruiting seasons. The density management study comprises adjacent 65-year old stands thinned in 1999 following 5 different regimes: untreated (^450 trees/ha), thinned (3 stands with ^300, ^200, or ^100 residual trees/ha), or regeneration-cut (0 residual trees/ha). During the first 4 years, 280 (chrono) and 211 (density) target species have been identified from a total of 4,100 collections and 515 total (322 target) species. Agaricales comprise ^70%, Russulales ^20%, Gomphales ^5%, Boletales ^3%, and Cantharellales 1.5% of the target species total. Cortinariaceae comprise ^84% of the Agaricales; *Cortinarius*, *Inocybe*, *Russula* and *Phaeocollybia* represent the most species-rich target genera. After thinning, density study post/pre-treatment stand species richness ratios were significantly depressed only in the two most heavily thinned stands. Absence of modern PNW monographs has dictated frequent identification re-evaluation and led to development of temporary identification keys based on individual research papers and extralimital monographs.

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548 - Fungal community analysis using environmental genomics

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We applied culture-independent methods developed for bacteria to assess fungal community diversity across three different forest types within the Duke Forest without relying on fruitbody collections. Samples representing litter, O, A, and B soil horizons were investigated by direct DNA extraction from 50 pooled samples and cloning of DNA libraries from PCR fragments for both variable (ITS) and conserved (18S) regions within the rDNA genes. Ca. 1600 ITS sequences were produced and sorted using BLAST searches. ITS sequences with > 80% similarity to fungal groups for which extensive taxonomic databases are already available were further analyzed phylogenetically. Two hundred 18S sequences were produced, and a global phylogenetic analysis was conducted to place them in a broader framework within Eukarya. Results showed that species diversity is extremely high, with a large proportion of unique sequence types. Fungi, especially ectomycorrhizal basidiomycetes, comprise 50-75% of eukaryotic diversity in all samples. We are currently in the process of developing automated databasing and bioinformatic tools to improve our ability to characterize fungal communities. Given the relative ease of sequence data collection, these tools should enable cross-platform comparisons of microbial biodiversity studies, including functional ecology and changes in microbial communities.

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549 - Studies in Rocky Mountain alpine *Laccaria* (Basidiomycota, Agaricales, Tricholomataceae)

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Documenting the diversity of gilled fungi in the North American alpine zone is a primary goal of the National Science Foundation Biotic Surveys and Inventories - sponsored Rocky Mountain Alpine Mycota project. As part of this project, a detailed study of the genus *Laccaria* was conducted using material collected at field sites above treeline in four mountain ranges located in the central and northern Rocky Mountains. Several *Laccaria* species are common components of the alpine mycota, appearing to be important ectomycorrhizal symbionts of the alpine dwarf willows *Salix reticulata* and *S. arctica* and shrub willows *S. planifolia* and *S. glauca*. Four taxa were identified on the basis of macro- and micromorphological characters: *L. montana*, *L. pumila*, *L.* cf. *bicolor*, and *L.* cf. *laccata* var. *pallidifolia*. Of these taxa, *L. montana* is reported only from the northern Rocky Mountain alpine sites, *L.* cf. *bicolor* and *L.* cf. *laccata* var. *pallidifolia* only from the central Rocky Mountains, and *L. pumila* from all four mountain ranges. *L. proxima*, collected at a subalpine site close to the northern field sites and in association with *Salix* shrubs, was not found above treeline. Ribosomal ITS DNA sequences are being analyzed to study biogeographic and phylogenetic patterns. The taxonomy, distributions, and ecology of Rocky Mountain alpine *Laccaria* species will be discussed.

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550 - Ribosomal DNA PCR-RFLP analysis of anaerobic fungi isolated from rumen and faeces

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Anaerobic fungi inhabit in the digestive tract of most herbivores and they can be isolated from gastro intestinal tact and faeces of these animals. In this study we aimed to identify anaerobic fungi by PCR-RFLP analysis of ITS region. Fresh, frozen (1 month) and fresh rumen digesta of 3 cows were used as fungal source. Fungal cultures (n=198 tubes) from highest dilution series of most probable number (MPN) technique, which gives rise to axenic cultures proliferate from a single cell in the lowest level of dilution, were used as DNA source. Primers ITS1F (CTT GGT CAT TTA GAG GAA GTA A) and BlastITS4 (TCC TCC GCT TAT TAA TAT GC) were used for PCR amplification. Successfully amplified samples (133/198) produced a single PCR product with ca 960 bp therefore it was not possible to identify the isolate at even genus level using size of the ITS region alone. Using of restriction enzyme *Dra*I, which digested all samples at least once, gave rise to 12 different ribotypes. Some ribotypes groups were found large (e.g. 45 isolates in one group) whilst few ribotypes included only a single isolate. Our results showed that PCR-RFLP analysis has a potential use in anaerobic fungal discrimination at least to the genera level.

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551 - The role of taxonomists in scientific reproducibility

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It is generally believed by the scientific community that the culture collections and herbaria are managed by trained taxonomists who ensure the purity and authenticity of the research materials prior to their supply. The quality of services provided by such centres are generally of high standard vis-à-vis those which lack the expertise. Unfortunately, the taxonomists have to prove, time and again, that their science is not old-fashioned and intellectually dull but is the bed rock of all biological research. Nevertheless, based on an appeal by a group of taxonomists and with scientific reproducibility gathering momentum, the editors of some reputed microbiology journals have made it a mandatory requirement of providing deposit details of working materials with recognized culture collection centres or herbaria before any paper is accepted for publication. We propose to discuss a few interesting cases encountered with our centre relevant to the points mentioned above and the options available with the editors of journals for the strict compliance of their latest guidelines.

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552 - The spectrum of macrofungi in beech forests under different air pollution pressure

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The base of our investigation is an assumption about affection of mycoflora structure by air pollution. The occurence of the ectomycorrhizal, saprobic and parazitic fungi was observed during three years at three beech stands. Conditions for fungi growth were nerly compatible on all stands, difference was only in degree of pollution pressure. Number of fungal species and fruitbodies, together with other characteristics of investigated stands (health condition of trees, soil analysis, climatic conditions), were analysed using mathematic-statistical methods. In this way factors affecting a fungi production in beech forest exposing to various pollution pressure was choosen. An important result of this work is a possibility to use the macromycetes for bioindication of various polluted beech forest stands: - The share of mycorrhizal species significantly decreases and the share of saprobic ones significantly increases at beech stands under pollution pressure; - The mycorrhizal species of genus *Cortinarius*, *Cantharellus*, *Hydnum*, *Ramaria* and *Tricholoma* are practically missing at these stands; - At the same time the number of saprobic species of genus *Clitocybe*, *Collybia*, *Coprinus* and *Lepista* expressively increases.

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553 - Refuges of heat-demanding polypore fungi near geothermal waters in Pribaikalia

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Refuges of polypore fungi which are located near geothermal waters of Pribaikalia, are distinguished by the degree of local heat-provision and the history of site evolution. Four types of refuges in the mountainous areas of Pribaikalia have been indentified. The fourth type includes sites in which all the nemoral complexes have have been preserved and concentrated in the warmer steppe landscapes. The site near Selenga river which includes broad-leaf associations of relics nemoral Japanese Elm (Pleshanov, Penzina, 1996). Species composition of the first zone is characterized by xerophylies: *Gloeophyllum sepiarium* (Wulf.:Fr.) Karst., *G. trabeum* (Pers.:Fr.) Murr., *Trametes ochracea* (Pers.) Gilbn.& Ryv. It is a zone of direct influence of hot springs. It is necessary to consider this biotope as an extreme for wood attaching associations - the wood is occupied by fungi for a short period, hypothetically, in the winter, when the spring water is natually cooler. The biotopes of the Selenga floodplain, which belong to the second zone of refuges, are distinguished by the maximum size of the fruiting bodies of the perennial polypore fungi in Pribaikalia, as well as a wealth of species diversity and structure of myco-community. Striking representatives of the thermophylic complex, which have been discovered in relict Elm forest, are *Trametes conchifer* (Schw.:Fr.) Pil. on fallen branches of Elm, and *Inonotus hispidus* (Bul.:Fr.) Karst., living on the trunks of live Elm trees.

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554 - New reports of polypores from the Dominican Republic, Greater Antilles

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Ciferri (1929a) and Murrill (1915) reported 17 species of polypores in 13 genera from the Dominican Republic according to modern taxonomy. We surveyed polypores in the Dominican Republic beginning in 1996. Decock & Ryvarden (2000) described *Perenniporia cremeopora* and Lodge, Ryvarden & Perdomo (2001) described *Antrodia aurantia* from the DR. Minter et al. (2001) listed 80 of our new records. In addition to the published records noted above, we report here six new species records for the Greater Antilles: *Antrodiella parasitica*, *Chaetoporellus latitans*, *Gloeoporus dichrous*, *Inonotus* cf. *micantissimus*,

Skeletocutis carneola and *S. vulgaris*. We report here an additional 30 species that are new records for the Dominican Republic: *Amylosporus campbellii, Antrodia malicola, A. sinuosa, Antrodiella hydrophila, Aurificaria luteo-umbrina, Ceriporia purpurea, Coriolopsis floccosa, Cyclomyces iodinus, C. tabacinus, Daedalea aethalodes, Fomitopsis spraguei, Gloeophyllum abietinum, G. mexicanum, Hapalopilus salmonicolor, Inonotus hispidus, Perenniporia* cf. *tephropora, Phellinus callimorphus, P. ferrugineovelutinus, P. linteus, P. merillii, P. orientalis, P. pini, P. robustus, Rigidoporus concrescens, Skeletocutis* cf. *carneogrisea, Trametes lactinea, Trichaptum perrottetii, T. sector, Tyromyces leucomallus* and *T. venustus*. Including the new records from this study, there are 133 species in 46 genera of polypores known from the DR. Part of each collection is deposited at JBSD and O.

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555 - Screening for lichenicolous fungi of *Usnea*

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Fungi were isolated from individuals of a species of *Usnea* (*U.* cf. *subfloridana*) at three sites in the European Alps, one of which was sampled thrice. Under semi-sterile conditions small pieces of the lichen were transferred in the field to various culture media, torn apart and spread over the agar plate. In the laboratory, growing hyphae were excised and transferred to fresh media until pure cultures were obtained. From these strains DNA was isolated and the SSU and ITS regions of the nrDNA were sequenced. The SSU sequences were aligned with about 1000 fungal sequences from GenBank and a phylogenetic tree, being presented, was calculated. A strain was assigned to a certain taxonomic group when its sequence clustered as ingroup in a clade consisting exclusively of taxa of the same taxonomic group according to the current classification. As a result, three of the 26 isolated fungi were identified as Basidiomycetes, whereas the remaining taxa belong to the Pezizomycotina (Ascomycetes). Of the latter ones 17 strains could be assigned to the following classes: Chaetothyriomycetes, Dothideomycetes, Eurotiomycetes, Lecanoromycetes, Leotiomycetes and Sordariomycetes and nine to the orders Chaetothyriales, Diaporthales, Eurotiales, Lecanorales and Xylariales. Five strains were identified at family level (Parmeliaceae, Trichocomaceae and Valsaceae). In addition, the nearest - yet sequenced - relatives of the isolated fungi were specified by an ITS nrDNA blast search in GenBank.

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556 - Endophytic fungi isolated from maize (*Zea mays*) and their possible role for biological control

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Endophytic fungi were isolated from leaves and stems of maize collected from plants growing in greenhouse and field conditions. Samples were collected after 20 and 40 days seed germination. Nine main genera of fungi were isolated and the most frequent genera found were *Colletotrichum*, *Fusarium* and *Acremonium*. From 1728 plant fragments quantitative significative differences were found being field plants about three times more intensly colonized by endophytes when compared to greenhouse plants. No significative differences were encoutered from number and genera of fungi collected from 40 days old plants when compared to 20 days old ones. Endophytes from the genera *Beauveria* and *Paecilomyces* were isolated both from fiels and greenhouse plants. The *Beauveria* isolates were classified as *B. bassiana* but molecular analysis showed that they differ from the main *B.bassiana* strains used in Brazil for insect biological control. *B. bassiana* isolates were tested against maize pest insects and against the bovine tick *Boophilus microplus*. Bioassays have shown that endophytic isolates were as effective as comercial strains using concentrations varying from 100 to 100 millions conidia/mL, the last being the most effective causing up to 75% mortality in comparison with the controls. Our result support the possible biological control role of endophytic entomopathogenic fungi as being suggested already by other authors.

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557 - New and interesting findings of macrofungi from the islands of Andros and Naxos (Cyclades, Greece)

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Reports on the biodiversity of macromycetes in islands and coastal areas of southeastern Mediterranean are practically non-existent. Investigation of typical biotopes of Andros and Naxos (the two largest islands of Cyclades in the Aegean archipelago), e.g. xerophytic heaths, sea-shore grasslands, dunes and maquis, resulted in significant records. The agaricoid taxa *Conocybe rickenii*, *Crepidotus epibryus*, *Lepista rickenii*, *Macrolepiota phaeodisca*, *Marasmiellus rosellus*, *Marasmius anomalus* var.

microsporus, *M. wynnei* f. *carpathicus*, *Melanoleuca polioleuca*, *Mycena zephirus*, *Panaeolus cintulus*, *Psathyrella microrhiza* and *Tubaria autochthona* were recorded for the first time in Greece, while *Ceratobasidium cornigerum*, *Mycoaciella bispora*, *Myxarium laccatum* and *Ramicola haustellaris* represent first reports for the respective genera in Greece. Of particular interest are records of lignicolous species from Mediterranean evergreen scrubs and frygana, i.e. *Hyphodontia juniperi* and *H. sambuci* on *Thymus capitatus*; *Peniophora lycii*, *P. pithya* and *P. tamaricicola* on *Pistacia lentiscus*; *Aleurodiscus dextrinoideocerussatus*, *Hyphodontia pruni*, *Peniophora lycii*, *Steccherinum fimbriatum* and *S. ochraceum* on *Genista acanthoclada*; *Aleurodiscus minor* and *Lentinellus omphalodes* on *Cistus* sp.; *Gloeophyllum abietinum*, *Perenniporia rosmarini* and *Peniophora piceae* on *Juniperus phoenicea*; and *Dacrymyces lacrymalis* and *Pluteus nanus* f. *nanus* on *Nerium oleander*.

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558 - BIOTEC Culture Collection (BCC) in Thailand

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BIOTEC Culture Collection is a specialized collection formally established in 1996 under the National Science and Technology Development Agency (NSTDA). The principle objective is to provide safe deposition of cultures for researchers; to preserve and maintain microorganisms isolated from Thailand for future use; and to manage strain data information. Another major role is to supply cultures and related information for an in-house screening programme for drug discovery from microorganisms. The collection comprises 10,000 isolates fungi from various sources and habitats: insect fungi (1,618), aquatic freshwater and marine habitats (1,324), rice blast fungi (1,148), soil including leaf litter and humus (973), wood-decay Xylariaceae and Basidiomycota (898), plant seeds (389 strains), endophytic fungi (754), lichens (247) and taxa from other sources (1,090). The collection also includes bacteria and actinomycetes (1,789) and yeasts (342). The preservation methods used are freezing at -80 °C, in a vapor phase of liquid nitrogen at -170 °C; freeze-drying and under liquid paraffin oil. The essential strain data of cultures being maintained are used to create a required database and produce the catalogue of cultures. On-line database is now available at URL: http://bcc.biotec.or.th/.

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559 - Taxonomic verification of basidiomycetes cultures in the LE (BIN) collection

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The Komarov Botanical Institute Basidiomycetes Culture Collection - LE (BIN) maintains over 1000 strains of 428 species of aphyllophoroid, agaricoid and gasteromycetoid macromycetes collected mainly in Russia and former USSR. Conservation of Basidiomycetes diversity *ex situ* with a special interest in rare and endangered species as well as cultural maintenance of mushroom taxa with biological activity, especially those could be used in medicine and industry, is one of our primary goals. Taxonomic verification of Basidiomycetes cultures maintained in the culture collections is of great importance. In the process of starting and transferring living cultures error can be introduced in two ways. First, cultures can be obtained from incorrectly identified basidiomata or the identification has been done according to an out of date classification. Recent studies on *Flammulina* and *Pleurotus* species enable us to carry out taxonomic verification of LE (BIN) cultures from these genera by mating study according to the biological species concept. Second, replacement of a Basidiomycetes culture with a a different species, mainly from the group of imperfect fungi, can occur during the strain isolation or subculturing. This often happens in culturing of slowly growing and ectomycorrhizal fungi. To veryfy a cultural strain's identity and purity detailed studies of cultural characters, fruiting of strains in culture and molecular analysis are being used in the LE(BIN) Collection.

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560 - Contribution of hyphal length in predicting P influx in maize and groundnut under field condition

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When P is deficient in soil solution, the critical root parameter controlling its uptake is its surface area. Hyphae of mycorrhizal fungi have the potential to greatly increase the absorbing surface area of the root. Modified gridline intersect method was employed for extracting hyphae from the soil and quantifying length under field conditions in a Benomyl treated and non treated soil with the following three P levels: P 0,P 50 and P 400mgkg-1 soil as single super phosphate. To asses the importance of mycorrhizal hypha nutrient uptake model was used. Wettable powder of the fungicide Benomyl was mixed thoroughly to 15 cm soil depth at 500 kg ha-1. Treated plots were covered by paddy straw for two weeks to avoid excessive heating. There was significant interaction between benomyl treatment and phosphorus application on hyphal length. P 0 recorded maximum hyphal length and was significantly higher as compared to other P application levels for both the crops. Maximum hyphal length was observed for maize crop than groundnut. When hyphal length was included in model calculations it gave better prediction of P influx in to the roots of maize and groundnut. When hyphal length was not included in model calculation, under prediction of P influx was more affected at lower P levels. It indicates that P solubilization by root exudates might have played more important role than AM in P uptake by maize and groundnut.

561 - Rare taxa in Estonian lichen flora - are they really rare?

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The recent checklist of lichenized and lichenicolous fungi of Estonia (Randlane & Saag 1999), which is mainly based on herbarium material, contains 851 species from 200 genera. Latest data available (http://www.ut.ee/lichens/fce.html) indicate the presence of 909 species in Estonia. In the checklist, the distribution of taxa in five regions of Estonia and their frequency according to six frequency classes has been presented. Rare species with 10 or less localities form 64% of the entire Estonian lichen flora. The analogous figure for vascular plants in Estonia is 36% and for bryophytes - 45%. The unusually large share of rare lichen taxa is partly due to the great diversity of different habitats and substrata suitable for lichenized fungi. Still, these data might be misleading because of the unsufficient recording of different species. Several new localities have been reported for 'rare' species during the last three years; six lichens that were considered to be extinct have been re-found. The analysis of world-wide distributional patterns of taxa would help us sort out these species which are locally really rare (and therefore also endangered) because they occur at the periphery of their range. In Estonia, e.g. *Arctoparmelia incurva*, *Caloplaca jungermanniae*, *Flavocetraria cucullata* occur at their southern limits while *Cladonia convoluta*, *Flavoparmelia caperata*, *Phaeophyscia chloantha* are recorded at the northern limits of their distribution.

562 - Conservation and valorisation of *Pleurotus* spp. germ plams

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Germ plasm collection must assure the conservation of genetic diversity also in view of their possible technological application. To be exchanged and used, germ plasm must be characterized by an univocal fingerprint able to assure its identity and stability. This is an important condition for comparing the results obtained in different studies. *Pleurotus* spp. are widespread populations among them, *Pleurotus eryngii*, *P. e. ferulae* and *P. e. nebrodensis* are typical Mediterranean populations. The International Bank of Edible Saprophytic Mushrooms of Italian National Council of Research owns 36 isolates of *P. ostreatus*, 313 of *P. eryngii*, 48 of *P. e. ferulae* and 22 of *P. e. nebrodensis*. These isolates were collected in view of their applications in biotechnologies as agents able to transform agricultural wastes into both an edible biomass and starting material for high value products extraction. Polysaccharides in particular 3 β glucans have been purified. The isolates, supplied by Research Institute donors, have been genetically characterised by RAPDs fingerprint. Moreover genetic approaches aimed at evaluating population biodiversity and genetic relationship among species are running with molecular markers as microsatellites, AFLP, and RFLP of amplified ITS and IGS rDNA region. A good level of variability in *P. eryngii* and in the correlated population/species *P. e. ferulae* and *P. e. nebrodensis* have been evaluated. A similar behaviour has been made evident also in *P. ostreatus*.

563 - Comparative diversity of vesicular arbuscular mycorrhizas in New Zealand vineyards under organic and conventional management regimes

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Many economically important plant species form complex symbioses with vesicular arbuscular mycorrhizal (VAM) fungi. Considerable research data indicates that these fungi impact on productivity, nutrient acquisition, pathogen defence and ecosystem stability. In natural systems, grapevines are normally infected with VAM and these associations have been shown to improve grape growth. In this study, soil samples were removed from the base of three common grapevine rootstock varieties at two South Island vineyards, one under conventional management, and the other under organic production (certified in 2000). Spores were collected by wet sieving, the genera identified by microscopic examination and expressed on a per gram basis. The individual VAM species present were determined using a combination of morphological criteria and sequencing of the ribosomal DNA. Root fragments were also cleared, stained and assessed for VAM colonisation. Implications of VAM diversity in New Zealand vineyards and the effect of management systems on population structures are discussed.

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564 - Diversity and conservation of fungi on Danube flood-plain forests

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River floodplain forests become extremely endangered in last century and there are only scarce data on fungi of those habitats. Field research on biodiversity of macrofungi in two Danube islands, Sihot' and Sedláckov ostrov (city of Bratislava) resulted in 211 species of Ascomycota and Basidiomycota. Following taxa recorded during our research are, according to the 3rd edition of the Red List of Slovak fungi, endangered: *Ceriporia purpurea*, *Crepidotus crocophyllus*, *Hypoxylon ticinense*, *Hyphodontia latitans*, *Marasmius minutus*, *Ossicaulis lignatilis*, *Oxyporus latemarginatus*, *Oxyporus philadelphi*, *Phlebia ryvardenii*, *Rhodotus palmatus*, *Scutellinia legaliae*, *Scutellinia sinensis*, *Spongipellis fractipes*, *Trichophaea variornata*, *Trichophaeopsis bicuspis*.

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565 - Different approaches on rice blast disease in Italy: an *in vitro* assay for assessing pathogenicity of the Italian *Pyricularia grisea* collection

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Blast disease, caused by *Pyricularia grisea* (teleomorph: *Magnaporthe grisea*), represents the main threat to rice crop. Blast pathogen population is divided into races, or lineages, defined according to virulence spectrum on differential rice cultivars; unfortunately, pathogenicity of races identified in a country cannot be compared with that of races in other countries, and *in vivo* screening procedures are both complex and time-consuming. Italian lineages have been only partially investigated, and a lot of work is still needed as many (125) rice cultivars are grown in Italy. A survey on a collection of *P. grisea* isolates, currently in progress in our laboratories, is the first step to characterise the Italian population. To date 103 strains have been isolated, and are available for further analysis. In order to characterise fungal isolates different methods (greenhouse and molecular screening, *in vitro* assays) could be used. An *in vitro* test was set up using partially purified elicitors, instead of viable pathogen, and rice calli. Suspension cultures of two rice cultivars (Gigante Vercelli and Maratelli, resistant and susceptible to blast, respectively) were used. In all cases a sharp rise of hydrogen peroxide production was found soon after elicitor addition. Interestingly, both control and elicited rates were 3 to 5-fold higher in the tolerant cultivar than in the susceptible one, thus strengthening the possibility to use this model to simulate *in vivo* infection.

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566 - Wild mushrooms: valorisation and conservation

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In recent years, in Portugal, the harvest of wild edible mushrooms has intensified greatly motivated by commercial valorisation of this resource in the international markets. The reduction of the mycological patrimony, and biodiversity in general, is mentioned as the main consequence of this process. The absence of regulation that discipline the harvest and commercialisation of wild edible mushrooms, as well as the inexistence of a global strategy that promotes the conservation, management and sustainable use of the fungus species and its habitat, are considered as the main factors that contribute to this situation. In this area, representatives of several entities under the coordination of the Instituto da Conservação da Natureza (Nature Conservation Institute) elaborated a proposal of intervention that created guidelines and indispensable measures to conserve and regulate the mycological resources. Among the several measures, suggested are highlighted: urgent regulation of the harvest, reception, concentration and commercialisation of wild edible mushrooms; creation of a good practices code; implementation of measures to promote the knowledge on portuguese fungal diversity and monotorising of particular interesting species; promotion of training courses, sensibilisation and environmental education which emphasize the ecological and economical importance of fungi. The present work intends, in a summary form, to promote the main aspects that resulted from this initiative.

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567 - Measuring macrofungal diversity in four Portuguese protected areas

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The struggle to bring mycological patrimony to the list of issues involved in biological research and conservation

prioritiy, and as an important item to the management of protected areas, is recent in Portugal. The increasing perception that the harvest of wild mushrooms has greatly intensified in recent years and the negative influences both on general biodiversity and on mycological diversity that it might have, and also the general lack of knowledge on Portuguese fungal patrimony, were the main reasons to initiate a project involving four protected areas in Central and Northern Portugal coordinated by the Portuguese Institute for the Conservation of Nature. This project involves the measurement of macrofungal diversity in several priority habitats listed in the Habitats Directive using a common sampling method. The macrofungal data will be complemented with environmental and vegetation parameters necessary for the assessment of the micota's ecological context.

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568 - Foliicolous lichens of La Réunion

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The foliicolous lichen flora of the French island La Réunion was investigated based on 113 leaf samples collected at seven localities. They represent different types of rain forest at altitudes from 160 to 1700 m. 27 species were identified, of which 16 are new to La Réunion. *Tricharia amazonum* is reported for the first time outside the neotropics, and *Porina atriceps* and *Badimia pallidula* are new to the African continent with adjacent islands.

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569 - Vertical distribution of ectomycorrhizal fungi in a podzol profile

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Ectomycorrhizal (ECM) fungi form symbiotic associations with roots of woody plants and are important in nutrient uptake in boreal forests. Podzols are highly stratified soils. Most studies of ECM species composition focus on the fine roots in the organic soil. The variation in ECM community structure between different soil horizons is thus poorly understood. We studied the vertical distribution of ECM roots in a podzol soil in the north of Sweden. In three continuous soil columns, seven horizons were distinguished and sampled, from the top organic layer to the bottom parental material at 50 cm depth. Root tips from each horizon were sorted into morphotypic groups. These were verified and identified by sequencing of the rDNA ITS region. Root tip density decreased from the O horizon down to the E2 horizon but increased again in the B horizon before declining to low values in the C horizon. Half of the root tips were found in the mineral soil. The degree of ECM colonization varied from 60 to 98%, but did not follow a clear pattern with respect to depth. We distinguished 22 ECM taxa, half of these were found only in the mineral horizons. The abundance of taxa in different horizons was analysed by correspondence analysis. We found a significant relationship between ECM taxon and soil horizon. The different horizons in a podzol profile represent different environments and the specific distribution of certain fungi could be explained by their functional role in the system.

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570 - Leprose lichens in Estonia

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Over 60 usually sterile sorediate crustaceous lichen species have been registered from Estonia. Such species occur in many genera which ordinarily produce ascocarps. Majority of the constantly asexual taxa are gathered into g. *Lepraria* and g. *Leproloma* (*Lichenes Imperfecti*), some of them have tentatively been assigned to other genera (*Fuscidea pusilla* Tonsberg, *Lecanora norvegica* Tonsberg et al.). The first checklist of Estonian lichens published in 1970 included 8 *Lepraria* species, only one of them - *L. incana* - belongs to that genus nowadays. Primitive morphology and often complex secondary chemistry of representatives of the leprose growth form makes their identification complicated and chemical analysis (TLC) essential. Currently 11 *Lepraria* and 1 *Leproloma* species are reported from Estonia, occurrence of lately described *Lepraria atlantica* Orange is in question.

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571 - Experimental study for increasing productivity of *Boletus edulis* s.l. in Italy

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Species grouped together as *Boletus edulis* s.l. are the mushrooms in highest demand and best known in Italy: they are a typical minor forest product of the hill and mountain belt of the penisula, of major economic importance in some cases surpassing the commercial value of timber. More than 140 ectomycorrizhal fungi are regarded as edible but only a small percentage have been successfully cultivated. For the other species, including the highly prized penny bun mushroom, no technique of stable inoculation on a regular basis in the host plant has jet been found. This means that the entire demand for this product, which amounts to more than 70,000 tons/year in Italy is met by mushrooms gathered in the wild. The present study proposes to clarify the ecological requirements of these species through manipulation of certain environmental parameters, in order to provide indications for environmental management aimed at conserving the ideal habitat of this culinary delicacy. The study area consists of four reforestations in two age classes (30 and 60 years) of *Abies alba* Miller on the Mt. Amiata (Tuscany, Italy). This area is historically famous for its mushrooms, but has recorded a decline in production in recent years. Two years of preliminary studies have shown that certain conditions are necessary for increasing productivity of *B. edulis* s.l. in these woods: forest age must not exceed 50 years, forest density should be intermediate and litter should be maintained.

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572 - An update on the biogeographical distribution area of the genus *Anthracoidea* (Ustilaginomycetidae) in light of new species

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Fungal samples loaned from herbaria were examined with light and scanning electron microscopy. The material consisted of various plant parasitic fungi on inflorescences of *Carex* spp. from all over the world. Most of the fungi were identified as belonging to the smut genus *Anthracoidea*. After spore morphological comparisons, the conclusion of the study was that several *Anthracoidea* species new to science can be described. The smut flora of Europe is well known and hence, new species from European material could not be found. All other areas are poorly studied despite of some published local smut surveys. New species could be found from Asia, North and South America. The distribution areas of host plants and their biodiversity are very likely to have a profound effect on their parasitic fungi. Compared to Europe many parts of Asia and America have a much richer sedge flora but still fairly few species of *Anthracoidea* have been found. Reasons for this are certainly manifold. Numerous species occurring on related sedge plants are reported to be common to e.g. Europe and Asia or Europe and North America. However, *Anthracoidea* flora of Central Asia is seemingly very unique and differs from Northern or Eastern Asian flora. On the other hand, some sedges may bear different smut species but they occur in different geographical areas. Some interesting examples will be presented with area maps.

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573 - Mycorrhizal interaction in the wild relatives of grain legumes in Palney Hills of Western Ghats of South India

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Pulse crops have certain uniform features which together make them indespensible if not difficult to replace. In India, as many as 28 wild legumes are known to be consumed as pulses by different tribal communities. The importance of legumes with arbuscular mycorrhizae are well defined. But available information on wild legumes is highly limited, especially in Indian scenario. The diversity of mycorrhizal fungi associated directly with wild legumes is not well understood. Estimation of mycorrhizal fungi in rhizosphere has little relationship with colonization of roots by individual species. Our presentation will discuss a complete analysis of three interactions including growth and biochemical variations with reference to natural landscape conditions.

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574 - Entolomatoid fungi from Korup National Park, Cameroon

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The Korup National Park consists of ancient, mainly lowland, rain forest in the South West Province of Cameroon and is acknowledged to be one of the most species-diverse areas in equatorial Africa. Macrofungi were collected in April - May 1996 and March - April 1997, at the beginning of the rainy season. As a result of these visits, an initial three papers have been published on the clavarioid fungi, heterobasidiomycetes, and corticioid fungi of the National Park. Several collections were made of entolomatoid agarics, representing at least 20 taxa. These are now being examined at the Royal Botanic Gardens, Kew, and provisional information, including descriptions and photographs, is presented here. It is hoped this work will result in the identification of some previously undescribed taxa and will provide essential baseline data for the better understanding of fungal biodiversity in the African wet tropics.

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575 - Micromycetes isolated from air and soil in an Italian carsic cave

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Caves are an hostile habitat to biota: do not support plant life and few animal species are adapted to live inside. Very little investigation has been devoted to fungal ecology of caverns and it is believed that no species is peculiar of this habitat. Micromycetes present in an Italian carsic showcave, situated at Toirano (SV), have been studied. Sixteen sites were sampled in a distance of about 400 metres from the entrance. Air sampling was carried out by settle plate technique and by surface air sampler (ParTrap Fa 52); soil analysis by hair baiting technique. Statistical analysis of the results, involving chi-square, has been performed. A total of 76 species, belonging to 47 genera, was recorded. *Alternaria alternata*, *Aureobasidium pullulans* var. *pullulans*, *Cladosporium cladosporioides*, *C. herbarum*, *Penicillium brevicompactum*, *Trichophyton terrestre* and *Simplicillium lamellicola* (abundant because it is involved in the process of stalactite growth), were dominants. Among the sporadic species, should be noticed *Conidiobolus coronatus*, mostly saprotrophic but also insect parasite and causal agent of nasal granuloma in man. Comparison between the number of species and the different methodological techniques used results statistically significant. In conclusion, mycoflora in a cave can be rather diversified; that can be mainly due to the atmospheric circulation and the presence of animals or their debris.

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576 - Chemotaxonomic groups in species of *Phyllosticta* based on the cell wall polysaccharides

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Phyllosticta is a Coelomycetous fungus producing pycnidial fruit bodies and causing leaf spot diseases in plants. It is a dimorph fungus having both perfect and imperfect states in their life cycle. Studies on the various species of *Phyllosticta* were collected from South India has been taken with a view to build up a genetic resources collection in our centre. For this purpose, fungal collections were made from various places in and around Tamilnadu, South India. So far more than 50 different species of *Phyllosticta* were collected and 30 of them were successfully grown in culture. In the case of *Phyllosticta*, identification of species is merely based upon the host specificity. But it is worthwhile to study the composition of cell wall polysaccharides along with the combination of morphological characters make an advantage in identifying the higher taxa at species level. In the present study cell wall Polysaccharides have been proved as chemotaxonomic markers used in the determination of natural groupings in species of *Phyllosticta*. In order to find out the chemical composition and structures of polysaccharides (alkali extractable & water-soluble) of cell wall and culture filtrate of the fungi were carried out with the above isolates. The inter specific relationship of different species of *Phyllosticta* was compared for the species studied based on the polysaccharide type of the cell wall. The results and significance of the findings are discussed in detail.

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577 - Mycorrhizal and ethanopharmacological value of some threatened orchid species of Shevaroy's, S. India

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Orchid mycorrhizas are symbiotic organisms, which establish an obligate symbiotic association with the roots of orchids. The orchid mycorrhizas represent the most widespread and the large majority of orchids must have evolved with compatibility system towards the fungal symbionts. In India, more than 2000 species of orchids have so far been reported in which mostly belong to epiphytic form received poor considerations than terrestrial form. All the threatened species of orchid under study penetrated an intracellular fungus called pelotons. The fungal hyphae may traverse various layers of velamen before entering into passage cells. From velamen itself the hyphae forming pelotons followed by exodermal cells and finally it reaches the interior cortex. Different histochemical, histoenzymological and fluorescent techniques were applied on peloton of the species viz. *Bulbophyllum kaintense* (Weight) Reichb.F., *Coelogyne nervosa* A.Rich and *Gastrochillus acaulis* (Lindley) Kuntz. However, antibacterial activity of organic and aquous extracts (leaf, root) of the former was carried out against different bacterial strains. From this study we can conclude that these threatened orchid species showed high antibacterial activity against the tested bacteria. These findings strongly imply that the involved orchid species is a major source for different ailments. Supported by: Department of Science and Technology, Government of India, No:SR/FTP/LS-217/2000.

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578 - Micromycetes in book depositories

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The micromycetes inhabiting air and the surface of the books were studied in 7 large book depositories of the National Library of Russia (Saint-Petersburg). The study was performed during 1995-2001. Micromycetes were isolated from numerous characteristic points and investigated in vitro. Total concentration of airborne fungal spores were examined. Furthermore physical characterististics of studied lodgings measured too. Total of 100 fungal species were recorded. The list included species belonging to 33 genera, mainly from Deuteromycetes. Some of them had a wide occurance, whereas for some species it was the rather limited. The dominant and the rare species were indicated. Most likely the distinction of the fungal microcommunities in the studied book depositories usually were resulting from presence of accidental species. Season changes on fungal composition of revealed microcommunities were also recorded. Considerable fluctuations of concentration of airborne fungi depended upon ecological factors variation. Between them were tempaterature, humidity and chemical properties of paper type. There were determined the conditions increasing the content of viable and conversely, factors completely inhibiting the concentration of micromycetes in book depositories.

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579 - Biodiversity of keratinophyles in Heritage Taj City- Agra (India)

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Abstract In present investigation 360 soil samples from urban and rural areas of Agra, were analysed. Out of which 296 were recorded positive for urban area and 238 for rural area. The percentage prevalence of dermatophytic and keratinophilic fungi were calculated 82.2% and 66.7% for urban and rural areas respectively. Total 21 species were recorded and isolated from baits amended soil collected different sites, out of which 9 sps were common in both sites. However, their prevalence varies with respect to sites. The major fungal species isolated from urban sites includes *Chrysosporium* (11 sps), *Trichophyton* (5 sps), while from rural area includes *Chrysosporium* (5 sps), *Trichophyton* (4 sps), *Myceliophthora* (2 sps), *Epidermophyton* (1 sps) and *Microsporum* (1 sps). The maximum percentage prevalence of *C. carmichaelii* was observed in urban are (22.2%). While in rural areas *M. vellerea* was recorded. Thus, it is concluded that maximum prevalence of dermatophytes were recorded in rural area due to prevailing unhygienic conditions as compared to urban areas.

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580 - Biodiversity of arbuscular mycorrhizal fungi in Indonesia

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This paper reviews the results of several studies on the biodiversity of arbuscular mycorrhizal (AM) fungi in a wide range of ecosystems in Indonesia, including natural forest, agricultural crops, plantation crops, botanic garden and peat swamp forest. *Glomus* and *Acaulospora* are the most common genera found in those ecosystems. At least 33 spesies of AM fungi in the 39 locations surveyed were described. *Glomus* with 17 species comprises *G. aggregatum, G. albidum, G. citricolum, G. clavisporum, G. etunicatum, G. fasciculatum, G. fuegianum, G. geosporum, G. lacteum, G. macrocarpum, G. manihotis, G. microaggregatum, G. microcarpum, G. multicaulis, G. rubiforme, G. sinuosum* and *G. versiforme*. *Acaulospora* with 10 species comprise *A. bireticulata, A. delicata, A. foveata, A. longula, A. mellea, A. rehmii, A. scrobiculata, A. spinosa, A. tuberculata*, and *A. walkeri*. *Scutellospora* with 4 species comprise *S. calospora, S. heterogama, S. pellucida*, and *S. projectura*. The genera *Entrophospora* and *Gigaspora* are represented with one species each, *Entrophospora infrequens* and *Gigaspora gigantea*. *Acaulospora walkeri* and *Scutellospora projectura* are the two new species reported from Indonesia. The number of AMF species varies from ecosystem to ecosystem, suggesting that their biodiversity can be influenced by soil conditions, cultural practices and vegetation types.

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581 - Diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana

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Endophytic fungi were isolated from living symptomless leaves of 12 tree species from two locations in the Iwokrama Forest Reserve, Guyana. 64 fungal morphotaxa were characterized from 2492 cultures, which were derived from a total of 2520 sample units. Species of *Colletotrichum*, *Nodulisporium*, *Pestalotiopsis* and

Phomopsis were most frequently isolated. Colonization was greater in samples from the midrib than in those from laminar tissue, and slightly greater at the tip of the lamina compared with the base of the leaf. In contrast to studies in temperate ecosystems, no distinct fungal communities were identified for individual plant species, suggesting that the degree of host preference is low. The implications for estimation of fungal diversity in tropical systems are explored.

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582 - Conservation status of the Agaricales, Boletales, Cantharellales, Lycoperdales, Phallales and Russulales of South Australia

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The conservation status of 443 species of Holobasidiomycetidae known from the State of South Australia in southern Australia has been evaluated. The World Conservation Union Red List criteria endorsed in 1994 were found to be inappropriate as the decision rules are based on quantitative threshholds of population size, distributional ranges, rates of decline and extinction risk. For most of the past 70 years there has been no taxonomic mycologist working on the Agaricales, Boletales, Cantharellales, Lycoperdales, Phallales or Russulales of South Australia. Therefore few quantitative data were available. We adopted a modified version of the system used to prepare the list of Rare or Threatened Australian Plants (ROTAP). In the ROTAP system threat is qualitatively assessed according to available expert knowledge. Species known only from the holotype, lectotype or neotype collection constitute 22% of the taxa studied. Ectomycorrhizal fungi comprise 35% of the mycoflora studied. Fifty-six species (13%) have been introduced from overseas. Of these 19 taxa are ectomycorrhizal with exotic trees. More than half of the taxa (57%) was described from a collection from South Australia. However, only 34% of the taxa are known from conservation reserves in South Australia. A remarkable 26% of the total taxa studied are known from one small national park of 840 ha close to Adelaide. The importance of specimen based collections rather than observational surveys cannot be over emphasised.

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583 - Host determinants of mycorrhizal colonization and functioning in maize (*Zea maize* L.)

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Enzymes related to metabolism of AM fungi i.e. alkaline phosphatase, acid phosphatase and malate dehydrogenase and host defence mechanism i.e. peroxidase were measured in mycorrhizal and non-mycorrhizal roots of four maize genotypes namely HIM 29, VL 90, VL16 and CM129 differing in mycorrhizal responsiveness. There was remarkable enhancement in Enzymes related to metabolism of AM fungi i.e. alkaline phosphatase, acid phosphatase and malate dehydrogenase and host defence mechanism i.e. peroxidase were measured in mycorrhizal and non-mycorrhizal roots of four maize genotypes namely HIM 29, VL 90, VL16 and CM129 differing in mycorrhizal responsiveness. There was remarkable enhancement in the level of enzymes related to mycorrhizal functioning in genotype VL 90 and HIM 129 while VL 16 and CM 129 did not respond as well in spite of considerable mycorrhizal colonization. The levels of mycorrhizal colonization as judged by arbuscular, vesicular to arbuscular senescence.

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584 - Biodiversity of arbuscular mycorrhizal fungi (AMF) in different agroclimatic regions of India

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Arbuscular mycorrhizal fungi (the symbiotic soil fungi forming association with plant roots) are an important component of biodiversity as about 80% of the world's plants are associated with AM fungi. India is a land of many climates and varieties of soils affording scope for much diversity. The diversity of AMF was studied in 10 different wheat growing regions of India as wheat is the second important food crop of India. This study would then help in exploiting the potential of these fungi in sustainable agriculture, particularly in wheat. The rhizosphere soil samples were collected from wheat fields. The mycorrhizal and soil parameters were analyzed and the trap cultures were raised from the collected samples for their further multiplication. The mycorrhizal parameters analysed include: total AMF spore count, species diversity of AMF, dominant AMF species, percent AMF colonization inside the roots and the infectivity potential of the AMF isolate. The soil parameters include analysis of pH, electrical conductivity, % total nitrogen, available phosphorus and available potassium. The spore count, root colonization, species diversity, dominant species and inoculum potential of AM fungi were found to vary with the agroclimatic region as well as the soil nutrient conditions. The number of species in a given region ranged from 1-20.

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585 - Long term decomposition studies of wooden test blocks at Khao Yai National Park, Thailand

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In a survey of wood decay, 4 woods (*Dipterocarpus alatus*, *Xylia dolabriformis*, *Alstonia scholaris* and *Anisoptera oblonga*) were immersed in two streams: Tad Tha Phu flows year-round; km29 is ephemeral. These were recovered each month over 1 year. 236 spp. of fungi were found. Importantly, after 1 year decomposition had barely begun. Using the same sites the work was extended to examine other woods and to allow for recovery of up to 10 yr. Work after 1 yr allows direct comparison with the preliminary study. 5 spp. were used: *Buchanania latifolia*, *Mangifera caloneura*, *Sindora siamensis*, *Stereospermum neuranthum* and *Tectona grandis*. *Buchanania* and *Tectona* supported the highest diversity (20+ spp.) while *Mangifera* and *Sindora* had <20 spp. each after 1 year. Diversity was higher at km29 (16-28 spp.) than at Tad Tha Phu (11-22 spp.). This pattern held for each wood except *Sindora* where 16 spp. were found at km29 and 19 at Tad Tha Phu. At km29 *Massarina thalassioides* and *Nectria* sp01 were found on all 5 woods while at Tad Tha Phu *Trichoderma* sp., *Candelabrum brocchiatum* and *Cancellidium applanatum* were on all 5 woods. After 1 year, diversity of fungi colonising was generally low. 30 spp. were found from the 5 woods at km29. However, 4 of the 5 woods had <10 spp. The exception was *Tectona* with 20 spp.: 11 being exclusive to *Tectona*. At Tad Tha Phu 21 spp. were recorded at 12 months: *Mangifera* had 4 spp. while the maximum of 10 was from *Sindora*. 6 of these were exclusive to *Sindora*.

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586 - Detecting diversity of ectomycorrhizal fungi in Douglas-fir dominated forests

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Surveys for ectomycorrhizal mushroom and truffle sporocarps over 4 fall and 3 spring seasons resulted in a large data set suitable for an analysis of the effect of sampling intensity on observed fungal diversity and abundance. An impressive 48 truffle and 215 mushroom taxa were collected from three replicate stands in each of three forest age classes (young, rotation-age, and old-growth) of Douglas-fir dominated stands in the Cascade Range of Oregon. Cumulative species richness was similar among forest age-classes but differed between seasons. Seventeen species accounted for 79% of the total sporocarp biomass. We investigate and present 1) how the observed diversity and abundance relates to sampling intensity, 2) the likely number of species extant in the study area but undetected by the survey, 3) the sampling intensity necessary to detect each known species, and 4) how the statistical power to detect a significant difference in fungal diversity or abundance varies with sampling intensity. Knowledge about methods for detecting the diversity of common and uncommon ectomycorrhizal fungal species is essential to maintaining the biological diversity of fungi.

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587 - Effect of ectomycorrhizal inoculation on the growth of *Cyclobalanopsis edithiae* seedlings

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Field collected soils and roots under a community of a native Fagaceae, *Cyclobalanopsis edithiae*, were used as an instant inoculum in this study. Acorns of *C. edithiae* of uniform weight and size were surface sterilized and sown on sterile sand for germination. The germinated seedlings were planted individually into a 1 L plastic pot containing 200 ml of field soil inoculum. An uninoculated control treatment was also set up. The pots were arranged in completely randomized block design and were placed in a temperature controlled unit at 25 °C. 5 seedlings were harvested from both the control and the inoculated pots for growth analysis every 8 weeks. Root collar diameter, shoot and root dry weight, leaf area, and the percentage of ectomycorrhizal colonization were measured and evaluated. The average colonization rates of the inoculated seedlings in the three harvests were 8.48, 14.28%, and 10.40%. No infection was observed in the control seedlings. The relative growth rate, root dry weight and total dry weight were significantly ($p<0.05$) higher in the inoculated seedlings than the control in all the three harvests. The result suggested that such low-cost and convenient inoculation method could be further explored for the production of Fagaceae seedlings in nursery condition.

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588 - Fungi from North-West Caucasus: from the mountains to the molecular level

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Soil microfungal communities were studied in 8 high-altitude ecosystems in Teberda Reserve (Karachaevo-Cherkessia, Russia): *Rhododendron caucasicum* bushes, alpine fens, alpine snow beds, *Geranium gymnocaulon* - *Hedysarum caucasicum* meadow, *Festuca varia* grassland, alpine lichen heath, moist and dry screes. The dominant species occurring in most soils were: *Pseudogymnoascus roseus* and its anamorph, *Mortierella parvispora*, *Cladosporium cladosporioides*, *C. herbarum*, *Mucor hiemalis*, *Tolypocladium inflatum*, *Penicillium simplicissimum*, and *Fusarium sambucinum*. Correspondence analysis showed that alpine fens and *Rhododendron* bushes had the most distinctive microfungal communities. The latter communities somewhat resembled those of podzolic soils under boreal forests. Some taxonomically interesting isolates were obtained. A taxon that was rather frequent in alpine fen soils was provisionally identified as *Leptodontidium* sp. Two groups of isolates differed slightly morphologically from one another. A detailed study including sequencing of ITS and 18S regions revealed that these fungi are rather unrelated to the type of the genus *Leptodontidium* but close to the teleomorph genus *Pseudeurotium*. Observation of conidiogenous structures of *Pseudeurotium* confirmed this affinity. A similar strain, CBS 326.81, had been isolated from potato meal factory sewage water in the Netherlands. Partial sexual reaction with infertile cleistothecia was observed between one Caucasian strain and CBS 326.81.

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589 - A preliminary investigation on coprophilous fungi in Northeastern Thailand

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Coprophilous fungi are associated with herbivore dung, playing an important role in recycling of nutrients in animal faeces. This group is a source of biocontrol agents, enzymes, antibiotics, etc. Surveys were made in Khao Yai National Park and areas in northeastern Thailand between May, 2001 and April, 2002. Over 150 isolates were made from 85 dung samples of sambar deer (*Cervus unicolor*), barking deer (*Muntiacus muntjak*), Asian elephant (*Elephas maximus*) and cattle. Of these, 52% were Ascomycetes and 10% their anamorphs. Basidiomycetes made up a second group with 25% of isolates while Zygomycetes accounted for only 12%. 14 genera were recognized with Ascomycetes accounting for 10 of these. The common genera were *Podospora*, *Coprinus* and *Pilobolus* which accounted for 54% of collections: most were successfully isolated. A succession was demonstrated. *Pilobolus* appeared after 3 days incubation, followed by the apothecial-forming ascomycetes such as *Ascobolus* and *Saccobolus* from 4-7 days. Perithecia of pyrenomycetes e.g. *Delitschia*, *Podospora* and *Sporormia* appeared from 7-14 days and were often associated with basidiocarps of *Coprinus*. These genera continued to fruit for a further 3 wks. Mitosporic fungi appeared late in the succession. Notable species were: *Coprinus*, *Delitschia pachylospora*, *Pilobolus*, *Podospora communis*, *Poronia gigantia*, *Saccobolus citrinus*, *S. thaxteri*, and *Zygospermella*.

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590 - Detection of fossil fungi in Cretaceous amber from Álava Northern Spain by different microscopy techniques

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Amber deposits dated from the Lower Cretaceous period were recently found at Álava, Northern Spain, being one of the more highly fossiliferous resin deposits. Although some reports on the characterization of fossil fungi exists, very few is known about their relationships with other fossil microorganisms. In this work we reported the use of different microscopy techniques to obtain simultaneously structural, ultrastructural and analytical information about the entire amber microscenosis and their individual components. A very well preserved microcenosis, which corresponds to a semiaquatic habitat, including different types of protozoas, microalgae and fungi was present in this amber. By scanning electron microscopy using secondary electron (SEM-SE) and backscattered secondary electron detectors (SEM-BSE), we found an abundant mycelium of hyaline and coenocytic hyphae in close association with numerous zooflagellates (probably fungal feeders). The elemental distribution spectroscopy microanalysis during SEM-BSE revealed Fe in the hyphae suggesting the nature of mineralization process. Using light and confocal laser scanning microscopy a basidiomycete mycelium with numerous clamp connections, crystals and encrusted cystidia like structures was detected in the amber outside section. Other hyphae, gametangia or sporangia like fungal structures and spores were abundant in this amber sample. Current work attempts to compare these fungi with extant and modern genera.

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591 - A preliminary survey of lichen fungi from Thailand

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The first published records of fungi from Thailand were lichens from Ko Chang Island at the turn of the last Century. They were neglected for the next 90 yr. However, the last 10 yr has seen increasing interest in Thai lichen fungi. Importantly, these are now being isolated and made available for further research. A lichen collection/isolation programme has been in place at BIOTEC for 2 yr. Natural forest was surveyed every 2 months. Forest was selected to

provide a range of micro-habitats and plant communities. Since October 2000, lichens were collected from 13 National Parks in Thailand. These sites were from the far south (Hala Bala at sea level) to the far north (Doi Inthanon at 2,565 metres above sea level). These collections yielded 1116 samples classified into crustose 80%, foliose 14%, fruticose 1%, others 2% and unidentified 3%. These have been identified to 2 orders including 14 families, 32 genera and over 150 species. Common genera of crustose lichens were *Graphis*, *Haematomma*, *Laurera* and *Lecanora*. Foliose genera included *Heterodermia*, *Parmotrema* and *Phyllopsora*. *Cladonia* and *Usnea* were common examples of fruticose genera. *Canoparmelia* and *Hypotrachyna* were notable rarities of foliose lichen. Isolations were made from ascospores by direct streaking of spores and spore discharge.

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592 - Lignin degrading enzymes of Xylariacious fungi in Thailand

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Thailand supports a rich diversity of xylariaceous ascomycetes, with most occurring on wood, although a number have been found on leaves, elephant dung, fruits and seeds, while others have been shown to be endophytes of forest trees (Thienhirun, 1997; Whalley et al. 1995). Nineteen genera in the Xylariaceae have been documented for Thailand with a total of 91 species. However, some 15 *Hypoxylon* and 30 *Xylaria* species await description (Thienhirun, 1997). Members of the Xylariales are active degraders of timber in nature and have been shown to cause soft rot and white rot attack of wood. However, little is known about the enzymes they produce. We have screened 51 strains of xylariaceous fungi over the past 2 years for their lignin degrading enzymes. For this study we have used Poly R-478, ABTS and azure-B medium to screen for general lignin degrading enzymes, laccase and peroxidase activity, respectively. Ninety four percent of the isolates gave positive results for laccase, 21% positive for peroxidases, while 43% decolourized the dye Poly-R 478.

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593 - Fungal diversity on woody substrates in Scandinavia

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Species diversity patterns have been derived from species-specific information of more than 1500 species of Ascomycetes, Heterobasidiomycetes and Basidiomycetes. Several functional associations to woody debris were defined, including primary parasite, primary saprotroph, secondary myco-parasite, secondary myco-saprotroph, and mycorrhiza species (using wood for sporocarp production). Most species are probably primary saprotrophs, but all functional associations are represented. Most species are associated to either coniferous (40%) or hardwood hosts (40%), but many are host generalists (20%). Most taxonomic groups exhibit this pattern, but at least the Ascomycetes show an elevated species richness on hardwood hosts. The degree of decomposition of the wood is important and four patterns were found: early decay preference (less than 10%), medium decay preference (50%), late decay preference (15%) and no decay preference (25%). These proportions varied across taxonomic groups. The dimension of woody substrates is a third factor that many fungi respond to. One group prefer small dimensions (twigs and thin stems) and another is strongly associated to trunks of large dimensions, but the majority of the species (60%) show no strong dimension preference. There are additional factors that wood-inhabiting fungi respond to like sun exposure/shade, part of the tree and standing/lying. For these factors there are major knowledge gaps that prevent compilation of diversity patterns.

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594 - Lichenicolous fungi in Estonia

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The history of floristical investigation of lichen-habiting fungi in Estonia is short. Until the 1990s, only two lichenicolous fungi (*Athelia arachnoidea* and *Rimularia insularis*) were known from Estonia. During the last five years, 69 species from 47 genera of these fungi have been registered from Estonia. Most of the lichenicolous species are ascomycetes (45 species); fewer are deuteromycetes (23) and basidiomycetes (7). The most species-rich genera are *Abrothallus* (5 species), *Licheniconium* (4), *Muellerella* (3), *Stigmidium* (4) and *Tremella* (4). Most species are categorized as very rare i.e. known from one or two localities in Estonia. Still, there are some exceptions. The distribution area of the basidiomycete *Athelia arachnoidea*, an aggressive parasite of different protococcoid algae and lichens, has extended since the 1940s. Since the 1990s, this species is especially widespread in towns, roadsides etc. The deuteromycete *Vouauxiella lichenicola*, a parasite of *Lecanora chlarotera* and allied taxa, was reported as very rare in 1999. However, the species is now found in about 30 places and its distribution partially coincides with the distribution of *Lecanora chlarotera*.

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595 - Interesting ascomycetes on Juncaceae and Apiaceae collected in the Czech Republic

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Saprotrophic ascomycetes and mitosporic fungi on Juncaceae and Apiaceae in the Czech Republic were collected and studied during the years 1997-2001. Eighty-five species belonging to 56 genera were found. Fifteen of these species are reported from the Czech Republic for the first time. They include representatives of Helotiales (*Hysteropezizella diminuens*, *Mollisia fusco-paraphysata*, *Naeviopsis carneola*, *Scutomollisia stenospora*), Pleosporales (*Lophiotrema grandispora*, *Nodulosphaeria mathieui*, *N. spectabilis*), Mycosphaerellales (*Mycosphaerella hypostomatica*), and mitosporic fungi (*Arthrinium cuspidatum*, *Chalara urceolata*, *Coremiella cubispora*, *Dendryphiella vinosa*, *Phragmocephala prolifera*, *Pyricularia juncicola*, *Stachybotrys cylindrospora*). The most interesting species are discussed and illustrated.

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596 - NITE Biological Resource Center (NBRC), a newly established biological resource center in Japan

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According to the report of OECD, biological resource centers (BRCs) are an essential part of the infrastructure underpinning life sciences and biotechnology. NITE, a non-profit institute under Ministry of Economy, Trade and Industry, has established a new biological resource center, NBRC with the following concept: NBRC is to preserve and supply the cultures of reference microorganisms including fungi, yeasts, bacteria and archaea as an essential function of a culture collection. NBRC isolates, characterizes and develops the original biological resources for its own collection. In addition, NBRC also provides DNA and clones based on the genomic sciences. To access the appropriate resources, NBRC develops advanced databases containing application data and taxonomic data. As the so-called intelligent administration, NBRC have to take care of the ownership of the biological resources to encourage the deposit of valuable resources. Convention for Biological Diversity should be considered when biological resources are transferred between countries. Based on these, NBRC intensively make international cooperative studies with the resource countries especially in the South East Asia to produce benefits for both sides.

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597 - Taxonomical investigations of Hungarian protected fungi

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In the last decades fungal taxonomy has undergone enormous change. The traditionally used morphological description of species has been completed with molecular and interfertility results. We examined phylogenetic relationships of the holotypes of fungi described in Hungary using morphological and molecular methods (DNA sequencing). Species of *Agaricus*, *Leucoagaricus*, *Macrolepiota*, *Lepista* and *Cortinarius* genera were investigated. In some cases our results confirmed, in others questioned the isolation of the examined species from their near relatives.

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598 - Diversity of fungi on wild fruits in Hong Kong

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The postharvest diseases of cultivated fruits have been well studied, however, there have been few surveys of the diseases of wild fruits. Small fleshy fruits are of special interest due to the potentially conflicting relationship with frugivorous birds and microbes. The fruits need to attract dispersers after ripening but repel microbes. In the current survey, 15 species of wild fruits were examined from the mature stage to the senescent stage for visible symptoms and signs of diseases in the field and after incubation. Half of the fruit samples were also surface-sterilized to eliminate surface fungi. A total of 104 fungal taxa have been isolated so far, including 21 ascomycetes, 57 coelomycetes and 24 hyphomycete. The most common ascomycete genera were *Gaeumannomyces*, *Guignardia* and *Massarina* spp. and the most common mitosporic genera were *Cladosporium*, *Colletotrichum*, *Penicillium*, *Pestalotiopsis*, *Phoma*, and *Phomopsis* spp.

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599 - Species of *Cordyceps* attacking Hymenoptera in Thailand

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In Thailand 88 *Cordyceps* spp. are known: Coleoptera (15 spp.) and Lepidoptera (28 spp.) hosts dominate with 9 spp. from Hymenoptera. 3 spp. (*C. sphecocephala*, *C. humberti* and *Cordyceps* sp.) are off wasps and 6 off ants. Of these 6, 4 are named - *C. irangiensis*, *C. myrmecophila*, *C. pseudolloydii* and *C. unilateralis*. 5 spp. have *Hymenostilbe* anamorphs; 4 *Hirsutella*. In contrast to coleopteran and lepidopteran *Cordyceps*, those off Hymenoptera are hard to isolate. Usually, *Hirsutella* are isolated easily while *Hymenostilbe* is considered problematic. With hymenopteran *Cordyceps* the converse holds. The anamorph of *C. unilateralis* is *H. formicarum*: the most common *Hirsutella* in natural forest in Thailand, it was isolated once during a 10 yr period. A programme to isolate this species has produced 3 more isolates. Conditions needed to induce a vegetative colony from ascospores are under investigation. However, for suborder Ophiocordyceps evidence suggests that ascospores act as carrier vehicles - the role being to produce spores that germinate to form vegetative colonies. Hymenopteran *Cordyceps* with *Hymenostilbe* anamorphs are assigned to suborder Neocordyceps. Molecular work (with limited species) shows evidence of long-branch attractions. Circumstantial evidence suggests a link between the hymenopteran *Cordyceps* with *Ophiocordyceps* appearing ancestral to *Neocordyceps*.

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600 - A preliminary study of the prevalence of endophytes in leaves and seed pods of *Colophospermum mopane* (Leguminosae) in Botswana

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Colophospermum mopane (Mopane) is a deciduous tree or multi-stemmed shrub dominating the vegetative structure in parts of Southern Africa. It is browsed by wildlife and is utilised by local people for domestic animal fodder and firewood. Mopane is the primary host plant of the 'mopane worm', the caterpillar state of *Imbrasia belina* which is harvested by local people and forms an important source of protein in their diet and provides income. No previous studies have been carried out on the fungi associated with this regionally important tree. Young (soft green), mature (leathery green), senescent (leathery yellow) and dead (brittle yellow-brown) leaves, and mature seed pods, were collected directly from selected trees. Tissue samples were dissected from the leaves and pods after surface sterilisation. The endophyte assemblage was dominated by species of *Alternaria* and *Phoma*, and to a lesser extent *Phomopsis*. The members of the assemblage remained in similar proportions in different aged leaves, but increased in frequency with increasing leaf age. *Alternaria* spp. were proportionally less common in petiole than lamina tissue. TEM studies on seed pods, which yielded endophytes, were carried out to investigate the role of the endophytes in degradation of lignified parts of the pericarp. Lignocellulolytic enzyme production by the endophytes was assessed with qualitative assays.

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601 - A preliminary report on a moss pathogenic heterothallic *Pythium* sp. from Spitsbergen Island, Svalbard

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In the course of studies on moss pathogenic fungi in arctic zone, compatible isolates of heterothallic *Pythium* sp. were frequently isolated from dying moss (*Sanionia uncinata*) in Ny-Ålesund (79°N, 12°E), Spitsbergen Island, Svalbard. The fungus caused discoloration of the moss after artificial inoculation at 0.5 and 10 °C. Morphological analysis of the isolates showed the following features. The main hyphae were up to 6 µm wide. Oogonia were mostly terminal, globose, smooth-walled, 18-30 µm in diameter. Antheridia were terminal, 1 to 5 per oogonium, sac-like and diclinous. Oospores were single, aplerotic, rarely plerotic, globose, 16-26 µm in diameter. The thickness of the oospore wall was up to 1 µm. Sporangia were globose, mostly terminal, occasionally intercalary. Diameter of encysted zoospores ranged from 10-11 µm. Optimal growth of mycelia occurred at 25 °C, the minimum temperature for the growth was 0.5 °C, and maximum was 28 °C. The rDNA internal transcribed spacer sequences of the isolates were different to those of *Pythium* spp. in the GenBank database. The results suggested that a new heterothallic *Pythium* sp. caused the discoloration of moss occurred on Spitsbergen Island, Svalbard.

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602 - Distribution of macrofungi in the forest in Taiwan

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The distribution of macrofungi at the long-term ecological research site in the Guandaushi forest in the middle Taiwan was ecologically studied. Several experimental plots were set up for the study. The important macrofungi genera were *Mycena*, *Marasmius*, *Xylobolus*, *Trametes*, *Russula*, *Coriolus*, *Lactarius*, *Amanita* and *Stereum*. Most of these macrofungi occurred in July during the summer period. A lesser amount of macrofungi grew in March during the winter period. *Mycena* and *Marasmius* were the common macrofungi that occurred both in summer and winter. The *Russula*, *Coriolus*, *Lactarius*, *Amanita*, *Armillaria*, *Fomitopsis*, *Ganoderma*, *Microsporus* and *Rhodophyllus* species were present in the summer period. *Xylobolus*, *Trametes*, *Stereum* and *Peniophora* fungi were found in the winter. The macrofungi distribution in the experimental plots varied. The occurrence and diversity of the macrofungi depended upon the type of predominant vegetation. The plants favorable to macrofungi were *Blastus cochinchinesis*, *Cinnamomum randaiense*, *Helicia rengetiensis*, *Syzygium buxifolium*, *Engelhardtia roxburghiana*, *Smilax china*, *Cunninghamia lanceolata*, *Nephrolepis ariculata* and *Plagiogyria dunnii*. The unfavorable plants to fungi were *Illicium arborescens* and *Diplopterygium chinensis*.

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603 - South African National Collection of Fungi: collections and services

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The National Collection of Fungi of South Africa is internationally accredited and affiliated at the World Federation of Culture Collections as PPRI (for the living culture collection) and PREM (for dried reference material). Both collections contain important type material and researchers are encouraged to deposit reference material in these collections to ensure the expansion of this invaluable national asset to the benefit of the scientific community. The types contained in PREM are integrated onto a database. The rest of the almost 57 000 specimens will shortly be incorporated as well. The list of types is available electronically. The specimens are available on loan for a period of six months to researchers nationally and internationally. About 4500 specimens are contained in the PPRI collection. This list is also available electronically. Both collections are managed by the staff of the Mycology unit of the Plant Protection Research Institute (ARC-PPRI), an institute of the Agricultural Research Council. Apart from managing the above mentioned collections, the unit also provides the following services: processing of infected material, isolation and identification of fungi, fungal analyses including testing of efficacy of fungicides, provision of biological information, as well as presentation of mycological courses such as basic laboratory techniques and taxonomy of major groups.

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604 - Biodiversity of fungi on reed (*Phragmites australis*)

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Reed is a dominant emergent macrophyte in many wetland ecosystems all over the world. Fungal communities associated with this high productive grass are demonstrated to be taxonomically diverse. A field survey in Belgium (mainly Schelde estuary) of Fungi occurring on reed during growth and decomposition results in about 230 taxa of which 40% Ascomycetes, 10% Basidiomycetes, 30% Coelomycetes and 20% Hyphomycetes. 1 Basidiomycete represents a new species, about 13 species (14%) of the Ascomycetes are undescribed; in the Mitosporic Fungi this proportion is probably even higher. A compilation of our research and world literature available on phragmiticole Fungi leads to the hypothesis that the total number of fungi occurring on reed will exceed 650 species. This poster presents results which will be published in a paper on biodiversity of fungi on reed. This biodiversity study is part of a broader ecological study concerning succession of fungal species and a monitoring of ergosterolconcentration (fungal biomass measure) during decomposition.

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605 - Towards a Mediterranean check-list of fungi: macromycetes from beech woods of Sicily and Greece

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The importance to increase the knowledge on macromycetes diversity in southern Europe has been repeatedly stressed by the OPTIMA (Organization for the Phyto-Taxonomic Investigation in the Mediterranean Area) Commission on Fungi, which considers the check-list of Mediterranean fungi as its main goal for the near future. In recent times, in Sicily (South Italy) and Greece many investigations on fungal diversity and distribution were carried out. According to recent literature data, 1248 and

1300 species of macrofungi have been recorded in Sicily and Greece respectively. As regards beech woods in particular, 402 taxa are reported from Sicily (which account to 35% of the total number of fungi recorded from Italian beech woods), whereas an equivalent number of fungi (i.e. 405 taxa) were found to occur in beech woods of Greece. In addition, the two investigated areas presented 137 taxa in common. Among the most representative genera, *Amanita*, *Boletus*, *Russula* and *Tricholoma* are prevalent in Sicily, whereas *Cortinarius* and the corticioid fungi are quantitatively more represented in Greece. Noteworthy is the occurrence of species strictly linked to the thermophilous beech woods such as *B. fragrans* and *B. satanas* (only in Sicily), and *B. aereus*, *B. radicans* (in both areas examined).

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606 - Biodiversity and distribution of aeroaquatic fungi

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Aeroaquatic fungi are an ecological group of fungi inhabiting shallow stagnant to slow-flowing freshwater bodies. They grow on submerged plant litter and often show pronounced ability to survive micro-aerobic conditions. However, their dispersal units are only produced if the substrate is exposed to the air; they consist of complicated, usually multicellular structures of an astonishing morphological diversity, which entrap air and are therefore well buoyant. Buoyancy is often enhanced by warts and hydrophobic encrustations. Dispersal takes place on the water surface. Up to data, about 90 aero-aquatic species are known world-wide. Most species have been recorded exclusively from temperate climates (more than 75%), comparatively few from the tropics (less than 20%), and very few were found both in tropical and temperate habitats (about 5%). Many species have a wide geographic distribution. Recent investigations revealed many new taxa even in comparatively well-studied Europe, indicating that its biodiversity is very imperfectly known world-wide. Systematically, the aero-aquatic fungi comprise members of the oomycetes, basidiomycetes and ascomycetes; however, for the majority of species teleomorphs are unknown. Concluded from known teleomorph stages and DNA sequence data, the largest aeroaquatic genera *Helicoon* and *Helicodendron* are polyphyletic, which may be also true of other genera. This clearly demonstrates that the aeroaquatic lifestyle evolved many times independently.

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607 - Utilisation of the population patterns and pathogenicity of soilborne and root-colonising fungi as bioindicators of soil health in agricultural soils

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Biodiversity and population dynamics of microbes change due to anthropogenic soil disturbances such as different agricultural land management practices. Mycological surveys, using several isolation methods, were undertaken to determine species diversity of soil and root-colonising fungi in pastoral, cropping, native grasslands and forest soils in New Zealand. Soilborne plant pathogenic species, such as *Cylindrocarpon* spp. and *Fusarium* spp., were frequently isolated during the surveys. Pathogenicity of these fungi was determined using axenically grown seedlings to provide an index whereby the relative pathogenicity of species could be compared between soil and land management types. Results indicated that more pathogenic species and strains were present in highly disturbed agricultural soils compared to relatively undisturbed native grassland forest and soils. These species and methods may have potential to be used as bioindicators of soil health and disease suppressiveness.

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608 - Ecology of rare species of *Hericium* in Britain

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The wood-decomposing species of *Hericium* are not common in Britain. *Hericium erinaceus* is protected under the Wildlife and Countryside Act (1981), reported as being 'vulnerable' in the British Red Data List, and is a UK Biodiversity Action Plan priority species. *H. coralloides*, *H. cirrhatum* and *H. alpestre* are all listed in the Red Data List as being 'vulnerable'. With the exception of *H. alpestre*, which is found decaying coniferous wood, *Hericium* spp. occupy the heart wood of deciduous trees and all species cause white rot. The ecology of these species of *Hericium* is little understood, thus we have examined their establishment following inoculation into felled logs and standing trees, how fungal community structure develops once these fungi have become established, and the outcome of interactions with other wood-rotting fungi. The work has revealed that it is relatively easy to establish these fungi by inoculation of colonised dowels, they are quite combative against other basidiomycetes, and they fruit readily. Preliminary evidence, however, suggests that spore germination may be poor. Thus, mode of colonisation may be an important factor contributing to their apparent rarity. These studies should provide the information required to conserve extant

Hericium populations and make recommendations for their artificial reintroduction, if necessary.

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609 - Encapsulation cryopreservation of *Serpula lacrymans* and the development of methods to identify molecular damage

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We present a generic encapsulation-dehydration method for the successful cryopreservation of building and Himalayan woodland isolates of *Serpula lacrymans* and *S. himantioides*. Mycelial cores from actively growing cultures were encapsulated in alginate beads and pre-treated for 72 h in a 0.75 M sucrose solution. Three desiccation methods were tested on the pre-treated beads: 4 h in a flow of sterile air while cooling the beads on ice (c. 19-26 °C), 4 h in a flow of sterile air without cooling the beads (c. 28-40 °C) and 18 h in a specially designed desiccator with silica gel (c. 22 °C). Desiccated beads were stored in liquid nitrogen for 30 min, then left to recover and the effect of treatment on the survival, lag phase, radial colony extension rate and morphology measured. A comparison of the different experimental methodologies employed clearly highlights the sensitivity of this approach to the operational parameters, in particular temperature. Results are described and compared for individuals of different origin and in view of the known temperature sensitivities of *Serpula* species. The development of molecular techniques such as ISSR-PCR to identify genetic damage accumulated during different storage and preservation treatments applied to *S. lacrymans* are described.

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610 - The biomarkers and fossils of cryptoendolithic Antarctic fungi and lichens

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Lithobiontic microbial communities living within are an example of survival in an extremely cold and dry environment. Any unfavourable change in external conditions can result in the death and disappearance of microbes, and this may be followed by the appearance of trace biomarkers and/or their microbial fossils. The results reveal the presence of inorganic biomarkers in the form of physicochemical bioweathering mineral patterns. The presence of Fe-hydroxide nanocrystals and biogenic clays around endolithic hyphae cells have been also observed. Others biomarkers, including deposits of calcium oxalates and silica accumulations, are clear signs of endolithic hyphae activity. The interior of the sandstone rocks reveal the presence of microbial fossils of cryptoendolithic lichens. These microbial fossils, detected for the first time within Antarctic rocks, contain well preserved relics of ultrastructural cytoplasm elements. Recognising internal cell structures within fossilised cells is proposed as a new criterion for defining the biogenicity of biomorphs. We suggest the use of SEM-BSE and EDS in situ microscopical and microanalytical investigation strategies, which make it possible to clearly determine inorganic traces of Antarctic endoliths. The implications of our findings are potentially significant for astrobiology because Antarctic cold and dry ecosystem are considered a terrestrial analogue for possible life on early Mars.

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611 - Study on the biodiversity of inoperculate discomycetes in Yamingshan, Taiwan

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Thirty sampling areas with 1 square meter had been constructed at the Silvergrass Grasslands and Arrow Bamboo Bushes near Hsiaoyukeng as well as Broadleaved Forest near Lujankenghsi in Yangmingshan National Park, Taiwan. One hundred and twelve samples including 43 from decayed twigs, 10 from arrow bamboo and 59 from silvergrass and other straws had been collected from the above sampling areas at the elevations of 510-830 m since August, 2000 to July, 2001. Eighteen species of Dermateaceae, 4 species of Leotiaceae, 3 species of Orbiliaceae, 4 species of Sclerotiniaceae, 18 species of Hyaloscyphaceae had been identified. The dominant species of the above three different areas of Lujankenghsi, Silvergrass Grasslands and Arrow Bamboo Bushes are respectively *Lachnum sclerotii*,*Lachnum* cf. *apala* and the species of *Mollisia*.

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612 - Fungi, rotted wood and beetles: Implications for native forest management in Tasmania

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Shorter rotation lengths of around 80 years in commercial native forests in Tasmania will eventually lead to the

elimination of large decaying logs on the forest floor. This poster describes an investigation of whether small trees, which could be left as a future decaying log habitat at the end of the rotation, are likely to follow the same successional pathways as larger logs, providing the full range of decay types and hence fungal and invertebrate biodiversity. The four objectives of the project were, in regard to *Eucalyptus obliqua* coarse woody debris (CWD) in Tasmanian wet sclerophyll forests: 1) To better understand the ecology of decomposing CWD, in terms of the associated wood decay fungi and saproxylic insects; 2) To investigate differences in fungal and insect biodiversity between small and large diameter logs; 3) To provide a checklist of the fungal and insect species dependent on *Eucalyptus obliqua* CWD in wet sclerophyll forests; 4) To look for direct associations between decayed wood, wood decay fungi and saproxylic insects; The ecological information from this research will contribute to assessing whether current forest practices are ecologically sustainable, developing appropriate indicators for monitoring forest diversity, and proposing prescriptions that ensure the conservation of these diverse wood decomposing communities.

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613 - Preliminary biodiversity inventory of dictyostelid slime molds in Taiwan

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Dictyostelid cellular slime molds are widely distributed in the world and there are most numerous and of the greatest variety in forest soils. Taiwan is an island located in the subtropics with many high mountains. Due to the vertical geographical and climatic complexity of this island, plant vegetation is luxuriant and the fungal biodiversity is abundant. The dictyostelid taxa were obtained from soils of various vegetations such as forest, grassland or cultivated field of Taiwan. Up to date, three genera and seventeen species have been recorded, namely: *Acytostelium leptosomum* Raper, *Dictyostelium aureo-stipes* Cavender, Raper et Norberg, *D. brefeldianum* Hagiwara, *D. clavatum* Hagiwara, *D. coeruleo-stipes* Raper and Fennell, *D. delicatum* Hagiwara, *D. giganteum* Singh, *D. lavandulum* Raper and Fennell, *D. macrocephalum* Hagiwara, Yeh et Chien, *D. minutum* Raper, *D. monochasioides* Hagiwara, *D. polycephalum* Raper, *D. purpureum* Olive, *D. rhizopodium* Raper et Fennell, *Polysphondylium pallidum* Olive, *P. pseudo-candidum* Hagiwara and *P. violaceum* Brefeld. Currently, *D. exiguum* Hagiwara was newly isolated from forest soils in Nantou County of Taiwan. The species was examined and illustrated in this text. Concerning ecological distribution and keys for the above eighteen species were also discussed and provided.

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614 - Preliminary assessment of diversity and productivity of edible mushrooms in savanna woodlands of Western Africa

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A simplfied method for preliminary assessment of the diversity and productivity of wild edible mushrooms in different types of savanna woodlands is presented. The results of a case study in Bénin (West-Africa) are given. Thirty edible macromycetes were found in 18 fixed plots from six Soudano-guinean vegetation types. The mushroom productions were assessed per species and per plot, using the number of produced carpophores and fresh weight (kg/ha). Edible macromycetes from the studied areas are for 86.6% ectomycorrhizal, mainly belonging to *Lactarius*, *Russula* and *Amanita*. The total edible macromycetes fresh weight production varies from 0.2 to 225 kg/ha per season, depending on the forest type. Open forests, i.e. savanna and woodland savanna, dominated by Caesalpiniaceae, yield qualitatively and quantitatively more edible taxa than the denser and rivulet associated forest types. *Lactarius gymnocarpoides* is the most common and abundant edible species in the savannas and woodland savannas. Specific productions of edible taxa are affected by host tree compositions reflected in the forest type. The study illustrates the importance of local indigenous knowledge, and indicates that the edible macromycetes from West-African savanna woodlands have substantial importance as a renewable, non cultivatable, non timber forest product.

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615 - Arbuscular mycorrhizas from the rhizosphere of *Macaranga denticulata* and their effects on the host plant at different levels of fertilizers

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The hill tribe (Karen) farmers in Mae Hong Son province of northern Thailand believe in beneficial effects of *Macaranga denticulata* Müll. Arg. on upland rice yield. The farmers manage the *M. denticulata* canopy in fallow plots of their rotational shifting cultivation in an acid soil (pH 4.9). Diversity of arbuscular mycorrhizal (AM) fungi was found 30 species from the indigenous rhizosphere of *M. denticulata*. The effects of indigenous AM fungi on growth of *M. denticulata* were assessed by growing the plants in pots containing soil (sandy loam and equal available P as indigenous soil, 3.5 mg/kg) that was set up to have pH 4.9 by mixing with soluble aluminium. AM colonization in roots of *M. denticulata* was highest (87.8%) in the treatment of no added N and P that was similar to AM colonization in the natural rhizosphere in wet season and lowest in the treatment of added N and P 50 mg/kg soil (51.0%). Spore numbers of AM fungi ranged from 13 to 37 spores/g fresh soil. In treatments with inoculated AM fungi the growth of *M. denticulata* was higher than in non-inoculated AM fungi. The highest growth of *M. denticulata* was obserbed in inoculated soil with 50 mg/kg soil of N and no added P (AM colonization, 70.4%).

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616 - Lichens on littoral of the White and Barents seas (Northwest Russia)

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Littoral lichen communities composed of species adapted to both periodic over wetting and high salinity are among the least studied by lichenologists. Investigations of littoral lichens along the extensive coastal strip of Russia are lacking. Lichen diversity of the White (Kandalaksha bay) and Barents (Dalnie Zelentsy) seas' coasts were studied during the field trips, supported by Federal Program Integratsia. Coinciding with data on British coasts three groups of lichensit were revealed: (1) coastal, (2) supralittoral, (3) littoral, with the latter being the least diverse but most specific. The littoral group is restricted to lower supralittoral and whole littoral zones. Barents coasts are dominated by lichen communities formed by *Verrucaria mucosa* in middle and lower littoral (were it is competing with red alga *Hildenbrantia* sp.), substituted by *Verrucaria maura* and satellite *Verrucaria* species in upper littoral. Both species form clear belts with coverage up to 98%. On the White sea coasts the same species are present, though with less developed coverage and *V. mucosa* does not extend into lower littoral. Additionally filamentous *Lichina confinis* is common in midlittoral on granite outcrops. In contrast with the former sea coast *Verrucaria* species here prefer littorals that are not exposed to direct waving. All littoral lichens are growing on basic rocks, and only *Pyrenocollema sublitorale* inhabits calcareous shells of Semibalanus balanoides.

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617 - Mycocoenological studies in beech-woods in Western Liguria (Italy)

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The work presents a study carried out in selected plots of several beech woods in Western Liguria (Italy) through five years. The zone investigated were the subject of some previous researches, devoted to study the mycological flora. From a phytosociological point of view and despite the limited diversity of the flora, the woods belong to *Trochiscantho-Fagetum* association (*Geranio-Fagion* alliance), quite common in Northern and Central Apennines of Italy. The macrofungi collected and identified amount to three hundred and fifty seven species. These species were attributed to the following five ecological groups: 173 ectomycorrizal fungi, 75 saprotrophic on dead wood, 60 saprotrophic on soil or humus, 47 saprotrophic in the litter, 2 parasitic on leaving trees. Gathered data point out that the mycological flora is rich in species which can be deemed strictly related to the beech, but they have a rather wide synecological relevance, occuring in all kinds of beech woods. Finally, some preliminary results are also reported, exploiting statistical analysis techniques based on both myco-floristic and enviromental data.

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618 - Filamentous fungi associated with the bivalve mollusk *Corbicula japonica* Prime

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The bivalve mollusk *Corbicula japonica* Prime (Corbiculidae) is a commercially valuable species and has unique pharmacological (hepatoprotective) properties. Mollusks were collected in the brackish water Ainskoye Lake (Sakhalin) on September 25, 2000. Adult individuals with shells 25 - 35 cm wide were selected. To remove sand and mud from the internal organs, animals were maintained for a day in flowing water. Then the mollusks were frozen and kept in a refrigerator at -18°C until the mycological investigation. Internal organs of the mollusks - mantle, muscles, gills, kidneys, digestive glands, male and female gonads - were prepared for the experimental study. Before inoculation to the liquid nutrient media, the organs of the mollusks were kept for two hours in sterilized distillated water with antibiotics to inhibit the bacterial flora. Several nutrient media were used: glucoso-yeast extract medium, Tubaki's medium, and others. We isolated and identified ten species of the filamentous fungi associated with the mollusk *Corbicula japonica*. They are *Mortierella longicollis*, *M. vinacea*, *M.* sp., *Mucor circinelloides*, *Gymnoascus* sp., *Trichoderma aureoviride*, *T. hamatum*, *Penicillium atramentosum*, *Aspergillus ochraceum*, and *Cladosporium sphaerospermum*. Growth of the yeast fungi was observed. Fungi were found in the digestive gland, in the female gonads, in the male gonads, in the mantle, and in the kidneys. Fungi were not found in the gills and muscles.

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619 - Coevolution between fungus-growing termites and *Termitomyces* fungi

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Termites of the exclusively Old World subfamily Macrotermitinae live in an obligate symbiosis with fungi of the genus *Termitomyces* (Basidiomycotina). Here we present phylogenies of both partners in this symbiosis (estimated using Bayesian analyses of DNA sequences). Our sample consists of 43 colonies of termites and their fungi (belonging to 32 termite species, covering 9 of the 11 genera) from three African and three Asian localities. Fungal sequences were obtained using comb material, basidiocarps and termite gut contents (using *Termitomyces* specific primers). For information on the taxonomic affiliation of the *Termitomyces* symbionts we also obtained DNA sequences from herbarium specimens of seven relatively well defined species. We show that the symbiosis has a single African origin and that secondary domestications of other fungi or reversals to a free-living state have not occurred. Host switching at low taxonomic levels is common and single termite species can have different symbionts. This is consistent with fungal reproduction, independent of termite reproduction (horizontal transmission), which is inferred to be the ancestral transmission mode. Specificity increases towards the higher taxonomic levels and the four main clades of fungus-growing termites are generally associated with specific clades of fungi. The inferred patterns of co-evolution challenge the hypothesis that vertical transmission is a prerequisite for maintaining advanced mutualistic symbiosis.

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620 - Species delimitation in the *Puccinia striiformis* complex

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Puccinia striiformis (Uredinales), the cause of yellow rust or stripe rust, is a species forming uredinia and telia on various species of Poaceae. It is assumed to be heteroecious, but the aecial host is not known. Currently, two varieties, *P. striiformis* var. *striiformis* and *P. striiformis* var. *dactylidis* are accepted by most authors. We sequenced the variable internal transcribed spacer (ITS 1+2) region and the more conservative 5.8S gene of the ribosomal DNA and found considerable differences among specimens on *Triticum* (Triticae), *Dactylis glomerata* (Poae) and *Poa pratensis* (Poae). These results correspond with a previous isozyme phenotype study and also with analyses of morphological features. Based on these differences we suggest a three-species system including *P. striiformis* on numerous hosts (mainly on Triticeae), *P. pseudostriiformis* sp. nov. on *Poa pratensis* (Poae), and *P. striiformioides* nom. et stat. nov. on *Dactylis glomerata* (Poae).

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621 - Nomenclatural and taxonomic status of some taxa in *Russula* sect. *Xerampelinae*

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Numerous validly published names in agarics have not been accepted by current authors. Due to short and insuficient original descriptions and missing type specimens their identity is doubtful. We have studied members of *Russula* sect. *Xerampelinae* with greenish pileipes those taxonomic delimitation and nomenclature are confusing. Only three taxa in that group are clearly delimited: *Russula clavipes* Velen., *R. cicatricata* Romagn. ex Bon and *R. schaefferi* Kärcher. *R. schaefferi* differs in having narrow terminal cels of generative hyphae in pileipes and spores with long spines. *R. clavipes* and *R. cicatricata* have terminal cells of generative hyphae in the center of the pileipes inflated. Illustrations are available for lectotypification (even Latin diagnoses are missing) of *Russula elaeodes* and *R. fuscoochracea* R. Schulz. Later name is superfluous (homonym of *R. fuscoochracea* Velen.) but *R. elaeodes* seems to represent a distict taxon. No type specimen was designated for *R. barlae*, *R. fusca*, *R. ochracea* and *R. olivascens* and original descriptions lack characters typical for the section. Another names, such as *R. citrinocincta*, *R. cookeiana* and *R. duportii* need also clarification. Complexe knowledge of the members of *Russula* sect. *Xerampelinae* and consequent rejection of several names are the only way how to stabilize the nomenclature and the taxonomy of this section.

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622 - Impact of rhizomorph structure on systematics of Hymenomycetes (Basidiomycota)

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Rhizomorphs can be used to confirm or to clarify relationships within Hymenomycetes (Agerer 1999, 2002, Hahn et al. 2001). Recent studies have shown that the final structure in combination with the ontogeny of rhizomorphs are important means to discern fungal relationships. Virtually all families of Boletales s. l. (Gomphidiaceae, Tapinellaceae, and Truncocolumellaceae excluded) have an identical type of rhizomorphs, the boletoid type (Agerer 1999). The recently by DNA-analyses repeatedly confirmed relationship between Gomphales, Geastrales and Gautieriales is characterized by ramarioid rhizomorphs (Agerer 1999, Hahn et al. 2001), which are unknown in any other fungal group. Agaricoid rhizomorphs (Agerer 1999, 2002) are typical of Agaricaceae and Lycoperdales. The unique rhizomorph structures are shown and their impact on Hymenomycetes systematics discussed. References: Agerer R (1999) Never change a functionally successful principle: the evolution of Boletales s. l. (Hymenomycetes, Basidiomycota) as seen from below-ground features. Sendtnera 6: 5-91. Agerer R (2002) Rhizomorph structures confirm the relationship between Lycoperdales and Agaricaceae (Hymenomycetes, Basidiomycota). Nova Hedwigia (subm.) Hahn C, Agerer R, Wanner G (2001) Anatomische und ultrastrukturelle Analyse von Ramaricium ochraceoalbum, einer seltenen Art der Gomphales und seine verwandtschaftliche Beziehung zu Geastrum und Gautieria. Hoppea 61: 115-125.

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623 - Preliminary results in assessing phylogenetic and biogeographic relationships among species of *Russula* subgenus *Ingratula*

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Phylogenetic and biogeographic relationships among species of *Russula* subgenus *Ingratula* are being studied using partial nuclear Ribosomal DNA Large Subunit (rnDNA LSU) and the complete Internal Transcribed Spacer region (ITS1, ITS2 and 5.8S rDNA gene). Species of *Russula* subgenus *Ingratula* are macromorphologically characterized by the tuberculate-striate margin of the pileus, the brownish-yellow extracellular pigments and the often disagreable or pronounced smell. Micromorphological characters vary broadly between groups of species. Based on these morphological features, species in this subgenus have been grouped into several sections and series, but their phylogenetic relationships had not been evaluated. Phylogenetic analyses of 35 LSU and ITS sequences, respectively, are being performed using Maximum Parsimony, Maximum Likelihood and Bayesian methods. The distinction between sections and between series is not supported in preliminary analyses. Species in the subsection *Foetentinae* are scattered in the cladogram, while species in section *Subvelatae* fall within two separate and unrelated clades. Sister taxon relationships for some taxa are suggested, e.g. neotropical *R. arcyospora* and cosmopolitan *R. laurocerasi*, American *R. pulverulenta* and European *R. insignis*. Test of Congruence for the combinability of the two data set together with sequencing of additional genes and additional taxon sampling are ongoing.

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624 - The '*Torula*' infections on *Cetraria* spp.

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The so-called sorediate forms of the lichen genus *Cetraria* was revised and found to be pathogenic conditions caused by parasitic hyphomycetes of which only one species had been formally described. The new genus *Erichseniomyces* with the type species *E. sorediella* comb. et stat. nov. and *E. islandica* sp. nov. are described as are the new species *Trimmatostroma ahlneri* and *T. danica*.

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625 - Locating the position of the Micareaceae within the Lecanorales (lichenized Ascomycota)

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The phylogeny of the Micareaceae and the genus *Micarea* Fr. was studied using 39 nuclear small subunit ribosomal DNA sequences, of which four were new. Phylogenetic analyses using maximum parsimony, maximum likelihood, and Bayesian inference were carried out. These resulted in 18 most parsimonious trees, of which one was corresponding to the 50% majority rule consensus tree from the Bayesian inference. The maximum likelihood tree was identical except for one collapsed branch. In all trees, *Micarea* adnata and *Byssoloma leucoblepharum* formed a strongly supported group. This study supports the placement of the Micareaceae together with the Pilocarpaceae inside the Lecanorales, as a sistergroup to the Bacidiaceae. Several null hypotheses concerning monophyly were tested: the monophyly of the family Micareaceae in the sense of Eriksson & Hawksworth, and the genus *Micarea* was rejected. *Scoliciosporum* A. Massal has sometimes been considered close to *Micarea*, but null hypotheses assuming monophyly together with the Micareaceae or the Bacidiaceae plus Micareaceae were rejected.

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626 - A Monograph of tribus *Myceneae* (*Mycena* excluded) in Europe

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Tribus *Myceneae*, in the tradition of the Flora agaricina neerlandica, vol. 2 (Bas et al. 1995) is characterized by fruitbodies with an omphalioid, mycenoid or tricholomatoid habit, a pileipellis in form of a cutis or ixocutis, sometimes with transitions to a trichoderm, with smooth, coralloid, diverticulate elements, amyloid or inamyloid spores, and white spore print. Revisions of all genera, except *Mycena*, are given, based on a thorough examination of material from all parts of Europe, including type-specimens and additional information. Particularly the genus *Hemimycena* appeared to be in need of a critical revision, because no serious monograph had been published after the *Mycena* monograph of Kühner (1938). Revision is hampered by the lack of type material, particularly of the species described from the (sub)alpine belt in Haute Savoie, France by Valla (1957). The present authors now accept 32 species, including 3 new ones. Type revisions result in a few name changes. In the difficult complex of *H. mauretanica*, a wide species concept is proposed. The genus *Xeromphalina* now is represented with eight species in Europe, including the North American *X. brunneola* and *X. campanelloides*. The genus *Rickenella* embraces after revision also the genus *Jacobia*, and is represented with 7 species.

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627 - Methods for the interpretation and the evaluation of spores in the genus *Cortinarius*

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In the genus *Cortinarius* sl. spores play a fundamental role in differentiating taxa, as to dimensions, form and ornamentations. In order to carry out an objective sporographic analysis, for each collection n spores (at least 32) have been measured with regard to length (L), width (l) and quotient (Q). Then, the analytic equation of the isoprobability ellipse at 68,26% level and the equations of the tangents to this ellipse have been calculated so as to obtain the length-width measures of six singular equiprobable spores, that is, the longest, the widest, the shortest, the narrowest and those which show the highest and the lowest Q, respectively. Starting from the basic spore, with given form and ornamentations, is thus possible to construct a sporogram with a highly descriptive effectiveness. The typology of ornamentations has been evaluated by observations made with both the optical and the SEM microscope. The results obtained have allowed us to find out some ranges based on the following features: ornamentation height [≤ 1 µm (smooth or hardly visible by the optical microscope); from 1 to 2 µm ; ≥ 2 µm]; development of the ornamentations on the spore surface (more concentrated towards the apex or in the upper half or more or less spread on the whole surface); warts form (rounded, pyramidal or spiny); presence or lack of hilar plage.

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628 - The taxonomy of the *Lecanora allophana* group using DNA data

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Lecanora allophana belongs to the large genus *Lecanora* of lichenized ascomycetes. Within the genus there are several groups of species and *L. allophana* belongs to the *L. subfusca* group, that is spread all over the world. *L. allophana* is also the type species of the genus. There are a number of species related to *L. allophana* that are more or less difficult to seperate from *L. allophana* using morphological characters. Therefore, there has been much doubt in some of the species of the group. Molecular studies, using nuclear ribosomal ITS data, show that most species in the group are well separated and that there are even more species than previously understood. However, within *L. allophana* and *L. horiza* the molecular data are not homogenous and indicates that there may be so called cryptic species within these taxa. Further studies of more genes will have to be carried out to settle this matter.

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629 - Application of pectic zymogram in the identification and genetic variation of *Fusarium* species

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The genus *Fusarium* includes several species which classified based on morphological characters. Different media are required to study the morphological characters of different species. Regarding molecular and biochemical techniques for identifying fungi, pectic zymograms have been used to characterized different fungi. Zymogram technique was used to identify species and form species of

Fusarium isolates. 318 isolates identified as the genus *Fusarium* were collected from different areas and hosts. Species of the isolates were identified based on morphological characters. The pectic enzyme solution was prepared for each isolate using liquid media containing citrus pectin as a sole carbon source. Electrophoresis was performed using pectin acrylamide gel. Several zymogram phenotypes were obtained for polygalacturonase and pectin esterase. In total, 12 zymogram patterns were determined for 318 isolates tested. The results showed that there is a considerable intraspecific variation for *Fusarium* species. There were 3, 5 and 2 zymogram electrophoretic patterns for *Fusarium oxysporum*, *F. solani* and *F. culmorum* respectively. However, there were only one zymogram pattern for *F. subglutinans* and also one for *F. equiseti*. Although the intraspecific variation based on zymogram was not correlated to the form species of *Fusarium*, the species of *Fusarium* were distinguished using this technique and there was no common zymogram pattern among species.

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630 - The lichen genus *Caloplaca* on Svalbard

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The Svalbard Archipelago hosts a highly diverse flora of high Arctic *Caloplaca* species with more than 40 species growing primarily on rock, soil, lignum and bone. A comprehensive study of the *Caloplaca* flora adds to the understanding of Arctic species distribution and clarifies the species concept of several formerly poorly understood species, e.g. *Caloplaca invadens*.

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631 - Molecular phylogeny of North American species of *Laetiporus* and related genera

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Relationships among species in the genera *Laetiporus*, *Phaeolus*, *Pycnoporellus*, *Wolfiporia*, and *Leptoporus* were investigated using nuclear 25S and ITS rDNA and mitochondrial 18S rDNA sequences. Members of these genera have poroid hymenophores and simple septate hyphae, and cause brown rots in a variety of substrates. Results based on parsimony and maximum likelihood analyses indicate that the genus *Laetiporus* as currently defined is not monophyletic. The ITS regions of all *Laetiporus* species examined were easily aligned, with the exception of *L. persicinus*. Analyses suggest that *L. persicinus* is no more closely related to other *Laetiporus* species than to species of *Phaeolus*, *Leptoporus* or *Pycnoporellus*. The six other *Laetiporus* species examined (*L. cincinnatus*, *L. conifericola*, *L. gilbertsonii*, *L. huroniensis*, *L. sulphureus*, and an undescribed species from the Caribbean) appear to make up a well-supported, monophyletic group. Results confirmed that *L. conifericola* and *L. huroniensis*, two species that occur on conifers, are very closely related but distinct species. The existence of a previously undescribed yellow-pored species from the Caribbean was also confirmed. This species may be more closely related to *L. gilbertsonii* than to any other species of *Laetiporus*. Two varieties of *L. gilbertsonii*, which are compatible in mating tests and differ only in pore color, were indistinguishable in these analyses.

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632 - Using parsimony networks, migration estimates and coalescence approaches to resolve the phylogeography of *Mycosphaerella graminicola*

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DNA sequences of nuclear-encoded loci can be used to determine the phylogeographical history of fungal populations. Four nuclear loci were sequenced in the fungal wheat pathogen *M. graminicola*. Two neutral non-coding RFLP loci and two nuclear single copy genes were selected. The coding genes were β-tubulin and 3-isopropylmalate dehydrogenase (LeuC). A total of 300 *M. graminicola* isolates originating from 14 wheat fields around the world were sequenced. All sequence loci showed useful diversity. The RFLP locus STS2 had 29 polymorphic sites in a 490 bp region, while STL10 had 31 polymorphic sites in a 1265 bp region. For the β-tubulin locus, 24 polymorphic sites were found among 366 bp sequenced. The leuC gene contained 65 polymorphic sites within the 870 bp region sequenced. Gene genealogies were generated for all sequenced loci following clone-correction for each locus/population. Trees were rooted using *Septoria passerinii*, the closest known relative of *M. graminicola*. Parsimonious networks were constructed using a nested cladistic approach, and migration rates between populations were estimated. A coalescence approach was used to determine the temporal scale of historical migration events among populations. All loci were also tested for neutrality and intergenic recombination. Our results suggest that the centre of origin for this pathogen is most likely in the Fertile Crescent, and that migration occurred first into the European continent and later into the New World.

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633 - A new winter-fruiting hyphomycete on wood of black locust (*Robinia pseudoacacia*) in the eastern United States

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A new hyphomycete, tentatively assigned to the genus *Cadophora*, is described based on studies of field collections and culture morphology, and analyses of the ITS region of ribosomal DNA. The fungus is found consistently on the undersides of decorticated logs, branches, and stumps of *Robinia pseudoacacia* in the eastern United States. Collections have been observed from Connecticut southward to Virginia and westward into Ohio. The fungus forms membranous, sporodochial conidiomata on decorticated wood of *R. pseudoacacia*. The conidiomata develop early in the winter and persist until spring. The fungus can be isolated from soil in the proximity of *R. pseudoacacia* and was once recovered from river silt in Maryland. Ribosomal DNA ITS sequences exhibit high homology with the ITS sequences of *Phialocephala fortinii*, various ericoid mycorrhizal fungi, and *Mollisia* species. Phylogenetic placement near *Mollisia* is consistent with the habitat on decorticated wood and darkly pigmented, phialidic conidiogenous cells that produce simple ameroconidia.

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634 - Expansion of the genus *Trichoderma*: addition of seven new species from Eurasia

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Species of the deuteromyceteous genus *Trichoderma* are cosmopolitan and typically soil-borne or wood-decaying fungi, some of them being economically important because of their production of industrial enzymes (cellulases and hemicellulases), antibiotics, and their action as biocontrol agents. In order to fully exploit this potential, we started a study of the global biodiversity of the genus. Thereby a number of unique strains were obtained which were culturally and genetically dissimilar to all of the about 40 known taxa of *Trichoderma*. Here we characterize seven of these strains as new species, all originating from Eurasia, and assign them to the established sections of the genus: *T. taiwanense* and *T. effusum* to section *Longibrachiatum*; *T. helicum*, *T. rossicum*, *T. velutinum*, and *T. cerinum* to section *Pachybasium*; and *T. erinaceum* to section *Trichoderma*. Parsimony analysis, using *ITS1* and *2* and *tef1* gene sequences, strongly confirm these placements and also clearly distinguish these new species from morphologically similar species.

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635 - Phylogenetic significance of adaptive structures of macromycete sporophors

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During last two centuries the approaches in study of fungi changed from the recognition of macromorphological characters to the microscopic and molecular ones nowadays. The last ones are available to the limited number of taxa with extrapolation of the morphologically similar species to the same group. New knowledge stimulates the diversity in interpretation of the data and reflects in multiplicity of the systems. New approach in regarding of fungi relationships consists in dividing characters to the groups of ecologically active, that connected directly with fungus adaptation to the environment (morphology of fruit body etc.) and ecologically inert (basidia and spores features, clamps etc.). As genetic relationships reflect in morphological similarity, and adaptation to the environment comes to be on the base of definite hereditary, modus variability being historically limited, it is possible to differentiate phylogenetic relationships and convergent likeness, as well as progress with the rise of evolutionary level and adaptive allogenetic evolution on the same evolutionary level. There are many examples to confirm this view. When evaluation of taxonomic characters in dependence on its adaptation to the environment agree with the molecular data it means the selected criteria are right, if not - attentive study to find the reason must be made and conclusions have to be more cautious.

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636 - Taxonomic monograph of *Fusicladium*, *Pollaccia* and *Spilocaea* (Hyphomycetes, *Venturia* anamorphs p.p.)

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The genera *Fusicladium* Bonord., *Pollaccia* E. Bald. & Cif. and *Spilocaea* Fr. (hyphomycetes) have recently been monographed (Ritschel 2001, Schubert 2001), based on light and scanning electron microscopical as well as molecular (rDNA ITS sequences) examinations, and includes comprehensive descriptions and illustrations of 40 *Fusicladium*, 7 *Pollaccia* and 5 *Spilocaea* species. *Venturia* and its anamorphs proved to be monophyletic. Based on a combination of morphological and molecular examinations and data, the generic delimitation and the species concept of *Venturia* anamorphs have been re-evaluated. Features suitable for the delimitation of species and other features unsuitable for this purpose have been pointed out. The structure of the conidiogenous loci (scars) and conidial hila, which is uniform within *Venturia* anamorphs, proved to be the basic characteristic for the delimitation of genera. Morphological and molecular examinations showed that the three anamorphic genera *Fusicladium*, *Pollaccia* and *Spilocaea* are not tenable. It is suggested to merge these genera under the well-known name *Fusicladium*, which is proposed to be conserved over the older name *Spilocaea*.

637 - Cultural and microscopic studies of *Schizophyllum commune* and *Grifola frondosda* (higher basidiomycetes) strains isolated in Ukraine

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The increasing interest in the cultivation of *Schizophyllum commune* and *Grifola frondosa* depends on their extraordinary medicinal properties. Biotechnological application of *S. commune* and *G. frondosa* mycelial cultures requires isolation of new strains from nature and more detailed studies of their morphological characteristics. Pure cultures of *G. frondosa* and *S. commune* were isolated from fruiting bodies collected on the territory of the Ukraine. Cultures were screened on different agar media at the temperature interval +4 - 38 °C. The growth rate of strains and enzyme tests on oxidases in cultures were determined. Fast growing strains, which have strong enzyme reactions were selected for the following investigation. Scanning electron microscopic studies showed that on hyphae of *G. frondosa* numerous clamp connections of medallion type were present. In old parts of colony often sprouting clamps and anastomoses are typical. In the younger parts of a colony thin ($\leq$ 1 mm wide) branched hyphae (dichohyphidia) are forming. Apical and intercallar chlamydospores are usual. In *S. commune* clamp connections of a classic shape are registered. Some hypha are densely covered with projections described by some authors as pseudoconidia or secretory conidia. Ellipsoid chlamydospores are often present. Teleomorph is typically formed during 5-6 weeks. Described characteristics are significant. For instance, clamps can be used for the control of purity and physiologic state under cultivation.

638 - Two new hyphomycetes from Spain

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Spanish mycobiota is considered highly diverse due to the regional variety of climate and vegetation. However, the hyphomycete check-list is still very reduced. Over recent years, we have conducted an extensive survey of those fungi from different habitats. Recently, we have found two undescribed specimens belonging to *Oidiodendron* Robak and *Phialophora* Medlar, isolated from litter and soil, respectively. Plant debris samples were placed into moist chambers, and soil samples were plated onto sterile Petri dishes which were moistened thoroughly with sterile distilled water and covered with small pieces of sterile wood (soil bait technique). *Oidiodendron myxotrichoides* sp. nov. is characterized by the presence of dark brown reticulate sporodochia, which resemble gymnothecia (ascomata) of *Myxotrichum*, and by its globose or subglobose arthroconidia. The presence of sporodochia constitutes a new character in *Oidiodendron*. *Phialophora vesiculosa* sp. nov. is characterized by possessing erect and branched conidiophores with branches ending in sterile vesicles, and by its mono- or polyphialidic, terminal or lateral conidiogenous cells with flared collarettes, from which spherical, brown conidia with a protuberant basal hilum are produced.

639 - Evolutionary relationships between aquatic anamorphs and teleomorphs: *Lemonniera*

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Mitosporic fungi have traditionally been classified based on their method of conidiogenesis, conidial secession, and conidium and conidiophore morphology. We wanted to determine whether these were important phylogenetic characters for a group of morphologically and developmentally similar aquatic hyphomycetes, or if this was due to convergent evolution. Cladistic analyses were performed on the 28S rDNA of eight species of *Lemonniera* and one species of *Margaritispora*. These two genera both have phialidic conidiogenesis and schizolytic conidial secession. *Lemonniera* has tetraradiate conidia and *Margaritispora* has unbranched conidia. Molecular analyses demonstrate that *Lemonniera* species are placed in two distinct clades: one within the Leotiomycetes; the other within the Pleosporales, Dothideomycetes. *Margaritispora* is placed with *Lemonniera* species within the Leotiomycetes. This demonstrates that for this group of species conidiogenesis, conidial secession and conidium shape are not phylogenetically informative, but are the result of convergent evolution.

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640 - Determination of biological species and analysis of genetic variability by RAPD of strains of *Pleurotus* subgenus *Coremiopleurotus*

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Pleurotus cystidiosus and *P. smithii* strains of different geographic origins were studied in terms of growth rate in culture, mono-dikaryotic matings and genetic variability determined by RADP in order to prove the occurrence of *P. cystidiosus* in South America. The present results show that the criteria used to separate the two species are unsatisfactory and that *P. smithii* should be considered a synonym of *P. cystidiosus*, thus extending the distribution of this species to Central and South America.

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641 - Phylogenetic relationships among the bunt fungi

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There are approximately 120 species of bunt fungi in *Tilletia* and related genera that parasitize members of the Poaceae. Most species are distinguished by teliospore ornamentation and host. A number of non-reticulate-spored species in *Tilletia* have been placed in other genera in the Tilletiales including *Neovossia*, *Conidiosporomyces*, *Ingoldiomyces*, and *Oberwinkleria*. Two economically important species, *T. indica* (Karnal bunt) and *T. horrida* (rice kernel smut) have been placed in *Neovossia* on the basis of tuberculate teliospore ornamentation, floret infection and the production of large number of non-conjugating basidiospores. Phylogenetic analysis of large subunit nuclear rDNA sequences shows that reticulate-spored taxa with hosts in the Pooideae, including *T. tritici*, *T. laevis* and *T. controversa*, form a well-supported monophyletic group (bootstrap >95%). *Tilletia indica* and *T. walkeri*, also on members of the Pooideae, form a well-supported group (100%) and group with reticulate-spored taxa and *Ingoldiomyces hyalospora* on hosts in the Pooideae (bootstrap >70%). A few reticulate-spored taxa and other tuberculate-spored taxa on non-Pooideae hosts, including *T. horrida*, fall in a separate, unsupported group although a number of well-supported lineages exist within this group. *Conidiosporomyces ayresii* groups strongly with *T. vittata*. Results suggest that host may be more phylogenetically informative than morphological characters.

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642 - Taxonomic revision of the lichen genus *Heterodermia* (Physciaceae) from China's mainland

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Thirty-one species of *Heterodermia* are recognized from China's mainland, based on examination of more than 1000 specimens kept in HAMS. Of which, *H. orientalis* and *H. sinocomosa* were described as new (Chen, 2001), and *H. galactophylla*, *H. pseudosquamulosa* and *H. rubescens* are added to Chinese lichen flora. The previous records of *H. erinacea* and *H. granulifera* are proved to be mistaken reports. They are actually *H. pellucida* and *H. isidiophora* respectively and should be excluded from Chinese lichen flora. The previous records of *H. albicans*, *H. barbifera* and *H. dactilyza* from China are doubtful. Biogeographically, *H. boryi*, *H. comosa*, *H. diademata*, *H. flabellata*, *H. isidiophora*, *H. leucomela*, *H. lutescens*, *H. obscurata*, *H. podocarpa*, *H. propagulifera*, *H. pseudospeciosa* and *H. speciosa* are tropical and/or temperate. *H. angustiloba*, *H. dendritica*, *H. firmula*, *H. fragilissima*, *H. microphylla*, *H. orientalis*, *H. pellucida*, *H. pseudosquamulosa*, *H. rubescens*, *H. sinocomosa*, *H. subascendens* and *H. togashii* are eastern Asian or southern Asian. Of which *H. orientalis*, *H. pseudosquamulosa* and *H. sinocomosa* are endemic to China. *H. dissecta* and *H. hypoleuca* are disjunctive in eastern Asia and eastern North America. *H. pacifica* occurs in Pacific region. The geographical affinities of *H. galactophylla*, *H. hypochraea*, *H. hypocaesia* and *H. japonica* are still uncertain. *H. corallophora*, *H. incana*, *H. pandurata* and *H. spinula* recorded in Taiwan of China have not been found in China's mainland.

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643 - Some merosporangiferous Mucorales indigenous to dungs of house mice in Taiwan

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During studies of coprophilous fungi indigenous to dung of house mice, some merosporangiferous Mucorales were isolated. By the use of corn meal agar (CMA) and malt extract agar (MEA) media as well as technique favor the isolation of merosporangiferous fungi in Taiwan. Dung of samples of mice in houses or garages was collected directly into glass vials for transport to laboratory. The materials then were dispended into aseptic damp chambers prepared by moistening of filter paper discs in Petri dishes having depths of two centimeters or they were put directly onto

CMA plates. The author selected *Mucor rouxianus* (Calmette) Wehmer as the standard host for culturing member of *Dimargaris cristalligena* van Tieghem and *Circinella umbellata* van Tieghem & Le Monnier for *Syncephalis nodosa* van Tieghem, *Dispira cornuta* van Tieghem and *D. simplex* Benjamin. Samples of house mice dung were assayed for species of representatives of the merosporangiferous Mucorales and identified as following species, namely, *Syncephalis nodosa* van Tieghem, *Dimargaris cristalligena* van Tieghem, *Dispira cornuta* van Tieghem, *D. simplex* van Tieghem, *Coemansia breviramosa* Linder and *C. interrupta* Linder. All of above listed six species were illustrated and described as new record to Taiwan.

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644 - A comparison of *Peronospora farinosa* isolates from *Chenopodium album* and *Spinacia oleracea* using morphological and molecular characteristics

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Downy mildews of any chenopodiaceous host are currently treated as a single species, *Peronospora farinosa*. Microscopic examination of the Korean isolates from *Chenopodium album* (CAI) and *Spinacia oleracea* (SOI) revealed that they are morphologically different. The conidia are minutely papillate in CAI, but non-papillate in SOI. The ultimate branchlets of CAI are mostly longer than those of SOI. Sequence analyses of the internal transcribed spacer region 1 of ribosomal DNA showed that they are clustered into two different groups. SOI shared only ca. 91% nucleotide sequence homology with CAI, but showed ca. 94% homology with *Peronospora corydalis* isolates. On the other hand, CAI had higher sequence homology with *Peronospora destructor* isolate than with SOI. Consequently, we believe that downy mildews from *C. album* and *S. oleracea* could be considered as separate species.

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645 - Taxonomic revision of *Uromyces* species on cultivated legumes in Japan

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Cultivated legumes are important crops throughout the world. Three *Uromyces* species; *U. appendiculatus*, *U. vignae*, and *U. viciae-fabae*, including varieties on cultivated legumes, have been reported in Japan. Although these species and varieties were classified mainly by morphology of the teliospores and urediniospores, their morphological delimitation is obscure. Therefore, the morphological characteristics of teliospores and urediniospores were clarified and molecular phylogenetic analyses based on the sequences of ribosomal DNA regions were carried out. More than 300 specimens collected from various areas of Japan were used for morphological observations. We examined the morphological characteristics of teliospores and urediniospores by light and scanning electron microscopy. Based on these observations, specimens were classified into three groups. About 80 specimens were selected from the specimens used for morphological observations and sequences of LSU rDNA (D1/D2) and ITS regions were analyzed. Specimens were separated into two clades in phylogenetic trees by the D1/D2 regions. However, they were separated into three clades in phylogenetic trees by the ITS regions. Correlations between morphological groups and phylogenetic groups based on these results suggest a revision of these species and varieties.

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646 - Identification of *Armillaria* isolates from Bhutan based on ITS and IGS-1 sequences

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Armillaria root rot is known to occur in conifer forests in Bhutan. The species causing this disease have, however, not been identified. The aim of this study was to determine the identity of field isolates collected from *Pinus wallichiana* and *Abies densa* at two localities in western Bhutan. DNA sequence data were obtained for the ITS and IGS-1 regions of the rRNA operon and compared with those of *Armillaria* spp. available on GenBank and our own database. IGS-1 sequences for isolates from *P. wallichiana* had the greatest level of similarity (98%) with sequences from the same DNA region of *A. mellea* subsp. *nipponica* from Japan. Although ITS sequence data are not available for *A. mellea* subsp. *nipponica*, sequences from this DNA region were most similar to the closely related *A. mellea* s.s. from Japan. Based on these findings, we believe that the isolates from *P. wallichiana* included in this study, represent *A. mellea* subsp. *nipponica*. ITS sequences for isolates from *A. densa* were most similar (99%) to *A. cepistipes* on GenBank. Parsimony analysis placed these isolates in a sister group to *A. cepistipes*, but also indicated that they are very closely related to *A. gallica* and *A. sinapina*. We believe that the isolates from *A. densa* represent *A. cepistipes*, but sexual compatibility tests are required to confirm this view, especially because *A. cepistipes* is closely related to *A. gallica* and *A. sinapina*.

647 - Molecular phylogeny of ascomycetes from the Helotiales

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This work presents a preliminary assessment of the phylogenetic relationships among species in the Helotiales based on rDNA sequence analysis. Except for the Sclerotiniaceae, the Helotiales have not been subjected to extensive molecular phylogenetic studies. We have analyzed the sequences of the ITS1-5.8s-ITS2 region from more than 100 strains corresponding at least to 42 species in the Hyaloscyphaceae and 32 in the Helotiaceae, with a special emphasis on the genera *Lachnum* and *Hymenoscyphus*. In addition, a subset of the species has been subjected to sequence analysis of the D1-D2 region of the 28s rRNA gene. The result of the cladistic analysis confirmed the heterogeneity of the taxa defined within the two families. The topology did not support the current morphology-based classification schemes of some of the taxa examined. In particular, a significant degree of incongruity was evident for several species in the genus *Hymenoscyphus* and less notably for *Lachnum* spp. The results are discussed in comparison with the morphological characters used to define taxa within the two families studied. The suitability of the ITS region for phylogenetic analysis of this heterogeneous group of fungi is also discussed.

648 - Lumping *Tricholoma sulphureum* and *T. bufonium*?

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The taxonomical status of *Tricholoma bufonium*, generally considered a rare species, has been a matter of discussion for a long time, some authors questioning the validity itself of the taxon and claiming that it is actually a mere variant of the closely related *T. sulphureum*. Others accept *T. bufonium* as a valid species on the basis of the constancy of the features distinguishing it from *T. sulphureum*, although, admittedly, intermediary forms exist. The two 'species' are clearly depicted in colour by some authors. We report the complete morpho-anatomical and molecular characterisation of the naturally occurring ECM type formed on silver fir by *Tricholoma bufonium*, as classified by sporocarp features. Molecular information has also been obtained for *T. bufonium* sporocarps collected on silver fir and oak and for fruitbodies classified as *T. sulphureum* collected on spruce and chestnut. Our data show that both ITS and LSU sequences were virtually identical in sporocarps referred to *T. sulphureum* and *T. bufonium* on the basis of colouration. However, strains from conifers and broad-leaved trees are to some extent different in sequence data. The taxonomic and ecological implications of our findings, together with already published information, notably the anatomical characteristics of *T. sulphureum* ECM on spruce, will be discussed.

649 - Cryptic speciation, evolution and morphological plasticity in metalliferous *Acarospora* (lichenised Ascomycota)

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The *Acarospora smaragdula* (Wahlenb.) A. Massal. and *A. rugulosa* Körb. species complexes are excellent models for studying the pattern of evolution and adaptation in a group where traditional taxonomic interpretation is complicated, and where molecular phylogeny can be utilised to investigate and test hypotheses of the evolution of morphological / anatomical traits. We are investigating the molecular phylogeny of populations of the *Acarospora smaragdula* and *A. rugulosa* complexes, to enable description of the pattern of evolution of metalliferous lichens in these groups, and to test the hypothesis that morphologically ± well-characterised taxa occurring on different metalliferous substrates are phylogenetic species. Here, we will present preliminary results from a pilot study focussing on *A. smaragdula* s. lat. and *A. sinopica* (Wahlenb.) Körb., utilizing well-known loci where fungal-specific PCR-primers are readily available (the nuclear ITS rDNA and mitochondrial SSU rDNA). Future directions, including extended phylogenies with additional markers, studies of the localisation and accumulation of metals in the lichens utilizing light microscopy, confocal scanning microscopy, SEM, mineralogical techniques and electron probe microanalysis, are briefly discussed.

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650 - The mating relationships of the S and F groups of *Heterobasidion annosum* in Northern Hemisphere

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Pairings made among homokaryons of *Heterobasidion parviporum* Niemelä & Korhonen (S group of the H. annosum complex) from Europe, Northeast China and Northwest North America, and the European H. abietinum Niemelä & Korhonen (F group) showed that the Northeast Chinese S has the strongest mating capacity among all the testers, being closely related with the European S and F, and also the North American S. The North American S is more readily compatible with the European F than with the European S, and it seems that the so-called North American S group should be referred to the European F group rather than the European S. Samples collected from North Yunnan (eastern foothills of the Himalayas, China) were paired with testers of the S group from NE China, with P, S and F groups from Europe, and S group from NW North America; the Yunnan stocks mated with all the testers by frequency over 90%. This suggests that the Yunnanese stocks have the largest effective population size.

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651 - Systematics and evolution of Gomphales (Basidiomycetes)

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The order Gomphales enclose eight genera in the world and *Ramaria* is the main one in relation to species number. Previous systematic arrangement in subgenus *Ramaria* was proposed by Frachi & Marchetti (2001, Fungi non delineati. 16). This subgenus was divided into ten sections only based upon the presence/absence of clamped hyphae and the basidiome colour. As part of a monograph of the Iberian Gomphales a phylogenetic study in Gomphales focused on *Ramaria* has been undertaken. Thirty-four species of *Ramaria* as well as other genera of Gomphales were included in the phylogenetic analysis based on ITS and 5.8S rDNA sequences. Moreover, phylogenetic analysis of the morphological characters were done on clavarioid basidiomes. The very variable ITS regions gave low bootstrap values but it can be deduced some considerations about the systematics and evolution of the Gomphales: 1) Subgenus *Echinoramaria* is monophyletic. 2) *Ramaria* is a genus related with several taxa among the Gomphales, *Gautieria morchellaeformis* is related with Section *Ramariae* and *Gomphus clavatus* with Section *Fennicae*. 3) *Ramaria bataillei* and *R. pumila* seems to be a bridge between the ectomycorrhizal and the saprobic *Ramarias*. 4) *Lentaria* and *Hydnocristella* are closely related with subgenus *Lentoramaria*. 5) The gelatinose texture of the fruitbodies seems to be a synapomorphy. 6) Clamp connections and fruitbody colour are very homoplasic characters and do not serve to divide sections among *Ramaria*.

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652 - Another canker-causing aerial *Phytophthora* from forest trees in California and Oregon

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Two distinct *Phytophthora* species cause similar symptoms on several western forest trees. *P. ramorum* causes lethal cankers (sudden oak death) on tanoak and coast live oak trees and foliar and dieback symptoms on other tree and shrub species in some western forests. A second *Phytophthora* species, previously undescribed, is occasionally isolated from lethal cankers on tanoak and coast live oak, and from foliar lesions on *Umbellularia* and other hosts, in areas where *P. ramorum* is also active. ITS DNA sequence indicates close relationship to *P. ilicis* (a foliar pathogen of holly) and *P. psychrophila* (newly described from European oak forest soils). It is homothallic with amphigynous antheridia, and has deciduous sporangia. It grows more slowly, with a lower temperature optimum, than *P. ramorum*. In log inoculation tests it is nearly as pathogenic to tanoak as *P. ramorum*. It does not infect holly leaves in leaf inoculation tests. In the forest it is usually associated with single killed trees, in contrast to the expanding patches of mortality caused by *P. ramorum*.

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653 - Endophytes from leafy liverworts: a molecular phylogenetic perspective

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It has long been known that leafy liverworts possess endophytic fungi, but these fungi have not previously been identified with precision. Here, I present results of molecular phylogeny as the means to identify these endosymbionts from selected leafy liverworts. I address possible explanations for the nature of such symbioses. I discuss hypotheses regarding liverwort structural adaptations for endosymbiosis, and explore methodology to test these hypotheses.

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654 - A molecular taxonomic revision of the genus *Pythium*

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The genus *Pythium* mainly consists of plant pathogens. Identification is based on morphology and is notoriously difficult due to variability and overlapping of characacters. Therefore a molecular approach was chosen to evaluate the existing taxonomy, and to find more reliable methods for identification and detection. All available species (approx. 100), represented by more than 500 isolates were included. One representative isolate of each species was used for sequencing of ITS and D1-D3 regions of the lsrDNA: all ex-type, neo-type, authentic or otherwise well defined representative strains. Sequences were used for both reconstruction of phylogeny and development of species-specific oligonucleotides. The latter were used in DNA-arrays for simultaneous detection of multiple pathogens in soil samples. All strains were used in studies of RFLPs of PCR amplified ribosomal IGS and ITS and RAPD patterns, to reveal intraspecific variation and to establish species boundaries. RAPD patterns were most variable; IGS patterns proved to be useful for identification. Studies were performed simultaneously. Phylogenetic trees showed which species were to be compared side by side on RFLP and RAPD gels; the latter patterns showed which additional strains should be sequenced in case of intraspecific variation. Results will be used to redescribe species based on molecular groups, to provide a database of molecular characters for identification and to develop specific probes for detection.

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655 - Elucidating the 'enigma' of *Aenigmatomyces ampullisporus* which is now found as a Zygomycetous fungus parasitizes a spermatophore of collembolan insect

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Aenigmatomyces ampullisporus Castañeda and Kendrick was described from Canada in 1993. Although the authors assigned it to the kingdom Fungi, they could not decide to which phylum it belonged. They gave it a name 'enigmatic fungus' and left its appropriate taxonomic position as a mystery to be resolved in future. From 1999 to 2002, I collected the fungus repeatedly from Japan, which was identified as *A. ampullisporus* in reference with the holotype specimen. As a result of observations, the 'enigma' was resolved as follows; 1) The 'host hyphae' as explained in the original description can no longer be a fungal structure, but are stalks of spermatophores of Collembola. 2) Spores infecting the head of spermatophores swelled to become hyphal bodies. While one of the hyphal arms elongates downward functioning as an anchor, the other arm elongates upwards bearing abundant sporogenous structures. In maturation, spermatozoon in a spermatophore gradually degrades and finally disappears entirely. 3) Spherical liquid-drops were stained in blue at the apices of spores when mounted in Lacto-aniline blue. 4) 'Oospores of the host' are directly connected with the hyphal bodies. Their pigmentation and ornamentation suggests that they are zygospores. Consequently, *A. ampullisporus* is not an Oomycetes-parasite but a zygomycetous fungus parasitizing the spermatophore of Collembola, and is probably a relative of holocarpoic amoeba-parasites of Cochlonemataceae, Zoopagales.

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656 - Inter- and infraspecific length variability in minisatellites in the ITS region of *Leccinum* (Boletales, Boletaceae)

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Phylogenetic relationships of the European species of *Leccinum* (Boletaceae) were investigated by maximum parsimony, likelihood analyses and Bayesian analyses of nrITS1-5.8S-ITS2 and 28S sequences. The separate gene trees inferred were largely concordant, and their combined analysis indicates that several traditional sectional and species-level taxonomic schemes are artificial. In *Leccinum*, the nrITS region ranges in size from 694 to 1480 bp. This extreme length heterogeneity is localized to a part of the ITS1 spacer that contains a minisatellite with tandemly repeated copies including the six-basepair GAAAAG motif. The number of tandem repeats varied from 2 to 6, but there were many repeat variants. Secondary structure analysis revealed that the ITS1 spacer in *Leccinum* appears to be still functional despite the presence of the minisatellite. Intraindividual sequence variation of the minisatellite was always smaller than between different species, indicating that concerted evolution proceeds rapidly enough to retain phylogenetic signal at the infraspecific level. In contrast, the evolutionary pattern exhibited by the major ITS1 repeat types found was homoplastic when mapped onto the species lineages inferred from the combined 5.8S-ITS2 sequences. The minisatellite therefore appears not to be useful for phylogeny reconstruction at the species level.

657 - Notes on the tribes *Leucocoprineae* and *Lepioteae* (higher basidiomycetes) of Israel mycobiota

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Agaricaceae (Fr.) Cohn has been of particular interest to scientists all over the globe for years due to peculiarities of its representatives ecology, biochemistry, biotechnological application. However, for some areas - Israel in particular - even the first step of study of this family - revision of species diversity - has not been done yet. Out of the four tribes of the family only *Agariceae* Pat. has been revised (Wasser & Binyamini, 1992; Wasser, 1996, 1998, 2002). The *Leucocoprineae* Sing. and *Lepioteae* Fay. in Israel tribes are in need of critical study and inventory of the species content. Analysis of existing fragmentary data in a number of articles (Reichart & Avizohar-Hershenzon 1955, 1959; Avizohar-Hershenzon, 1967; Binyamini, 1975; 1976; 1984) showed that up to now 15 species (9 of genus *Lepiota*, 1 of *Leucocoprinus*, 1 of *Chlorophyllum*, 1 of *Leucoagaricus* and 3 of *Macrolepiota*) are registered. We report of seven new for the Israel territory species and intraspecies taxa found during 2001-2002 growing seasons: *Chamaemyces fracidus* (Fr.) Donk.; *Chamaemyces fracidus* var. *pseudocastaneus* Bon et Boiff.; *Lepiota brunneolilacea* Bon et Boiff.; *Leucoagaricus littoralis* (Meiner) Bon et Boiff.; *Leucoagaricus wichanskyi* (Pil.) Bon et Boiff.; *Leucoagaricus carneifolius* (Gill.) S. Wasser; *Macrolepiota fuligineosquarrosa* Malenc.

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658 - Out of Gondwana? - Relationship between European and African *Lactarius* sp. of the subgenera *Lactifluus* and *Lactariospsis*

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The forests of tropical Africa are remarkably rich in Russulales species, particularly in species displaying what are considered to be conservative characters. Therefore, the hypothesis was put forward that the Russulales evolved on the Gondwana continent and spread from there to the northern hemisphere. The subgenera *Lactifluus* and *Lactariopsis* of the genus *Lactarius* belong to these supposedly primitive groups with a strong tropical African foothold, in terms of species numbers, infra-subgeneric diversity, and distribution. Both subgenera are also relatively well-represented in tropical lowlands of Central- and South-America. In contrast, only few and morphologically outstanding European *Lactarius* species were assigned to these subgenera. In molecular phylogenetic analysis, these species were indeed placed on very long branches, separated from the majority of European species that are comparatively closely related. Molecular phylogenetic analysis methods were applied to investigate the relationship between selected European and tropical African species of the subgenera *Lactifluus* and *Lactariopsis* and to test the provisional infra-subgeneric classification of African *Lactarius* species that was entirely based on morphological characters.

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659 - The phylogenetic position of the genera *Lepraria* and *Leproloma*

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The phylogenetic position of the entirely asexually reproducing genera *Lepraria* and *Leproloma* was investigated using sequence data from the ITS1-5.8S-ITS2 and small subunit (SSU) nuclear ribosomal DNA. Phylogenetic reconstructions were carried out using a Bayesian Markov chain Monte Carlo (MCMC) tree sampling technique and an alignment-free maximum likelihood distance method. The results indicate that most species currently referred to the genera *Lepraria* and *Leproloma* form a single, monophyletic group. This monophyletic group is sister to a well-known group of lichens in the Stereocaulaceae (Lecanorales, Lecanoromycetes, Ascomycota), namely *Stereocaulon* and *Muhria*. *Leproloma* is polyphyletic and nested within *Lepraria* s. str. A few species, however, do not belong in *Lepraria* s. str., viz. *Lepraria flavescens*, which belongs in *Lecanora*, and *Lepraria lesdainii* and *L. obtusatica*, the positions of which are currently unknown.

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660 - *Toninia* species in Hungary

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Species of *Toninia* are dominant in the sandy steppe and rocky grassland areas in Hungary. In the Hungarian lichen flora of Verseghy (1994) nine species were listed under *Toninia*: *T. candida*, *T. caradocensis*, *T. cinereovirens*, *T. coeruleonigricans*, *T. lobulata*, *T. toniniana*, *T. tristis*, *T. tumidula*, *T. zsakii*. A revision of the c. 300 Hungarian *Toninia* specimens was highly needed, since the *Toninia* monograph of Timdal (1991) was not considered by Verseghy. Three of the above species are now belong to other genera: *Hypocenomyce caradocensis* (syn. *T.*

caradocensis), *Mycobilimbia lobulata* (syn. *T. lobulata*), *Micarea melaenida* (syn. *T. zsakii*). *Toninia sedifolia* with c. 200 specimens is the most frequent species of *Toninia* in Hungary. One specimen of *T. alutacea* (under *T. intermedia* from 1933) and two specimens of *T. aromatica* (from 1917 and 1955) kept in BP were regarded as uncertain data by Verseghy. No recent collections could confirm their occurrence in Hungary. *T. physaroides* and *T. verrucarioides* were indicated with Hungarian distribution by Timdal (1991). *T. physaroides* must have been overlooked because of its similarity with *T. sedifolia*. Parasitic and lichenicolous lichens are generally undercollected in Hungary. Most of the recent specimens also belong to *T. sedifolia*. Nevertheless *T. diffracta* was identified from a recent collection as new to Hungary. [Supported by the Hungarian Research Fund (OTKA T030209) and 'Research Grant Bolyai János'.]

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661 - To morphological and anatomical variability of lichen-forming fungus *Xanthoria patietina* s. lat. (Teloschistaceae, Ascomycotina)

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Xanthoria parietina (L.) Th. Fr. is characterized by large thallus with plane and wide lobes. More than 40 infraspecific taxa of various status are hitherto described for this very polymorph species. However there are only a few morphological characters, which have being hitherto used for taxonomy of this group, namely presence of soredia or isidia, morphology of thallus or single lobes and ecological characters of habitats. The aim of this study is to select a number of correlated characters, which may be applied for taxonomy of the whole *X. parietina* group. Three morphological types of *Xanthoria parietina* were selected on the basis of results obtained. The first one, thallus is very small (up to 2 cm diam.) with very dissected narrow lobes which never overlapping by marginal zone (lobes of the second level are well developed). The second one, with large entire thallus (4-16 cm diam.), only slightly dissected at marginal zone, lobe margins not overlapping (lobes of the second level are absent). The third one, thallus is very large (10 cm and more in diam.), margin almost entire, almost undissected, lobe margins (if developed) overlapping. Involving additional material from other regions of Eurasia and the further comparative study of groups selected with type material of *X. parietina* and *X. microspora* B. de Lesd. will show taxonomical status of these groups mentioned.

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662 - Identification and intra-specific characterization of penicillia and aspergilli by Fourier-Transform Infrared Spectroscopy (FT-IR)

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Airborne microfungi are a matter of discussion in public and occupational hygiene, they can be allergenic, toxigenic or some may cause infections. To determine health hazards, reliable species identification and physiological characterization of isolates is needed. Conidial suspensions of airborne fungi from the genera *Aspergillus* and *Penicillium* were characterized using Fourier-Transform Infrared Spectroscopy (FT-IR). A new method was developed for the preparation of conidia. A database was established containing reference strains from culture collections as well as fresh isolates. The results obtained by cluster analysis were compared to data from chemotaxonomic investigations, where the production of secondary metabolites and the presence of mycotoxins in conidia has been investigated. The taxonomic relationship was reflected in the dendrograms resulting from the cluster analysis. Identification of newly isolated strains was possible by comparison to the database established. In some species, intra-specific variation concerning mycotoxin production occurred, which was equally reflected in the discrimination of strains. The new method seems to be particularly suited to be applied in different areas of applied and systematic mycology: microbial inventories, rapid identification of pathogens, biotechnology, quality control in culture collections. Moreover, the method proved to be a powerful tool in taxonomic studies.

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663 - Phylogeny of *Termitomyces* and related taxa based on nuclaer (nLSU) and mitochondrial (mtSSU) ribosomal DNA

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A phylogeny of the symbiotic fungi of the fungus-growing higher termites (subfamily Macrotermitinae) and related taxa is estimated with Bayesian methods. Our sample consists of 130 sequences of nLSU rDNA obtained from basidiocarps, termite nests and termite gut material and represents extensive geographic and taxonomic sampling. Results of the Bayesian analysis are in agreement with those of traditional maximum parsimony and maximum likelihood methods. Inclusion of mtSSU rDNA sequence data from several samples agrees with the overall topography of the phylogeny. The results confirm that *Termitomyces* constitutes a monophyletic group within Lyophylleae. *Podabrella* (*T. microcarpus*) is derived within *Termitomyces*. The proposed related genus *Sinotermitomyces* belongs to *Termitomyces* but does not constitute a monophyletic group. *Gerhardtia brunneoincarnata*, proposed to have affinities to *Termitomyces* due to absence of clamps, belongs to Lyophylleae, but does not belong to *Termitomyces*. There seems to be a geographical differentiation of clades as well as a differentiation in relation to host genera.

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664 - Characterisation of yeast communities in aquatic environmental samples by Temperature Gradient Gel Electrophoresis (TGGE)

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Several difficulties affect the characterisation of microbial communities in environmental samples. Traditionally, culture-dependent methods were used for studies of microbial diversity. However, this approach is too laborious to be used intensively. To overcome the disadvantages of the isolation step and the selective effect induced by culture media, several culture-independent approaches have been proposed. One of the most popular is PCR-DGGE/TGGE. This method is based on the PCR amplification of DNA fragments from the pool of total DNA extracted from a natural sample. The separation of the amplicons is done in a denaturing electrophoresis. In this study we optimised this approach for the detection and characterisation of yeasts in aquatic samples. Water volumes of 5 L were filtered and the DNA of the organisms present in the filter was extracted. Then, a set of primers specific for fungi and designed for the 26S rDNA, was used. Finally, the amplicons were separated using a TGGE apparatus. It was observed that yeasts were present in relatively low numbers, which difficulted their detection. Simultaneously, liquid medium enrichments were performed during periods of 1-12 days. After incubation, DNA was extracted and analysed by PCR-TGGE. The electrophoretic profiles obtained from enriched and non-enriched samples from different aquatic environments were compared and the composition of the yeast community was analysed by direct DNA sequencing of the TGGE bands.

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665 - *Nexomyces cubensis* gen. et sp. nov. from Cuban soil

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During the course of a study of soil ascomycetes from Cuba, an undescribed ascomycete was isolated from Ciénaga de Zapata (Matanzas province). Due to endemic, rare and endangered organisms, which can be found in this region, it has been declared a Biosphere Reserve by UNESCO. A bait technique was used for recovering the fungi. Petri dishes were half-filled with the soil samples and moistened with sterile distilled water. Several pieces of sterile wood (approx. 1x2 cm) were placed on the soil surface or partially buried in it. The new ascomycete *Nexomyces cubensis* gen. et sp. nov. is characterized by ostiolate and non-ostiolate setose ascomata and ellipsoidal, thick- and smooth- walled, brown to dark brown, opaque ascospores, which are umbonated at the apex with a protuberant apical germ pore. Due to its morphological features, this taxon was easily recognized as belonging to the Sordariales, although its exact taxonomic placement was difficult to ascertain. In order to establish its phylogenetic relationships, the ITS rDNA region sequence was compared with those of other genera of the Sordariales. Analyses of the sequences suggest that this taxon is close to Sordariaceae.

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666 - Phylogenetic boundaries in *Ramariopsis*

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The genus *Ramariopsis* was first described by Corner (1950) who separated from the genus *Clavaria* those species having branched fruit bodies, whitish colors and spiny hyaline spores. Petersen (1966, 1969 & 1978) proposed an emendation in order to include species with smooth spores and simple fruit bodies. The present work comprehends a phylogenetic analyses based on morphology, whitch pretends to clarify the phylogenetical boundaries of *Ramariopsis*. Thirty-six morphological character were considered for four species of *Ramariopsis* sensu Corner (1970) and six species of *Ramariopsis* sensu Petersen, eleven close outgroups from genus *Clavaria*, *Multiclavula*, *Clavariadelphus*, and *Scytinopogon*, included to test phylogenetical boundaries; three additional far outgroups were included for cladogram orientation purposes, a total of twenty-four species were included. Outgroups were selected by performing a phylogenetical prospection of the Clavariaceae family. *Ramariopsis* sensu Corner was found as a monophyletic group, with *Scytinopogon* as its sister group, which agree with Corner (1970) proposals. *Ramariopsis* sensu Petersen was rejected as a monophyletic natural genus.

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667 - Molecular study of the *Caloplaca saxicola* group on the background of morphological taxa

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Caloplaca (Teloschistaceae, Ascomycota) with more than 1000 species can be considered one of the most complex and diversified genus among crustose lichens. Its delimitation has been always problematic due mostly to the strong similarity between lobed species of *Caloplaca* and species of other genera within this family, such as *Xanthoria*. Among all lobed *Caloplaca* species, the *Saxicola* group has been the most controversial taxonomically. Here we report the results from phylogenetic analyses of the nuclear rDNA internal trancribed spacers (ITS) sequence data for 60 specimens belonging to the *Saxicola* group. Collections from Europe, North America and Asia are included. We have used these molecular data to compare the current phenotypic delimitation of certain species. Hence, a morphological comprehensive study of the group is provided with an emphasis on the enigmatic *C. arnoldii* s. auct., the phenotypically variable *C. saxicola* s. str. and the concept of *C. biatorina* in the different continents. A proposal for achieving a new and stable classification of this group will be presented.

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668 - Phylogeny of the genus *Arachnomyces* and its anamorphs

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Arachnomyces is a genus of cleistothecial ascomycetes that has morphological similarities to the Onygenaceae and the Gymnoascaceae but is not accommodated well in either taxon. The phylogeny of the genus *Arachnomyces* and related anamorphs was studied using nuclear SSU rDNA gene sequences. Partial sequences were determined from ex-type cultures representing *A. minimus*, *A. nodosetosus* (anamorph *Onychocola canadensis*), *A. kanei* (anamorph *O. kanei*) and *A. gracilis* (anamorph *Malbranchea* sp.) and aligned together with corresponding sequences of forty other ascomycetes. Phylogenetic analysis using maximum parsimony showed the genus *Arachnomyces* is monophyletic, includes the hyphomycete *Malbranchea sclerotica*, and forms a separate lineage within the Eurotiomycetes. All known anamorphs in this lineage are arthroconidial and have been placed either in *Onychocola* (*A. nodosetosus*, *A. kanei*) or in *Malbranchea* (*A. gracilis*). *Onychocola* is chosen as the most appropriate taxon for the arthroconidial states of *Arachnomyces* and thus *Malbranchea sclerotica* and the anamorph of *A. gracilis* are treated as species within *Onychocola*.

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669 - Analysis of 18S ribosomal RNA gene sequences reveals the phylogenetic relationships of the genus *Mycosphaerella*

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Mycosphaerella is one of the largest groups of plant pathogenic fungi with more than 1800 species names. Anamorphs associated with *Mycosphaerella* number more than 40, many of which also are economically important with large numbers of species. For example, *Cercospora* and *Septoria* contain more than 3000 and 1000 species names, respectively, most of which probably were derived from species with a *Mycosphaerella* teleomorph. Despite their common occurrence and high economic importance, members of this genus have been under-represented in phylogenetic analyses and the taxonomic position of *Mycosphaerella* is uncertain. Most taxonomists place the genus in the order Dothideales. However, this conclusion has not been supported by limited analyses of 18S ribosomal RNA gene sequences. To test whether the genus *Mycosphaerella* belongs in the order Dothideales, 18S rDNA sequences were obtained from five species representing the major groups within the genus identified in previous analyses of ITS sequences. All five species of *Mycosphaerella* clustered together but separate from species of *Dothidea* and formed a basal group to the other loculoascomycetes in a neighbor-joining analysis of 109 18S sequences. Species of *Dothidea* clustered with those of *Botryosphaeria*, another genus of uncertain taxonomic position. Inclusion of *Mycosphaerella* within the Dothideales was not supported by this analysis and a new order may be needed to accommodate species of *Mycosphaerella* and related genera.

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670 - Biogeography and species concepts - *Lentinus lindquistii* is *Lentinus tigrinus*, a circum-global species

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Lentinus lindquistii (Sing.) Lechner & Alberto (=*Pleurotus lindquistii* Singer) is known only from its type locality in Argentina. *Lentinus lindquistii* has been separated from *L. tigrinus* s. s. based on non-inflated generative hyphae and other slight morphological differences (i.e. spore size). We employed tester strains of *L. lindquistii* and single-basidiospore isolates (SBIs) of *L. tigrinus* (Bull.: Fr.) from 10 widely scattered geographic locations to ascertain congruence of morphological, biological, and genetical species concepts. SBIs of *L. tigrinus* were obtained from fresh material collected in the field and basidiomata produced *in vitro*. SBIs from each collection were paired with those of all other collections (n = 4 or 8) to ascertain sexual intercompatibility. Results showed complete compatibility among collections, indicating that all collections represented the same biological species. Our data demonstrate the morphological variability of two 'taxa' in the *L. tigrinus* complex. Sequence analysis of the ITS1-5.8S-ITS2 nrDNA suggests that widely scattered geographical populations of the *L. tigrinus* complex are genetically divergent. Although these populations have been separated long enough to allow sequence evolution, they are still capable of compatible mating.

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671 - Ascocarp impalement: a novel mechanism of dispersal in cleistothecial ascomycetes

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Ascomycetes have evolved a variety of mechanisms to ensure the dispersal of their meiospores by animals. Some of these include the production of sticky spores (e.g. Ophiostomatales) or even the development of the ascocarp directly on the body of an arthropod (e.g. Laboulbeniales), both ensuring that propagules are transferred economically from one suitable habitat to another. Here, we report a novel mechanism that has developed independently in two ascomycete lineages, the Leotiomycetes and the Eurotiomycetes. In representatives of these classes, namely *Myxotrichum* and *Auxarthron* respectively, the ascocarp is a cage-like cleistothecium with an internal mass of minute, pale coloured ascospores borne within a lattice of rigid thick walled hyphae. By exposing mature ascocarps of these two fungi to active Dipterans (Sarcophagidae and Calliphoridae) for 24 hours we demonstrated that this type of ascocarp is picked up through an impalement event in which the arthropod hairs pass between the spaces of the cleistothecial lattice, glancing or piercing the ascospore mass and affixing the entire ascocarp to the body of the vector. We suggest that this impalement mechanism is the driving force behind the convergence in ascocarp form in these cleistothecial fungi.

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672 - *Cryphonectria cubensis* resides in a genus outside *Cryphonectria*

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Cryphonectria cubensis is a serious pathogen of *Eucalyptus* spp., clove (*Syzigium aromaticum*) and *Tibouchina* spp. in the tropics and sub-tropics. In previous studies, sequence data from the ITS1/ITS2 regions of the ribosomal DNA operon, and LSU and SSU DNA, suggested that *C. cubensis* does not group together with other *Cryphonectria* spp. The aim of the present study was to pursue this question further and to determine an appropriate generic placement for *C. cubensis*. An expanded collection of *Cryphonectria* and *Endothia* spp. was included and we produced additional sequences for the ITS1/ITS2 regions and the beta--tubulin genes. Herbarium specimens, including type collections, of *C. cubensis* and other species of *Cryphonectria* were also examined in detail. DNA sequences revealed that a world-wide collection of *C. cubensis* isolates, grouped separately from *Cryphonectria* and *Endothia* species in a strongly resolved clade. The anamorph of *C. cubensis* is blackened, pycnidium-like and superficial, and the teleomorph has black perithecial necks extending beyond the stromatal surface. This is in contrast to *Cryphonectria* that has orange stromata with semi-immersed anamorphs and orange perithecial necks. Morphological characteristics support DNA-based analyses, showing that *C. cubensis* should reside in a genus separate from *Cryphonectria*. In contrast to previous views, anamorph morphology and colour appear to be more taxonomically informative than teleomorph structure and ascospores.

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673 - Reconsideration of the conspecificity of *Endothia eugeniae* and *Cryphonectria cubensis*

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Endothia eugeniae, a canker pathogen of clove (*Syzigium aromaticum*), was synonymised with the *Eucalyptus* canker pathogen *Cryphonectria cubensis*. This synonymy emerged from morphological studies, cross-inoculations and isozyme analyses. However, for stromatal morphology, the type description of *E. eugeniae* does not concur with that of *C. cubensis*. New collections of *Cryphonectria* have become available to us and the aim of this study was to consider the validity of the synonymy of these two fungi. Isolates of *C. cubensis* and *E. eugeniae* were compared based on DNA sequences from the ITS1/ITS2 region and beta-tubulin genes. Specimens of clove in Zanzibar and South East Asia, were also compared morphologically with specimens of *C. cubensis* from *Eucalyptus*. Phylogenetic analyses showed that the majority of isolates from clove grouped together with *C. cubensis* isolates. A smaller number of isolates from Indonesia, however, grouped separately from the *Cryphonectria* and *C. cubensis* clades. Two fungi with different tissue types were observed on clove bark. One of these resembled *C. cubensis*. The other fungus is a species morphologically similar to *Cryphonectria*. The *C. cubensis* anamorph was morphologically variable on clove, which might explain anomalies regarding the description of *E. eugeniae*. Our results suggest that the synonymy of *C. cubensis* and *E. eugeniae* is valid. However, there is another fungus, closely related to *Cryphonectria*, also present on clove.

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674 - The genus *Urocystis* (Ustilaginomycetes) in China

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For the genus *Urocystis* in China, Ling(1953) described 4 species and Wang (1963) published 11 species. In 1979 Tai Fanlan in his 'Sylloge Fungorum Sinicorum' recorded 13 species. So far a total of 36 species of the genus *Urocystis* are reported from China, distributed among 33 genera of 9 families of the host plants. These smut species are arranged alphabetically as follows: *Urocystis achnatheri*, *U. agropyri*, *U. agropyri-campestris*, *U. agrostidis*, *U. antipolitana*, *U. beijingensis*, *U. bolivari*, *U. brassicae*, *U. bromi*, *U. calamagrostidis*, *U. clintoniae*, *U. delphinii*, *U. dioscoreae*, *U. fischeri*, *U. granulosa*, *U. helanensis*, *U. hierochloae*, *U. irregularis*, *U. japonica*, *U. leersiae*, *U. magica*, *U. melicae*, *U. nevodovskyi*, *U. occulta*, *U. paridis*, *U. poae*, *U. primulicola*, *U. pulsatillae*, *U. qinghaiensis*, *U. ranunculi*, *U. rodgersiae*, *U. sorosporioides*, *U. stipae*, *U. tritici*, *U. ulei* and *U. yunnanensis*. Among them, *U. achnatheri*, *U. beijingensis*, *U. helanensis*, *U. qinghaiensis* and *U. yunnanensis* were recently discovered as new species, known only from type locality and possibly endemic.

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675 - Traditional infrageneric classifications of *Gymnopilus* (Agaricales, Cortinariaceae) are not supported by ribosomal DNA sequence data

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Fulvidula (*Gymnopilus* P. Karst.) was divided by Romagnesi (1942) into *Annulatae* and *Cortinatae* (= section *Gymnopilus* Singer). The first includes species with a persistent, membranous annulus and the second species with an arachnoid veil. Hesler (1969) elevated Singer's sections to subgenera and divided *Gymnopilus* into two sections: *Microspori* and *Gymnopilus*. Guzmán-Dávalos (1995) proposed a third section, *Macrospori*, within *Gymnopilus*. This classification has been accepted by most authors; even though the assignment of a species is oftentimes ambiguous. Here we test the monophyly of *Gymnopilus* and the appropriateness of the current classification system using ribosomal nuclear ITS1-5.8S-ITS2 sequence data. Phylogenetic analyses of 59 sequences (53 ingroup and 6 outgroup) were performed using maximum parsimony. Initial analyses support the monophyly of *Gymnopilus*. Its division into the two traditional subgenera was not supported, neither was the recognition of the three sections in subgenus *Gymnopilus*. Four well-supported clades were identified in the genus: 1) the *spectabilis-imperialis* group; 2) the *penetrans-sapineus* complex; 3) a clade formed by *G. underwoodii*, *G. validipes* and *G.* cf. *flavidellus*; and 4) the *dilepis-medius* group. Thus, while the presence of a membranous veil and the size of the basidiospores traditionally have been used to support infrageneric groups, these characters have been shown to be highly homoplastic, and of little use at this taxonomic level.

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676 - Species level identification of the barley pathogens in *Pyrenophora*: Morphological versus molecular evidence

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Pyrenophora teres and *P. graminea* cause two important foliar diseases of barley, net blotch and leaf stripe, respectively. *P. teres* comprises two pathovars (*P. teres* f. *teres* and *P. teres* f. *maculata*) differentiated by lesion type (net blotch and spotted net blotch). Molecular phylogenetic analyses were used to examine species concepts within *Pyrenophora*. The initial ITS sequence analysis revealed species-specific sequence motifs in the ITS1 region for most species sampled but failed to definitively differentiate among barley isolates at the species level. This result supported prior mating experiments suggesting that *P. teres* and *P. graminea* represent one biological species, despite morphological differences, and differing mode of infection and disease symptoms on a single host. Further parsimony analyses were performed using sequence data from three genetic loci for multiple strains. Several isolates were included whose *P. teres* pathovar identification had been confirmed in inoculation trials on the host and several strains of *P. graminea* newly isolated from distinctive leaf stripe lesions. Two distinct clusters corresponded to *P. teres* f. *teres* and *P. graminea*, while numerous strains variously identified but including those confirmed as *P. teres* f. *maculata*, were intermediate.

677 - Molecular and morphological characterization of a cosmopolitan root endophyte allied to the Leotiomycetes

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Sterile fungi isolated from surface-sterilized roots of the Ericaceae were hypothesized to be conspecific based primarily on restriction fragment length polymorphisms and provisionally named as Variable White Taxon (VWT). In preliminary re-synthesis trials with *Vaccinium myrtilloides* and *V. vitis-idaea*, two isolates tested did not form ericoid mycorrhizas. Several isolates of culturally similar fungi were obtained from roots of the Orchidaceae and Pinaceae and suspected of being VWT based on colony morphology and characters of the mycelium. To assess conspecificity and infer their phylogenetic affinities, partial nuclear ribosomal DNA sequences were determined. Parsimony analyses supported a species level distinction for VWT isolates and indicated that the taxon is closely related to, but distinct from, *Hymenoscyphus ericae* (Leotiomycetes). A new anamorph genus and species is proposed to accommodate this endophyte, which so far has remained sterile in culture, to facilitate information retrieval and discussion and provide a sound foundation for additional experimental work. Strains are identified using a suite of morphological characters in conjunction with ITS sequence data.

678 - *Knufia cryptophialidica*, close phylogenetic relative of *Coniosporium* spp. (Ascomycota, *incertae sedis*)

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When first described, the taxonomic position of *Knufia cryptophialidica* (monotypic genus, Hyphomycetes), isolated from galls on stems and branches of *Populus tremuloides*, was hypothesized to be related to the metacapnodiaceous sooty molds. Similar characteristics of the group include slow-growing, black, cerebriform growth on agar, the production of undifferentiated conidiogenous cells on moniliform hyphae, and phialidic conidiogenesis in some species. In a study focused on the identification and phylogenetic placement of a sterile black cerebriform fungus from *Sphagnum* leaves also suspected of being related to the Metacapnodiaceae, DNA sequences from the nuclear ribosomal RNA gene were determined for 4 strains of *Knufia cryptophialidica* for comparative purposes. Parsimony analyses indicated that *K. cryptophialidica* was distant from the sooty molds in the Capnodiales but closely related to *Coniosporium apollinis* and *C. perforans*, two species shown previously to form a strongly supported sister clade to the Chaetothyriomycetes. The *Coniosporium* spp. were isolated from stone and are superficially similar in morphology to *K. cryptophialidica*, but develop catenate muriform conidia from meristematic conidiophores.

679 - Hawaiian puffballs, earthstars and stinkhorns

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An annotated list of gasteromycetes found in the Hawaiian Islands including members of the Lycoperdaceae, Geastraceae, Nidulariaceae, Tulostomataceae, Phallaceae, Clathraceae, and Sclerodermataceae will be presented from collections taken over the past ten years on all the major islands. Alien habitats such as coastal *Casuarina* groves, coastal *Prosopsis* groves, cypress plantings, and hillside *Leucaena* thickets harbor a number of earthstars and stalked puffballs including *Tulostoma* and *Battarreoides*. The native mamane-naio vegetation zones on the leeward sides of the high volcanic mountains are also excellent habitats for xerophytic earthstars, *Battarrea*, and *Tulostoma*. Members of the Phallaceae including *Dictyophora*, *Mutinus*, *Pseudocolus*, and *Phallus* are found in agricultural and horticultural settings whereas *Aseroë rubra*, along with various species of *Scleroderma* are associated with *Eucalyptus*. *Vascellum*, *Bovista*, *Calvatia*, and *Dictyophora* are common inhabitants of grass on lawns, golf courses, and pastures. A pictorial summary of the individual species will be presented.

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680 - Population structure of *Septobasidium curtisii*, a fungal symbiont of scale insects

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Septobasidium curtisii lives symbiotically with scale insects on trees and is common in the southeastern United States. The fungus forms thin black mats of hyphae over colonies of scale insects infecting some but not all. Species of *Septobasidium* are thought to disperse only as spores infecting young scale insects, and subsequently, hyphae from infected insects fuse to form a single fungal colony. If this is true for *S. curtisii* then fungal colonies may be genetic mosaics, and populations may be differentiated on small spatial scales within a stand of trees or on a single tree in accordance with host insect population structure. To test these hypotheses we use DNA sequence data from multiple loci to determine patterns of genetic diversity in single colonies and across geographic and host ranges in *S. curtisii*. Sequence data from five loci from single spore isolates show that colonies are composed of a single heterokaryotic individual that produces recombinant spores. Preliminary data from collections across the southeastern United States indicate that population structure of *S. curtisii* and host insects are correlated but at a larger spatial scale than expected.

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681 - *Puccinia hemerocallidis*, cause of daylily rust, a newly introduced disease in the Americas

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Daylilies (*Hemerocallis* spp.) are the most important perennial flowering plant grown in the U.S. and are traded extensively by hobbyists. A rust of daylilies was introduced recently into North and Central America. *Puccinia hemerocallidis* is known on daylilies in Asia (stages II, III) with *Patrinia* (Valerianaceae) as the alternate host (stages 0, I), also native to Asia. Specimens from Costa Rica, Japan, Russia and the United States as well as previously collected specimens from its native eastern Asia were examined morphologically to confirm that this rust was *P. hemerocallidis*. In addition, the ITS region of the ribosomal DNA was sequenced from six representative fresh specimens from the Americas and Asia. The range of variability of certain morphological characteristics, including the ratio of non-septate to 1-septate teliospores, was greater than indicated in the current literature. Teliospore and urediniospore sizes varied among specimens but statistical analysis indicated that there was no significant difference in size between the Asian and the American spores. Three synapomorphies (including one indel) were present in the ITS region that distinguished the two Asian specimens from the four American specimens. However the ITS variation within the American or Asian specimens was approximately equal to variation between specimens from the two broad geographic areas. We conclude that the rust introduced into the Americas is *Puccinia hemerocallidis*.

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682 - Exceptional morphological diversification in *Cladia* and phylogenetic related lineages in Australasia

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Cladia is a genus of lichenized fungi with most of its species endemic to the Australasian region. Of the 14 described species in the genus only four occur outside this region. Three other lichen genera endemic to this region, *Heterodea*, *Ramalea* and *Thysanothecium*, share many anatomical features with *Cladia*, and its family Cladoniaceae in general, but have very distinct vegetative morphologies. In our phylogenetic analysis of three regions of the nuclear and mitochondrial rDNA, the species of the genus *Cladia* were divided into two clades. The first clade, containing *C. aggregata*, the type species of the genus, consisted exclusively of members of the *C. aggregata* complex. This complex of very similar, hard to distinguish species is characterized by rather irregular perforations and a dark green-brown to black color. The second clade, in some analyses a grade, includes regularly perforated species of *Cladia*, such as *C. ferdinandii* and *C. sullivanii*. The latter species are paraphyletic relative to *Heterodea*, *Ramalea* and *Thysanothecium*. On the basis of this result the name *Cladia* should be restricted to species of the *Cladia aggregata*-complex; the correct generic names for the remaining species requires further investigation. Our phylogenetic hypothesis is consistent with the recent diversification of these endemic 'genera' in Australasia. Their previous treatment as separated genera reflects the striking morphological evolution that has accompanied their diversification.

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683 - The genus *Gymnopilus* (Basidiomycetes, Agaricales) in the Czech Republic

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A project dealing with the taxonomy and distribution of the genus *Gymnopilus* in the Czech Republic was started in 2001. Up to now, 7 species were recognised. *Gymnopilus picreus* is common in mountains but scarce to rare in highlands and lowlands. *Gymnopilus bellulus* is found almost exclusively in natural to virgin montane forests. *Gymnopilus josserandii* (= *G. subsphaerosporus*) is very rare and grows in the same habitats like *G. bellulus*. *Gymnopilus flavus* is rarely found in forest meadows of Moravia. *Gymnopilus fulgens* is also rare. It was collected on peaty soil in a forest near Prague. *Gymnopilus spectabilis* is scarce in lowlands and hilly country. Concerning the group of *G. sapineus* and *G. penetrans*, my preliminary results show that there are no differences in microcharacters between collections with smooth and scaly pileus. For the present I agree with Høiland that there is only one species, namely *Gymnopilus sapineus* s.l. It is the most abundant species of *Gymnopilus* in the Czech Republic. The species growing on burnt places (*G. odini*, *G. decipiens*) are extremely rare or even absent in the Czech Republic as no collections are kept in Czech herbaria and no fresh fruitbodies were found in the year 2001. The taxonomic value of some interesting collections (e.g a whitish form of *G. spectabilis* or a species with the vinaceous red pileus growing on soil among mosses) will be evaluated in the future.

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684 - Delimitation of the genus *Ciboria* (Sclerotiniaceae, Helotiales, Ascomycota) based on rDNA sequence, morphology, ultrastructure and life history data

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The current delimitation of the genus *Ciboria* includes taxa with cup-shaped to plane to recurved, brownish, stipitate apothecia with an outer excipulum of globose cells, producing ellipsoid, hyaline, unicellular meiospores and substratal stromata, and lacking a macroconidial mitosporic state. Species of *Ciboria* infect a wide range of host plants and tissues, and is morphologically heterogeneous. Recent studies based on rDNA sequence data and ultrastructural data have indicated that the genus is polyphyletic. Here we present a more comprehensive study including new as well as previously published rDNA sequence data and phylogenetic evidence, and discuss the resulting phylogeny in comparison with available data on morphology, ultrastructure and life history. We conclude that the genus should be restricted to comprise only a group of taxa infecting and mummifying catkins of trees in the plant families Betulaceae, Corylaceae and Salicaceae. Another distinct group currently classified in *Ciboria* but characterised by production of verrucose ascospores, infects seeds of host in Betulaceae, develops very dense and hard stromata and appears to be obligate biotrophs. We conclude that this group deserves rank as a separate genus. Several taxa currently classified as *Ciboria* should be excluded from the genus, but their taxonomic status remain uncertain until additional phylogenetic evidence is made available.

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685 - Taxonomy of Rhytismatales in China

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The Rhytismatales are a well-known order of ascomycetes, which is widely distributed in temperate and tropical regions of the world. Some members of this order can cause considerable economic damage on trees. *Lophodermium seditiosum* which causes serious needle cast of *Pinus sylvestris* and *Ploioderma pini-armandii* causing withering of needles of *Pinus armandi*, for example, are known from Western China.The abundance of members of Rhytismatales has been documented for Europe, North America, and Australia, but in the past only few species were reported from China. Since 1990, however, many new members were described, especially from tropical and subtropical regions of China. A total of 17 genera and 117 species were documented by the end of 2000. As a continuation of this effort the authors made an expedition to the Yunnan Province located in Southwestern China and to the Anhui province in Southeastern China in 2001. A microscopic analysis of more than 120 collections revealed 12 genera, *Bifusella*, *Coccomyces, Colpoma*, *Davisomycella, Duplicaria*, *Hypoderma*, *Lirula*, *Lophodermium*, *Naemacyclus*, *Neococcomyces, Ploioderma*, and *Soleella* - represented by 38 species. Among these 16 are species new for science. Three genera and five species are recorded for China for the first time.

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686 - A re-evaluation of *Lojkania* Rehm, with an addition of two new species

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The circumscription of *Lojkania* is amended to merely include species with germ slits on ascospores. Additional to the germ slit, there are characters found to be remarkably correlated and consistent, which coincides with the new definition of this genus. The ascomatal wall is composed of characteristically thin sclerotial cells. Smooth ascospores are 1-septate and uniseriate-arranged in ascus. Asci exhibit different extent of development in an ascoma, and alike most bitunicate genera, have no ocular chamber at the tip. Accordingly, the segregations with its similar genera are, therefore, readily distinguishable. Two new species are hitherto erected in *Lojkania*. They are *L. incrassata* and *L. wushensis*.

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687 - Phylogenetic relationships between the genus *Saprolegnia* and related genera inferred from ITS sequences

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Phylogenetic relationships among the genera of *Isoachlya*, *Pythiopsis* and *Saprolegnia* (Saprolegniales, Oomycetes) were studied based on the sequence data of internal transcribed spacers including 5.8S ribosomal DNA regions. The genera share the saprolegnoid type of zoospore discharge, but they are different in some points. Thus, the zoospores of *Pythiopsis* show monoplanetic behavior, while those of the other genera are diplanetic. *Isoachlya* is distinguishable from *Saprolegnia* by its manner of zoosporangial renewal. Several species selected from three genera were sequenced. The data were analyzed with supplement data of *Achlya* spp. obtained from GenBank. The results of the neighbor-joining and the maximum parsimony analysis showed sequenced species separated into three clusters. Cluster I consisted of several species of *Isoachlya* and *Saprolegnia*. Cluster II included two species of *Pythiopsis* (*P. cymosa* and *P. terrestris*) and *S. megasperma*. It was discovered that the mucilage outer layer surrounding young oogonia is a common characteristic to all of them. Cluster III was composed of three species with eccentric oospore, i. e. *I. eccentrica*, *P. humphreyana* and *S. anisospora*. These results suggest that the orthodox generic classification reflects only the part of phylogenetic relationships among these organisms. We reexamine the phylogenetic value of the various taxonomical criteria that have been used in the orthodox classification of the family Saprolegniaceae.

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688 - Towards a monograph of the Parmulariaceae

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The family Parmulariaceae is widespread in the tropics and contains fungi with a large variety of morphological characters and host species. 31 genera and more than 100 species are included. Type species of the genera *Aldonata*, *Aldona*, *Aulacostroma*, *Coccodothis*, *Cocconia*, *Cycloschizon*, *Cyclostomella*, *Cocconia*, *Dictyocyclus*, *Dothidasteroma*, *Englerodothis*, *Ferrarisia*, *Hysterostomella*, *Inocyclus*, *Kentingia*, *Kiehlia*, *Pachypatella*, *Palawaniella*, *Parmularia*, *Parmulariopsella*, *Parmulariopsis*, *Parmulina*, *Perischizon*, *Polycyclina*, *Polycyclus*, *Protothyrium*, *Pseudolembosia*, *Rhagadolobium*, *Rhipidocarpon*, *Symphaeophyma*, *Thallomyces* and their synonyms have been studied. Illustrations with drawings and microphotographs have been prepared, and all genera re-described. Some characters, including ascospores in a mucous coat, asci with rostrate dehiscence, centrum immersed in a gelatinous layer which sometimes becomes blue in iodine and superficial mycelia with hyphopodium-like structures are useful as diagnostic features. Useful characters for generic delimitation include ascomata (position in the substrate, shape, outer wall, connection to the host), superficial mycelium (presence or absence, position, presence of hyphopodium-like structures) and ascospores (colour, shape, septa). Comparison will be made with the possibly related family Asterinaceae and a new concept for the Parmulariaceae introduced.

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689 - Molecular systematics of phylloplane yeasts: Pigmented species belonging to the *Erythrobasidium* lineage of the Urediniomycetes (Basidiomycota)

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A screening of the mycobiota on leaves from selected plant species (*Acer monspessulanum*, *Quercus faginea*, *Cistus albidus*, *Pistacia lentiscus* and *Osyris quadripartita*), collected at the 'Arrábida Natural Park', an ecosystem of Mediterranean characteristics in Portugal, yielded about 830 yeast isolates. Two isolation methods were used: the conventional method, based on plating of leaf washings onto solid medium, and the spore-fall method. About 30% of the isolates, a significant fraction of which producing ballistoconidia, presented the following characteristics: production of orange to red pigments, inability to produce

starch-like compounds, assimilation of D-glucuronic and/or protocatechuic acids but not inositol. These yeasts were assigned to the *Erythrobasidium* lineage of the Urediniomycetes using a combination of conventional phenotypic identification methods with molecular techniques: PCR fingerprinting (MSP-PCR) with primer m13 and rDNA sequence analysis (26S rDNA, D1/D1 domains). The results showed that some of the isolates corresponded, or were similar, to recognised species, viz. *Sporobolomyces coprosmae*, *Sp. salicinus*, *Sp. gracilis*, *Rhodotorula aurantiaca* and *Erythrobasidium hasegawianum*, whereas others seemed to represent undescribed species.

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690 - Evolution of mating type gene arrangement in the genus *Stemphylium* (Ascomycetes)

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In this study we are investigating distribution and arrangement of the mating type genes *MAT-1* and *MAT-2* in 96 isolates of the genus *Stemphylium*. The mating types were assessed with primer sets amplifying the diagnostic alpha and HMG boxes of the *MAT-1* and *MAT-2* genes respectively. For most of the isolates, both *MAT-1* and *MAT-2* genes could be demonstrated. In the genus *Cochliobolus*, which is closely related to *Stemphylium*, the occurrence of both mating type genes in one isolate is indicative of homothallism. In homothallic species of *Cochliobolus*, *MAT-1* and *MAT-2* are arranged in different ways. In *Stemphylium*, several species are known homothallics, including *S. vesicarium*, and the anamorph of the type species of *Pleospora*, *S. herbarum*. *MAT-1* and *MAT-2* were present in both *S. vesicarium* and *S. herbarum*. The remaining 94 isolates included in this study belonged to 17 other species, as well as a number of yet undescribed species. Some of these isolates contained only one mating type gene. The distribution of mating type genes and their arrangement was mapped into phylogenetic trees inferred from *ITS*, partial *GPD*, and *EF-1a* DNA sequences. It is generally thought that homothallism derived from heterothallism. To test this hypothesis, we used phylogenetic analyses to investigate whether heterothallism or homothallism was the ancestral state in *Stemphylium*, and how many times a switch from homothallism to heterothallism or vice versa occurred.

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691 - A preliminary catologue of the Saprolegniaceae of the Iberican peninsula

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The Saprolegniaceae are one of the lest known and investigated group of aquatic fungi in the Iberican Peninsula. According to the bibliographic data it was only known two genera over twenty of the orden. In view of the interest concerning to the data in this area, we have been undertaken a biosystematic study of the Saprolegniaceae in the Iberican Peninsula, as a Phd included in the project 'Flora Micológica Iberica'. Up to now samples has been colleted from different areas of West, North-East and South of Iberican Peninsula and species from five genera of these orden have been identificated: *Saprolegnia*, *Achlya*, *Dyctiuchus*, *Aphanomyces* and *Thraustotheca*.

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692 - Molecular and morphological comparisons of *Fusarium* species accommodated in the *Fusarium subglutinans* sensu lato complex

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A total of 13 species formed part of the *Fusarium subglutinans* sensu lato species complex at the time of this study. These species are pathogens of many hosts including pine, mango and pineapple but are morphologically similar. They are all characterised by typical *F. subglutinans* morphology including false heads, absence of chlamydospores and microconidia produced in polyphialides. The aim of the study was to distinguish between these species based on morphology and DNA sequences. Phylogenetic relationships were determined based on sequences from the histone, elongation 1α, and β-tubulin genes. Furthermore, sequences of the EF-1α gene were subjected to restriction analysis using four different restriction enzymes. Based on their recognition sites, unique EF-1α restriction patterns were generated for the species. The morphological characteristics that were informative included the origin of conidiophores on the aerial mycelium, conidiophore branching, the number of conidiogenous openings on the polyphialides, macroconidial septation and the presence or absence of sterile coiled hyphae. All 13 of the species in the *Fusarium subglutinans* sensu lato could thus be distinguished based on morphological and molecular characteristics.

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693 - *Ophiostoma kryptum*, prov. nom. from larch and spruce in Europe, similar to *Ophiostoma minus*

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Tetropium species (Coleoptera: Cerambycidae) are secondary, though important pests of their host trees in Europe. *Tetropium fuscum* and *T. castaneum* principally infest Norway spruce (*Picea abies*), while *Tetropium gabrieli* attacks primarily European larch (*Larix decidua*). Recently, the fungal associates of *Tetropium* spp. in Europe and Canada came under scrutiny after the invasion of *T. fuscum* into Atlantic Canada. Outside its native habitat in Europe, *T. fuscum* proved to be a potentially devastating pest and with it came *Ophiostoma tetropii*, a fungus that has previously not been recorded in Canada. An unknown species of *Ophiostoma* was isolated from Norway spruce, infested by *Tetropium* sp. on European larch, infested by *T. gabrieli* in Europe. Although similar to *O. minus*, this fungus could be distinguished from this species and is described here as *Ophiostoma kryptum* prov. nom. *Ophiostoma kryptum* is characterized by perithecia with short necks and a *Hyalorhinocladiella* anamorph as well as a *Leptographium*-like anamorph. Although *O. kryptum* and *O. minus* are morphologically very similar, molecular data, significant differences in their colony morphologies on OA and MEA and differences in their habitat and insect vectors distinguish them. *Ophiostoma minus* and *O. kryptum* thus represent another example of morphologically similar, yet genetically and ecologically separated species in the ophiostomatoid fungi.

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694 - Taxonomic re-evaluation of three related species of *Graphium*, based on phylogeny, morphology and ecology

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Graphium pseudormiticum was described in 1994 from the galleries of the bark beetle, *Orthotomicus erosus*, introduced into South Africa from Europe. This fungus is characterized by conidia with conspicious basal frills that are produced in false chains, a trait not observed in other species of *Graphium*. *Rhexographium fimbriasporum* was described in 1995 from *Ips typographus* on *Picea abies* and was distinguished from *Graphium* based on its apparently unique conidiogenesis. The conidia of *R. fimbriasporum* are produced in false chains and have conspicuous basal frills. In this study we compared *G. pseudormiticum* with *R. fimbriasporum* based on DNA sequences, morphology and ecology. We included a large collection of isolates, tentatively identified as *Graphium* spp., collected from *I. typographus* on *Picea abies*, *Ips cembrae* on *Larix decidua* and *Tomicus minor* on *Pinus sylvestris*. *Graphium pseudormiticum* and *R. fimbriasporum* were morphologically similar and based on rDNA sequence (ITS and 18S), phylogenetically closely related but different, grouping with species of *Graphium*. *Rhexographium fimbriasporum* will thus be transferred to *Graphium*, but remains distinct from *G. pseudormiticum*. Another fungus with conidia and conidium development similar to *G. pseudormiticum*, is one of the dominant associates of *I. cembrae* on *L. decidua* in Europe. This fungus is distinguished from *G. pseudormiticum* and *G. fimbriasporum* based on morphology and phylogeny and is described as *G. laricis* prov. nom.

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695 - Taxonomy of the lichen family Pertusariaceae in Thailand

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The methods to identify the crustose lichen family Pertusariaceae are composed with morphological and anatomical studies, chemical test by spot test, UV test, recrystallization, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). That can identify Pertusariaceae into two genera *Pertusaria* and *Ochrolechia*. The different distinctly characters of *Ochrolechia* from *Pertusaria* are well-defined prominent exciple, vivid colour disc and the chemical, gyrophoric acid with a little amount of others. While as *Pertusaria* has two forms of apothecia, disciform and verruciform without superfacial thallus and more chemicals, xanthones, orcinol depsides, depsones and depsidones. Species of *Pertusaria* are *P. albescens, P. amara, P. asiana, P. cicatricosa, P. cinchonae, P. coccopoda, P. commutata, P. leiocarpella, P. melaleucoides, P. patellifera, P. pertusa, P. scaberula, P. scutellifera, P. thwaitesii, P. tetrathalamia, P. tropica, P. velata, P. wattiana, P. xantholeuca and P. xylophyes*. Most of them are new records in Thailand. Many specimens could be new species. Species of *Ochrolechia* are during identification.

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696 - Phylogenetic relationships of *Phellinus* and allied taxa based on ITS1-5.8S-ITS2 sequences

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To elucidate phylogenetic relationships of the Hymenochaetales, sequences of the nuclear ribosomal ITS1-5.8S-ITS2 region from 96 strains were analyzed. The results showed that *Trichaptum* and *Schizopora* are closely related to the Hymenochaetales. But *Asterostroma*, *Phaeolus*, and *Vararia* are placed outside the Hymenochaetales. Total twelve groups were recognized as separate clades. Eight groups were same as those recognized by early mycologists: *Phellinus robustus* complex (= *Fomitiporia* Murrill 1907), *P. pini* complex (= *Porodaedalea* Murrill 1905), *P. igniarius* complex (= *Phellinus* Quélet 1886), *P. rimosus* complex (= *Fulvifomes* Murrill 1914), *P. ferruginosus* complex (= *Fuscoporia* Murrill 1907), *Inonotus radiatus* complex (= *Mensularia* Lazaro 1916), *I. hispidus* complex (= *Inonotus* P. Karst. 1880) and the eighth group (= *Inocutis* Fiasson & Niemelä 1984). Four groups were newly recognized in this study. The first group was temporarily named as *P. linteus* complex. *Hymenochaete* formed two separate groups that did not include any species of *Phellinus* and *Inonotus*. The last group, consisted of three *Phellinus* species and two *Inonotus* species, occupied a basal position of the main clade and clustered with *Schizopora* and *Trichaptum*. The present study confirmed that *Phellinus* is polyphyletic and, furthermore, rejected the widely acknowledged monophyly concept of *Inonotus*. The species of *Inonotus* were so intermingled with *Phellinus* species that the genus became topologically polyphyletic.

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697 - Secondary metabolite syndromes in the lichen genus *Letrouitia* (Ascomycota, Letrouitiaceae)

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Secondary metabolites from the lichen genus *Letrouitia* Haf. & Bell. were analysed with HPLC. Most of the species contained the anthraquinones parietin and fragilin, and a few also 7-chloroemodin. In addition, a number of additional secondary compounds were detected, presenting chemosyndromes that in most cases are characteristic for each species.

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698 - Biology and systematics of the Saprolegniaceae: A treatise

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We are pleased to announce the impending publication of a comprehensive treatise on the biology and systematics of the watermolds (Mastigomycota: Saprolegniaceae). This work critically analyzes the world literature on this family covering the period from 1750 through 1987 - citing >2800 references - with a brief excursion to 2000 in the systematic section. It includes 30 chapters (with 49 tables and 58 figures) dealing with important aspects of watermold biology and an additional 20 in which we present a comprehensive revision of the systematics. Keys to the family and genera are presented together with extensive illustrations of included taxa. This work will be available to the world scientific community at no charge on the worldwide web probably by August 2002. It can be accessed using the URL http://www.ilumina-dlib.org/ by selecting "advanced search". In the advanced search window, type "Padgett" in the "contributor search window", tic "book" in the "learning resource type" window directly below, then click on the "submit" button. We hope that our effort will stimulate renewed interest in this fascinating group of organisms.

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699 - Systematics of Australian *Dermocybe*

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The ectomycorrhizal basidiomycete genus *Dermocybe* is well represented in a diverse range of ecosystems on most continents. There has been a paucity of reliable taxonomic information for Australian species, and many collections of *Dermocybe* from Australia have previously been incorrectly assigned to Northern Hemisphere species. Several Australian species have considerable differences in pigment chemistry to their Northern counterparts, including a number of unique chemical compounds. Morphology and pigment chemistry (thin-layer chromatography) were used in phenetic analyses of 110 Australian *Dermocybe* collections. Nineteen taxonomic groups were identified at species level, of which 11 are undescribed. Representatives from 12 of these groups were combined with 25 other members of *Cortinarius* and *Dermocybe* from the Northern and Southern Hemispheres in parsimony analyses using ITS rDNA sequence data. Trees show *Dermocybe* as monophyletic, but *Cortinarius* as paraphyletic. *Dermocybe* aff. *sanguinea* from the Southern Hemisphere is sister group to the clade of Northern Hemisphere dermocybes. Northern Hemisphere members of *Dermocybe*, with the exception of *D. olivaceopicta*, form a separate clade to the Southern Hemisphere taxa. Southern Hemisphere *Dermocybe* are consistently placed basal to Northern Hemisphere members.

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700 - Anatomical and molecular characterization of *Tomentella* ectomycorrhizae

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The members of the genus *Tomentella* (Thelephoraceae) are among the most widespread ectomycorrhizal (ECM) partners of trees in needle and deciduous forests all over the world. Although being abundant in the mycorrhizosphere, fruitbodies appear rarely and are usually overlooked. That is the reason why identification of mycorrhizae of this group is mainly carried out using molecular tools (e.g. DNS sequence analysis). Investigation of morphological and anatomical characteristics using the methods introduced by Agerer (PhC, Nomarski microscopy, SEM and histochemical tests) is also useful in determining ECMs but more precise results can be got by combining morphological and molecular methods. Up to now only few tomentelloid ECMs have been described comprehensively. From the continental deciduous forests of the Hungarian Plain several different morphotypes of tomentelloid mycorrhizae have been isolated and characterized by Agerer's methods. The majority of them have been detected in this territory for the first time. Molecular methods (PCR and sequence analysis of the rDNA ITS region) were successful in identification, comparing ITS-sequences of mycorrhizae with those included in DNA sequence databases of fruitbodies. Results of molecular identification and morphological-anatomical characterization of tomentelloid ECM morphotypes collected in drought-adapted oak and poplar forests, forming symbioses with *Quercus robur*, *Q. cerris* and *Populus alba* trees, have been demonstrated.

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701 - Phylogeny of rDNA its region in smut fungi

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Nuclear ribosomal ITS regions were amplified from spore samples of several *Anthracoidea* Brefeld species collected from nature. PCR products identified as anthracoideal showed unexpectedly large length variation among the species. Two species viz. *A. aspera* (Liro) Kukkonen and *A. fischeri* (P. Karst.) Kukkonen with a considerable size difference were taken for sequence analysis. ITS products were cloned into pGEM-T vector and sequenced. For full reliable bidirectional sequencing of the longer ITS fragment additional internal primers had to be designed. By sequence alignment it was found that 358 bp length difference of the sequences was caused by single gap in the ITS1 region. To evaluate taxonomic position of the genus *Anthracoidea* among the Ustilaginales multiple alignment was generated with other smut ITS sequences downloaded from GenBank. It became soon evident that comparison had to be expanded to more distantly related groups. The sampling of fungal sequences was biassed to cover much of the biodiversity of plant parasites, especially species which had been formerly or are presently recognised as smuts. Alignment revealed that the highly conservative and slowly evolving 5.8S rRNA distinguishes smuts on grasses and sedges of the present subclass Ustilaginomycetidae from all other fungi. Phylogenetic analyses will be presented and taxonomic status of the genus *Anthracoidea* will be discussed.

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702 - Phylogenetic studies of some *Terfezia* and *Choiromyces* species

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A phylogenetic study of the genus *Terfezia* was undertaken based on analysis of the ITS and the 5 prime end of the 25S rDNA regions. Phylogenetic analysis of *Terfezia* and *Choiromyces* spp. (Terfeziaceae) revealed that all but one of the *Terfezia*species form a single clade, while all but one of the *Choiromyces* species group, as expected, with *Tuber melanosporum* (Tuberaceae). Several discrepancies detected between the two trees indicate a separate evolutionary path for the two regions studied. *C. echinulatus* and *Terfezia pfeilii*, both from the Kalahari Desert, are closer to each other than to any other species, a point which will be discussed further. Closer analysis of a group of sequences obtained for *T. boudieri* suggests that this group comprises at least two distinct though morphologically indistinguishable species. Further analysis of a mycelial isolate from *T. boudieri* fruit-bodies exhibiting a double ITS profile belonging to one of the two proposed species provides tentative evidence for long-term in vitro subsistence of an ascomycetous dikaryon.

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703 - New systematics in xanthorioid lichens

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The genus *Xanthoria* has been delimited on its foliose thallus morphology and the presence of upper and lower cortical layers. The morphology and anatomy of the genus have recently been described in several papers, and molecular phylogenetic studies have shown it to be polyphyletic. One of these lineages is the *X. fallax* (or *ulophyllodes*) group. The *Xanthoria fallax* group is characterised primarily by having bacilliform spermatia and well-developed rhizinae. Furthermore, many of the species produce soredia. At present the group includes the seventeen species. Molecular studies based on nuclear ribosomal DNA suggest that the *X. fallax* group is a separate lineage diverging at the very base of the genus *Xanthoria*, and HPLC-studies of the anthraquinones in *Xanthoria* also pointed at particular characteristics of the *X. fallax* group. The strong indications that the *X. fallax* group was circumscribed by a number of different and unrelated character sets prompted us to perform a closer study of the morphology, anatomy, secondary chemistry, and molecular phylogeny of the group. The monotypic, recently described genus *Xanthomendoza*, was included in the study as it was known from molecular studies also to have a basal position relative to *Xanthoria*. Apart from the new generic scircumscription of the xanthorioid lichens many new species, especieally from the southern hemisphere are being been described.

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704 - Phylogeography and molecular variation in *Serpula lacrymans* and *S. himantioides*

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The dry rot fungus *Serpula lacrymans*, predominantly found in houses, and its sister taxon *S. himantioides*, mostly occurring outdoors, have global distributions in temperate regions. The phylogeography of both species were investigated on a global scale using DNA-sequences from three target loci; the nrDNA internal transcribed spacer (ITS) region, and partial sequences of the betta-tubulin (*btub*) and translation elongation factor 1 alpha (*efa*) genes. Preliminary data suggest the existence of two varieties of *S. lacrymans*, one predominantly occurring indoor, and one growing in the wild. Little genetic variation was found in worldwide *S. lacrymans* ITS, *btub* and *efa* sequences of the domesticated variety; obtained ITS sequences from Canada, China, Europe, Mexico and New Zealand were identical to ITS GeneBank sequences from Himalaya and Australia. In *S. himantioides*, on the other hand, extensive sequence variation was found in all three target loci. The preliminary data suggest that the domesticated *S. lacrymans* variety has experienced a bottleneck event coupled to a recent worldwide spread-out.

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705 - Genotypes of *Lobaria pulmonaria* along an East - West transect in Eurasia

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Lobaria is a conspicuous foliose lichen species. The species was common in pre-industrial periods in humid temperate and boreal regions of the Northern Hemisphere, and cooler parts of the Tropics. Unfortunately this species suffered a tremendous decline during the past decades and the species is now considered endangered in most parts of Central Europe and other industrialised countries. North-Eastern Asia is considered a diversity hotspot of *L. pulmonaria* and related species. In Switzerland, recent studies based on ITS I revealed genetically diverse and impoverished populations. A geographic differentiation among the local populations was not detected within this small geographic area. In this paper we will test the hypothesis that the genetic diversity of *L. pulmonaria* is higher in the Far East of Russia compared to more western populations. Specimens were collected in Sakhalin, Northern Urals, Komi Republic (Russia), Ukrainian Carpathians (Ukraine), Finland, Norway, Switzerland, France and Portugal. We analysed a fragment, which included the internal transcribed spacer ITS1, the 5.8S ribosomal RNA gene, and parts of ITS2. The fungus-specific primer ITS1-F and the recently developed *L. pulmonaria* specific primer nu-ITSII-137-3' were used to amplify the fragment.

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706 - Survey of ophiostomatoid fungi in Austria, Central Europe

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Fungi belonging to the ascomycetous genera *Ceratocystis*, *Ceratocystiopsis* and *Ophiostoma* and related anamorph genera such as *Leptographium*, *Pesotum* and *Graphium* are also known as the ophiostomatoid fungi. Ophiostomatoid fungi are dispersed by bark beetles (Coleoptera: Scolytidae) and other phloem feeding and wood boring beetles or by air-borne and rain-splash inoculum. Since 1992 the assemblages of ophiostomatoid fungi associated with bark beetles on Norway spruce, *Picea abies* (*Ips typographus*, *Ips amitinus*, *Pityogenes chalcographus*, *Hylurgops palliatus*, *Hylurgops glabratus*, *Dryocoetes autographus*), European larch, *Larix decidua* (*Ips cembrae*), Swiss stone pine, *Pinus cembra* (*Ips amitinus*), Scots pine, *Pinus sylvestris* and Austrian pine, *Pinus nigra* (*Tomicus piniperda*, *Tomicus minor*, *Ips sexdentatus*), elm, *Ulmus* spp. (*Scolytus* spp.) and European beech, *Fagus sylvatica* (*Taphrorychus bicolor*) have been studied in Austria. The mycobiota of *Tetropium* spp. (Coleoptera: Cerambycidae) on spruce and larch was also investigated. In addition, a small number of ophiostomatoid fungi were isolated from conifers and hardwoods without signs of insect infestation. In total, 40 species of ophiostomatoid fungi were isolated. These included 3 *Ceratocystis* spp., 3 *Ceratocystiopsis* spp., 22 species of *Ophiostoma*, 5 *Leptographium* spp., 6 *Graphium* spp. and 1 *Pesotum* sp. This ongoing study has greatly improved our knowledge of the occurrence, hosts and the vectors of ophiostomatoid fungi in Austria.

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707 - Phylogenetic analyses of four taxa of *Fusarium*, based on partial sequences of the translation elongation factor-1 alpha gene

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Phylogenetic relationships between four *Fusarium* species were studied using parts of the nuclear EF-1α-gene as a phylogenetic marker. Sequences from 12 isolates of *F. poae*, 10 isolates of *F. sporotrichioides* and 12 isolates of *F. langsethiae* Torp & Nirenberg ined. yielded 4, 5 and 5 genotypes respectively. In addition we included one isolate of *F. kyushuense*. The aligned sequences were subjected to neighbor-joining, maximum parsimony and maximum likelihood analyses. The results from the different analyses were highly concordant. The EF-1α-based phylogenies support the classification of *F. langsethiae* as a separate taxon in the section *Sporotrichiella* of *Fusarium*, as the closest sister taxon to *F. sporotrichioides* while *F. kyushuense* is the sister taxon to *F. poae*, corresponding well with the ability of the former taxa to produce T-2 and HT-2 toxins. In contrast morphological characters indicate a closer relationship between *F. langsethiae* and *F. poae* on the one hand, and between *F. sporotrichioides* and *F. kyushuense* on the other hand.

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708 - Chlamydospore formation of *Entoloma clypeatum* f. *hybridum* on mycorrhizas and rhizomorphs associated with *Rosa multiflora*

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Chlamydospores of *Entoloma clypeatum* f. *hybridum* were described on the mycorrhizas and rhizomorphs associated with *Rosa multiflora*. Pinkish mycelia were observed around rhizomorphs and mycorrhizas of *E. clypeatum* f. *hybridum* associated with *R. multiflora*. Rhizomorphal connections with fruiting bodies were traced to identify the colored mycelia. They were thick walled with roughened surface, ellipsoid with marginal segments, 12-16 x 5-7 Âµm (including segments), and hyaline to pinkish color. Hyaline, roughened-surface and swollen cells were terminally observed in vegetative hyphae with clamp connections. Surface view was the same both in the swollen cells and the spores. Two spores arranged in a chain were also observed. Fragmented clamp connections were observed on several hyphal tips. Developmental pattern of chlamydospore seems to be the *Nyctalis* type. This is the first report on chlamydospore formation on the mycorrhizas in entolomatoid fungi.

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709 - A putative hybrid or introgressant between *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* from Austria, Central Europe

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The ascomycete fungi *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* have been responsible for the two destructive epidemics of Dutch elm disease since the early 20. century. Although a strong reproductive barrier operates between these two species, natural hybridization between them has been reported (Brasier et al., 1998, Mycol. Res. 102, 45-57). During recent surveys of the Dutch elm disease pathogens in Austria an unusual *Ophiostoma* isolate was obtained from a twig sample of a diseased elm tree. This isolate has an unique colony morphology neither resembling that of *O. ulmi* nor that of *O. novo-ulmi*, but similar to that of certain *O. ulmi* x *O. novo-ulmi* laboratory generated hybrids (Kirisits et al., 2001, Forstwiss. Cbl. 120, 231-241). In laboratory crosses with authenticated isolates of the Dutch elm disease pathogens this strain proved to be sterile as recipient (female), while it behaved like *O. novo-ulmi* ssp. *americana* as donor (male) in crosses with both subspecies of *O. novo-ulmi* as recipient. The DNA sequences of the *cerato-ulmin* gene and the *colony type* gene of this unusual isolate were also identical to those of *O. novo-ulmi* ssp. *americana*. The unusual colony morphology of this *Ophiostoma* isolate and its female sterility gives suspicion that it could have been arisen from hybridization between *O. ulmi* and *O. novo-ulmi* or from introgression of *O. ulmi* into *O. novo-ulmi*. Examination of this isolate is continuing in order to prove its possible interspecies hybrid origin.

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710 - Hybridization between the subspecies of *Ophiostoma novo-ulmi* in Austria, Central Europe

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The second epidemic of Dutch elm disease has been caused by the ascomycete fungus *Ophiostoma novo-ulmi*. *O. novo-ulmi* is separated into the subspecies *novo-ulmi* and *americana*, both of which occur in Europe. We have recently presented evidence for recombination between two genes in isolates of *O. novo-ulmi* from Central Europe, indicating hybridization between *O. novo-ulmi* ssp. *novo-ulmi* and *O. novo-ulmi* ssp. *americana* (Konrad et al., Plant Pathology 51, 78-84). In the present study a geographically diverse population of 99 isolates of *O. novo-ulmi* from Austria was investigated. Isolates were examined for their fertility response as donor (male) to authenticated strains of *O. novo-ulmi* spp. *novo-ulmi* and ssp. *americana*. They were also studied by PCR-RFLP of the *cerato-ulmin* (*cu*) gene and the *colony type* (*col1*) gene. In 71 isolates fertility tests, as well as PCR-RFLP of *cu* and *col1* gave consistent results. 49 and 22 isolates were identified as ssp. *novo-ulmi* and ssp. *americana*, respectively. 23 isolates showed recombination between *cu* and *col1* and were thus identified as hybrids between ssp. *novo-ulmi* and ssp. *americana*. In further 5 isolates the fertility tests and the molecular analyses gave conflicting results and these isolates are suggested to be subspecies hybrids as well. This study has shown that a large portion of isolates of *O. novo-ulmi* from Austria consists of hybrids between ssp. *novo-ulmi* and ssp. *americana*.

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711 - Analysis of pathogenicity and molecular markers in sexual hybrids of *Phytophthora infestans* and *P. mirabilis*

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Isolates of *Phytophthora infestans* and *P. mirabilis*, originating from the presumed centre of origin of *P. infestans* (Toluca valley, Mexico), were crossed *in vitro*. Both species are heterothallic, and can act both as male and female partners in the cross. Viable offspring was obtained with an increased *in vitro* growth rate and abundant sporulation. DNA-fingerprinting (AFLP) showed that the F1 progeny consisted of true sexual hybrids. The offspring was (mildly) pathogenic on potato and *Mirabilis jalapa*, the respective hosts of *P. infestans* and *P. mirabilis*, and on tomato. F1 isolates were successfully crossed to obtain F2 and back-cross offspring. In F2 and BC populations, pathogenicity on tomato and potato (and possibly on *M. jalapa*) was a segregating trait. Mitochondrial DNA haplotype was checked for all offspring and almost all isolates contained the *P. mirabilis* type mitochondrial DNA, indicating that *P. mirabilis* acts preferably as the maternal partner. By analysing the molecular make-up and host specificity of *P. infestans*/*P. mirabilis* F1, F2 and BC hybrids, the possible role of hybridisation in the speciation process of *Phytophthora* species in Toluca valley could be assessed.

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712 - *Lewia* teleomorph of *Alternaria triticicola* from oat grain in Norway is now described

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Lewia triticicola Kosiak & Kwaúna ined., the teleomorph of *Alternaria triticicola* isolated from oat grain in Norway, has recently been described. During a survey of *Fusarium* and *Alternaria* species in Norwegian grains of reduced quality, a fungus with characteristics of *Alternaria triticicola* Vasant Rao isolated from surface disinfected oat (*Avena sativa* L.) kernels has been identified. The oat sample was collected from Volden in Trøndelag, Norway in September 1998. Stored on slant agar at 4 °C in darkness for a year, the fungus produced ascomata. The ascomata were single or often gathered in groups, 2-3 mm in diameter, submerged mainly in the surface layer of the medium. Single-ascospore cultures produced anamorphic and teleomorphic states by the same isolate. This is the first report on the production of a teleomorph by *A. triticicola*. The teleomorph of *A. triticicola* was named *Lewia triticicola* Kosiak & Kwaúna ined. Although the fungus originated from *Avena* and not *Triticum*, the specific name *triticicola* is retained to conform with that of the anamorph, as is conventional for *Lewia / Alternaria* relationships. *Lewia* Barr & Simmons species are known as teleomorphs of *Alternaria* Nees: Fries. Including this new species, five species of *Lewia* connected with *Alternaria* anamorphs have been described so far. Apart from *L. infectoria*, which is a common inhabitant of Gramineae, no other *Lewia* species have been described from cereals.

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713 - A comparison of *Metarhizium* isolates from natural forest with those from agricultural ecosystems: A preliminary study

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Metarhizium is one of the most studied of insect fungi. However, most isolates are derived from agro-ecosystems. We posit that the rate and pattern of evolution of *Metarhizium* spp. from natural forest may prove different from those of agricultural origin. To test this hypothesis a molecular approach was adopted using selected strains from the natural forests in Thailand compared with sequences of key isolates from agricultural strains downloaded from the Genbank. A region ITS-5.8S rDNA was sequenced for isolates of *Metarhizium* from forests in Thailand. With the inclusion of strains from nature, the sequence data further confirms the distinct separation of the *Metarhizium* isolates into two clades which are well supported by high bootstrap values: the *M. anisopliae* and *M. flavoviride* clades. The *anisopliae* clade formed a sister group to a species from forest off Coleoptera which is provisionally identified as a *Metarhizium* anamorph of *Cordyceps taii*. No *Cordyceps* state has been linked with the *flavoviride* clade. Long considered an Homopteran clade, it was notable that two isolates from nature, but off cockroaches, grouped within *flavoviride* sensu lato. Significantly, forest *flavoviride* from Homoptera (including leafhoppers and adult cicadas) formed the base of this clade. Current evidence presented here suggests the *flavoviride* clade has host-fidelity while the *anisopliae* clade demonstrates host-promiscuity.

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714 - The plant tree, roots and clades: mycorrhizae and plant phylogeny

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Most plants obtain at least some nutrients through hyphae of mycorrhizal fungi, and these symbioses appear to date back to the early days of plants' land colonization. Since then, several mycorrhizal types have evolved, each type allowing exploitation of different nutrient pools and habitats, yet limiting the symbionts' ranges to areas of mutual occurrence. As mycorrhizae play important roles in community and ecosystem ecology, understanding the evolution of different mycorrhizal states allows researchers to connect plant evolution, community and ecosystem ecology. Recent substantial advances in our understanding of plant phylogeny and mycorrhizae in general prompted a re-evaluation of previously published reports. We mapped available mycorrhizal data onto current phylogenetic models of the plant kingdom. The resulting trees show some interesting patterns, such as a concentration of ectomycorrhizae in the Eurosids, and they also reveal significant gaps in our knowledge. These trees, overlays, and evolutionary commentary are available on the web (http://www.wisc.edu/botit/MPP/Index.htm or http://www.botany.wisc.edu/) to provide a practical tool for teaching about mycorrhizae to systematists and ecologists of all levels. These comparative data also provide suggestions for future research leading to elucidation of the biological mechanisms underlying evolution of these symbioses.

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715 - Incipient sympatric speciation of a fungal endophyte after host shift

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Host shifts which promote adaptational changes are considered to be important in processes of sympatric speciation. Here, we report on sympatric host races of the fungal parasite *Epichloë bromicola* (Ascomycota, Clavicipitaceae) which presumably emerged after the shift from the grass *Bromus erectus* to other *Bromus* hosts. The life cycle of *E. bromicola* infecting *B. erectus* is characterized by a conspicuous sexual state (stromata) that enables the fungus to transmit contagiously, and which prevents seed production of the host. Asexual strains infecting the two woodland species, *B. benekenii* and *B. ramosus*, are asymptomatic and transmit vertically by seed. Analyses of AFLP fingerprinting and of sequences of the *tub2* and *tef1* genes of isolates from the three *Bromus* hosts collected in Switzerland and nearby France revealed that isolates are genetically differentiated according to their host and that the seed-transmitted isolates are derived from ancestral, sexual strains on *B. erectus*. These sexual strains appeared to be compatible with all three hosts, while seed-transmitted isolates from *B. benekenii* and *B. ramosus* were host specific on these two hosts. However, experimental matings indicated still unrestricted interfertility among isolates of all three hosts. We suggest that isolates infecting *B. benekenii* and *B. ramosus* represent long-standing host races or incipient species, which emerged after host shifts and which are evolving through host-mediated, disruptive selection.

716 - Molecular characterization of red yeasts from aquatic environments in Patagonia, Argentina

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Main features of aquatic environments of glacial origin in Patagonia (Argentina) are low pollution level and ultra-oligotrophy. Eight of these aquatic reservoirs were surveyed for the occurrence of red yeasts. Subsurface water samples were filtered and used for colony counting and yeast isolation. A set of 64 isolates was characterised based on the mini/micro satellite-primed technique (MSP-PCR). DNA fingerprinting with M13 and (GTG)5 primers allowed the grouping of 34% and 56% of all isolates, respectively. Comparison of representative fingerprints of each group with those of type strains of various pigmented yeast, made possible the quick identification of an 87% of isolates. The 26S rDNA (D1/D2 domain) sequence analysis was performed for one representative of the remaining unidentified groups. *Rhodotorula mucilaginosa* was found in almost all samples and represented ca. 50% of the isolates studied. Less frequent species corresponded to 6 genera and 16 species including five new yeast species of the genera *Rhodotorula* (2), *Cryptococcus* (1) and *Sporobolomyces* (2). Total yeast counting usually ranged 1-150 CFU l-1 and rarely exceeded 200 CFU l-1. In contrast with other studies on yeast biodiversity in freshwater, red yeasts did not often predominated in our water samples. This study represents the first report on red yeast occurrence and biodiversity in North-western Patagonia.

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717 - Polyphasic taxonomy of the genus *Rhizopus*

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Rhizopus Ehrenberg, established by Ehrenberg in 1820, was monographed by Schipper & Stalpers, who divided the genus into three groups, the *R. stolonifer*-, *R. oryzae*-, and *R. microsporus*-groups, according to characteristics of the sporangial apparatus and growth temperatures. Thereafter, the characteristics of morphology, growth temperature and the data from mating experiments became the basic criteria for species delimitation of *Rhizopus*. In the zymogram, three distinct groups, the same as those obtained by traditional methods are recognizable. On the other hand, a number of specific taxonomic problems were addressed. Then, sequencing of 28S rDNA D1 & D2 regions, G+C content, and DNA-DNA hybridization were proceeded. The analyses of 28S rDNA sequences resolved the *Rhizopus* strains into three divergent phylogenetic clades consistent with the morphological characteristics and zymogram. The results of G+C content and DNA-DNA hybridization support the recognition of *R. azygosporus* and *R. reflexus*. Taxonomy of the genus *Rhizopus* was reevaluated.

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718 - Phylogenetic analysis of selected *Aschersonia* (Hypocrella) species and their allies

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Despite its potential in biocontrol of scale insects and whiteflies, *Aschersonia* (teleomorph: *Hypocrella*) has seldom been included in phylogenetic studies. Nevertheless, it sits in an important position in the family of Clavicipitaceae. In this study, we investigated the phylogenetic relationships among *Aschersonia* (*Hypocrella*) isolates and species from related genera based on several genes. Parsimony analysis based on molecular data shows that *Aschersonia* (*Hypocrella*) isolates form a monophyletic clade, which is sister to an insect pathogen clade including *Cordyceps* and allies. A third clade represents grass endophytes. Within the *Aschersonia* clade, *A. aleyrodis* and *A. goldiana* form a clade; while another isolate referred to *A. goldiana* exists in another clade that includes *A. andropogonis*. Some authors have considered *A. aleyrodis* and *A. goldiana* to be conspecific, while others treat them as separate species based on morphological characters, especially pigmentation. We intend to clarify the relationships between these two species using molecular data and detailed morphological study.

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719 - A revision of the terverticillate Penicillia

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Some of the most common fungi in stored foods are the terverticillate *Penicillia*. Correct identification of these *Penicillia* is important in order to predict and avoid fungal spoilage and mycotoxin contamination of foods, feeds and in indoor environments. A revision of these species is overdue. We have chosen a polyphasic approach for classifying and cladifying these *Penicillia* (approximately 50 species), using a series of morphological, physiological and secondary metabolite data for classifying and beta-tubulin sequencing for cladifying them. Effective identification keys can then be developed after this important step. In general classification and cladification pointed to the same species and species series (natural series). For example the series *Olsonii* is unique both concerning morphology, physiology and profiles of secondary metabolites and this is in agreement with cladistic analysis of beta-tubulin sequence data. Image analysis of colonies and chromatographic data or other rapid methods combined with chemometrics can also be used at a later stage once the classification is clear. Examples of the use of the different methods will be given.

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720 - *Tuberculina*: relatives of rusts parasitize rusts

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The morphology, ultrastructure, molecular phylogeny, and infection biology of some species of the hyphomycetous genus *Tuberculina* Sacc. were examined. The presented results indicate that (1) *Tuberculina* belongs to the Basidiomycetes and is closely related to the Uredinales, (2) *Tuberculina* species are parasites on the haplontic stage of their rust hosts and show a distinct mode of interaction, and that (3) the commonly used species concept within the genus does not meet all aspects of the phylogeny inferred from molecular data.

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721 - Identification of a basidiomycetous fungus with high ability to degrade dioxins

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During screening for efficient biodegraders of various recalcitrant organopollutants, including dioxins, an interesting fungus, which possesses a high ability to degrade toxic dioxins, was isolated from white rot of a broad-leaved tree from Kyushu Island, Japan. An isolate of this fungus was inoculated on a sterilized log of *Quercus acutissima*. After four months of incubation, it produced a resupinate and poroid basidioma on the inoculated log. The basidioma is microscopically characterized by having monomitic hyphal system, clampless hyphae and clavate basidia, and by lacking cystidia. These morphological characteristics and the partial sequencing data of 18S rDNA indicate that this fungus belongs to the genus *Ceriporia* (Basidiomycetes). Among the described species of the genus, the fungus is microscopically similar to *C. alachuana* and *C. ferruginicincta*. However, its basidiospores are broader than those of *C. alachuana* and narrower than *C. ferruginicincta*. Further, the specimens of *C. alachuana* and *C. ferruginicincta* have distinctly poroid hymenial surface with small angular pores (3-8 and 6-8 per mm, respectively), whereas hymenial surface of the present fungus is variable in shape, i.e., poroid with larger pores (2-6 per mm), lacerate to dentate. These results strongly indicate that the present fungus is an undescribed species belonging to the genus *Ceriporia*.

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722 - Phenotypic and molecular variability amongst isolates of *Ceratocystis fimbriata*, causing coffee tree canker in Colombia

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One of the most important diseases that reduces the profitability of coffee plantations in Colombia is coffee tree canker. This disease, caused by *Ceratocystis fimbriata*, has increased in severity and incidence in recent years, resulting in substantial losses to resource-poor farmers. The aim of this investigation was to consider the relatedness of *C. fimbriata* isolates from different Colombian coffee-growing regions, and thus to provide a foundation for future development of resistant coffee varieties. Fifty isolates from 11 provinces were characterized based on morphology and pathogenicity. In addition, isolates were compared using RAPD and microsatellite markers. DNA sequence comparsions for the ITS region, Mat-2 HMG Box and partial β-tubulin gene, were also undertaken. Results of all comparisions clearly showed that Colombian isolates of *C. fimbriata* are highly variable. All molecular parameters used for these comparisons also showed that *C. fimbriata* isolates from Colombia, reside in two major phylogenetic lineages. We believe that these probably represent distinct taxa, which we are in the process of characterizing further. Isolates had different levels of pathogenicity on coffee, with some causing death of more that 90% and others killing less than 5% of inoculated plants. There was also a clear pattern to the distribution of the most pathogenic strains within coffee growing areas. Our results represent the first intensive study of this plant pathogen in Colombia.

723 - Phylogenetic relationships within the Boletales (Basidiomycotina)

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The Boletales is a morphologically diverse order of Basidiomycetes that includes poroid, gilled, resupinate and epigeous gasteroid fungi. The work presented here is included in the Flora Micologica Iberica project. The main purpose was to establish the relationships, based on morphological, molecular and chemical data, between the Boletales. Although, the main collections belong to the Iberian Peninsula, material from all over the world were included to compare with the Iberian ones. The phylogenetic analyses, based on sequences of the ITS regions including the 5.8S rDNA, allow to arise the following conclusions: a) The boletoid, agaricoid, secotioid and gasteroid basidiomes appear in different groups. The genera with gills (*Chroogomphus* (Singer) O.K. Mill, *Gomphidius* Fr., *Hygrophoropsis* (J. Schröt) Maire ex Martin-Sanz, *Neopaxillus* Singer, *Omphalotus* Fayod, and *Paxillus* F.) included in three families according to Hawksworth et al. (Dictionary of the fungi, 1995) appear in five clades. Only the family Gomphidiaceae is monophyletic. b) The family Rhizopogonaceae is not monophyletic. *Alpova* C.W.Dodge is not included between the species of the genus *Rhizopogon* Fr. & Nordholm but within the clade of the *Boletus* species. On the other hand, *Truncocolumella* Zeller is closely related to *Suillus* species. c) The family Suillaceae should include not only *Suillus* and *Gastrosuillus*, but also *Truncocolumella* and *Rhizopogon*.

724 - *Morchella conica* fruited in several waves a season in one site in Northern Israel

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Morels (*Morchella* spp.) are known to grow in a wide range of habitats, in various places around the world, usually for one extended wave of three weeks per season in one site. However, in Northern Israel we have located morel fruitbodies (*M. conica*), which appeared in several waves from December until the end of April in one site. The morels emerged on roadside, nearby various types of trees. We have observed and recorded the changes in the mushroom population size and the distribution of fruitbody size, as well as changes in weather conditions. Over 150 initials, up to 1.5 cm long were counted in the beginning of the first wave, but only less then half survived 2 days later, some continue to develop up to 10 cm long. A correlation was found between cold rainy days followed by warm dry days and between fruitbody initiation and development. These observations suggest that *Morchella* species are adjusted to their geographical location, and can fruit for several waves if weather conditions permit.

725 - *Leptographium* species isolated from bark beetles infesting Japanese red pine in Japan

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A survey was conducted to accumulate knowledge on the biogeography of *Leptographium* and their association with beetles in Japan. Seven species of *Leptographium* were isolated from bark beetles infesting Japanese red pine in Japan. They were *L. pini-densiflorae*, *L. lundbergii*, *L. serpens*, *L. wingfieldii*, *L. yunnanense*, *Leptographium* sp.1 and *Leptographium* sp.2. *Leptographium* sp.1 was similar to *L. neomexicanum* but different in the respect of having more ellipsoidal conidia. *Leptographium* sp.2 resembled *L. procerum* but was distinguishable with well-developed base of stipe. These unidentified species are considered to be undescribed. *L. serpens* and *L. yunnanense* are firstly recorded in Japan. Others are already-known species in Japan. *Leptographium* sp.1 was isolated from *Hylastes pulmbeus* together with *L. serpens*. *L. lundbergii* and *Leptographium* sp.2 was isolated from five beetle species, *Tomicus piniperda*, *T. minor*, *Hylurgops interstitialis*, *Hylastes paralleus*, and *H. plumbeus*. *L. pini-densiflorae* was isolated from *T. piniperda*, *T. minor*, *Cryphalus fulvus* and *Orthotomicus angulatus*. *Leptographium wingfieldii* and *L. yunnanense* were isolated exclusively from *T. piniperda*.

726 - The Genera *Gymnopus* and *Rhodocollybia* in the southern oak forests of Costa Rica

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Floristic and monographic studies in the genera *Gymnopus* and *Rhodocollybia* in Europe and in northeastern United States have been compiled recently (Halling, 1983; Antonín & Noordeloos, 1997). Such studies have been scarce, however, for these collybioid species collected in tropical locations. Morphological studies on specimens collected in the oak forests in the Talamanca Mountains of southern Costa Rica have revealed eight species in

Rhodocollybia and 18 in *Gymnopus*. Most represent previously described tropical - subtropical species like *G. neotropicus*, *G. lodgeae*, *G. fibrosipes*, and *R. turpis* reported from South America and the Caribbean, but some are considered to be of temperate origin, such as *G. confluens*, *G. dryophilus*, and *R. prolixa* var. *distorta*. Presence of such diverse fungal taxa from the north and south, especially those associated with oak forests, put perceivable importance to the biotic exchange that took place over the Central American Isthmus in recent geologic times. Sequences from the ITS1-5.8S-ITS2 nuclear ribosomal DNA region were obtained for most of these tropical specimens and have been used to generate a phylogenetic tree. Costa Rican species, including newly proposed species, are highlighted to show their relationship to those from temperate locations. Current taxonomic placement of these taxa based on morphological characteristics is also analyzed.

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727 - Ascomycetous mitosis in basidiomycetous yeasts: its evolutionary implications

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In budding cells of ascomycetous yeasts, mitosis occurs in the parent, while in basidiomyceteous yeasts it occurs in the bud. However, in the basidiomycete *Agaricostilbum pulcherrimum* mitosis occurs in the parent and parent-bud junction. To test whether *A. pulcherrimum* has a novel mitotic pattern, investigations of additional yeasts in the Agaricostilbomycetidae (Urediniomycetes) utilized immunofluorescence localization of freeze-substituted material to visualize mitosis. In *Bensingtonia yuccicola* mitosis occurred in the bud, as in all other basidiomycetous yeasts studied to date, but in *Stilbum vulgare* it occurred in the parent. *Stilbum vulgare* also exhibited predominantly binucleate yeast cells. Nuclear rDNA sequence data showed that *A. pulcherrimum* and *S. vulgare* are more closely related to each other than to *B. yuccicola* in the Agaricostilbomycetidae. Evolutionary implications of these mitotic patterns will be considered.

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728 - *Uredo* sp. nov., a first rust species on the Penaeaceae

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In the context of our surveying and mapping from rust fungi within the BIOTA-project Southern Africa, we have found a new rust fungus in the BIOTA-observatory near Olifantsbos in the Cape Peninsula National Park. The host is *Saltera sarcocolla* (L.) Bullock. Till this day there is no other rust fungus known neither from the genus *Saltera* Bullock, which is endemic in the SW Western Cape, nor from the family Penaeaceae, which is endemic in the S and SW Western Cape and S Eastern Cape in South Africa (1, 2). 1. Crous, P. W., Phillips, A. J. L., Baxter, A. P. 2000. Phytopathogenic fungi from South Africa, 1-358 2. Bredenkamp, C. L. 2000. Penaeaceae. In O. A. Leistner (ed.), Seed plants of southern Africa: families and genera. Strelitzia 10: 440-442.

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729 - Ongoing speciation within *Cryptococcus neoformans* revealed by global patterns of genetic variation

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Taxonomy within *C. neoformans* is an open question, with 3 varieties and 5 serotypes currently recognised. Variety *grubii* (serotype A), var. *neoformans* (serotype D) and the hybrid serotype AD) correspond all to the teleomorph *Filobasidiella neoformans* var. *neoformans*, and var. *gattii* (serotypes B, C) corresponds to the teleomorph *F. n.* var. *bacillispora*. A global epidemiological study using PCR-fingerprinting with a minisatellite specific primer (M13) and RFLP analysis of the orotidine monophosphate pyrophosphorylase (URA5) and phospholipase (PLB1) genes revealed ongoing speciation within *C. neoformans*. All 3 typing techniques divided over 1000 clinical, veterinary and environmental isolates from around the world into 8 molecular types (VNI serotype A, var. *grubii*; VNII serotype A, var. *grubii*; VNIII serotype AD hybrid; VNIV serotype D (var. *neoformans*); VGI, VGII, VGIII and VGIV serotypes B and C, var. *gattii*. VNIII, AD hybrid isolates revealed two RFLP patterns one corresponding to VNI, VNII and VNIV and the other to VNII and VNIV suggesting different recombination events between var. *grubii* and var. *neoformans* leading to diploid or triploid strains, having two or three different copies of the respective genes. The variation found in the PCR-fingerprinting profiles and RFLP patterns lies within a range comparable to that between established species in other fungal genera, indicating that evolution and speciation within the cryptococcal complex is an ongoing process.

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730 - New approach to an old problem - resolving the *Peltigera canina* species complex (Peltigeraceae, lichenized Ascomycota)

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The *Peltigera canina* species complex represents the most recently derived section within the genus *Peltigera*. Morphology and secondary compounds were the only taxonomic evidences used to circumscribe the species forming this complex of highly polymorphic group of foliose lichens. To evaluate the putative morphospecies within the *canina* complex, maximum parsimony (MP) and maximum likelihood (ML) phylogenetic analyses were conducted on separate and combined ITS and LSU nrDNA sequences for 17 recognized species and eight potential taxa, represented by 65 individuals. Patterns of variation in length and structure found within a hypervariable region of ITS1 were used as supplementary data for delimiting genetic boundaries among closely related species. In addition to 20 coded characters derived from ambiguously aligned portions of alignments (INAASE), 24 coded discrete characters were provided by the hypervariable ITS1 marker. Based on optimal topologies derived from MP and ML searches we found complete concordance between phylogenetic and morphological species circumscription for 13 *Peltigera* species from this complex. Phylogenetic distinctness between North American and European populations of the morphologically uniform species *P. degenii* was detected and highly supported. Our results confirm recognition of three newly proposed undescribed species. No evidence for recombination was found in the ITS and LSU regions within the *P. canina* complex.

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731 - PCR-RELP analysis of intergenic spacer(IGS) of ribosomal DNA among *Fusarium oxysporum* formae speciales

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Nuclear rDNA provides useful inter- and intra-specific polymorphism eukaryotic organism. The intergenic spacer (IGS) or non-transcribed spacer (NTS) lies between the large subunit and small subunit encoding regions of consecutive cistrons. Closely related species may show considerable diversity in IGS of few reflecting both length and sequence variation. *Fusarium oxysporum* has one of the broadest host ranges of many plant pathogenic fungi and over go different formae speciales of *F. oxysporum* are recognized. Variation within the intergenic spacer (IGS) of the ribosomal DNA gene for twnety-two strains of *F. oxysporum* and its formae speciales was examined by PCR, compled with RELP analysis. The length of the amplified IGS region was about 2.6 Kb in all strains except *F. oxysporum* f. sp. *cucumerium* from Korea and *F. oxysporum* f. sp. *niveum* from Japan. Those two strains were 2.5 Kb long. Restriction digestion of IGS-RELP regions by *EcoR*I, *Nru*I, *Hinc*II, *Sal*I, *Sma*I, *Bge*II, *Hind*III, *Xho*I, and *Kpn*I gave rise to nine IGS haplotypes among all strains. Cluster analysis based on the prosence or absence of comingrating restriction fragments show the two groups based on 44% genetic similarity. These results demonstrated that analysis of IGS showed some difference within and between *F. oxysporum* formae speciales.

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732 - Variability analysis of the Fumonisin producing species *F. verticillioides* using the IGS region

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F. verticillioides, included in the so-called *Gibberella fujikuroi* species complex, is one of the most important source of fumonisins contaminating food products because of the widespread occurrence of this fungus on maize, among other crops. Detection methods rely on the previous knowledge about the degree of intraspecific variability. In this work we have analysed the partial sequence of the intergenic spacer of rDNA (IGS) of a wide sample of *F. verticillioides* strains from different geographic location, hosts, mating type and fumonisin production. considering the incidence of asexual reproduction. The analysis revealed the existence of two groups of strains: fumonisin producing and fumonisin non producing strains which would indicate that some divergence is taking place within *F. verticillioides*. Within the group of fumonisin producing strains no clustering of isolates regarding geographic origin nor host could be detected. The variability observed was compared with data from other *Fusarium* species taking into account their mode of reproduction.

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733 - Contribution to the knowledge of *Termitomyces* (tropical basidiomycetes) from Cameroon: Ecology and systematics

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Species of the genus *Termitomyces* (Agaricales, Pluteaceae) are good edible tropical mushrooms characterized by a small to very large pileus generally bearing a more or less prominent umbo called perforatorium and a central and solid stipe producing a more or less long underground pseudorrhiza. Their other main characteristic is that they are exclusively symbiotic species living in symbiosis with termites on or around termitaries built by the latter. They arise from fungus-combs within these termitaries. The first description of species growing in Cameroon was done by Heim in 1952 and the author reported just 8 species in his account. A field study recently carried out enabled us to describe 14 species of *Termitomyces* from Cameroon among which 4 new and 5 new forms of *Termitomyces striatus* (3) and *Termitomyces subclypeatus* sp. nov. (2). The new species and forms described are *Termitomyces grisumbo*, *T. mboudaeïna*, *T. subumkowaani*, *T. subclypeatus* forma *bisporus*, *T. subclypeatus* forma *tetrasporus*, *T. striatus* forma *grisumbo*, *T. striatus* forma *subperforiata* and *T. striatus* forma *tricystidiata*. Also as shown in this colour poster with species described mostly in their natural habitat, the description of 5 other species (*Termitomyces mammiformis* Heim, *T. aurantiacus* Heim, *T. striatus* forma *annulatus* (Beeli) Heim, *T. schimperi* (Pat.) Heim, *T. letestui* (Pat.) Heim and *T. microcarpus* (Berk. & Br.) Heim previously described by Heim in 1952 is revised and completed.

734 - Lichenicolous lichens: Independent lines of evolution? - evidence from *Acarospora stafiana*

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Several common lichenized fungal genera, such as *Acarospora*, *Buellia*, *Caloplaca*, *Lecidea* and *Rhizocarpon*, comprise lichenicolous as well as free-living species. An implicit assumption of recognizing lichenicolous species is that they represent independent lines of evolution. Molecular approaches offer a powerful tool for testing the phylogenetic relationships among lichenicolous and independent taxa. This is a preliminary investigation of *Acarospora*, and focuses on *A. stafiana*, a relatively common lichenicolous species on *Caloplaca trachyphylla* in western North America. We analysed nrDNA ITS sequences of *A. stafiana*, its host, other free-living *Acarosporae*, other *Acarospora* hosts, a lichenicolous fungus (*Stigmidium epixanthum*) occurring on *A. stapfiana*, and a *Candelariella* sp. that appears at least facultatively lichenicolous on the same *Caloplaca*. The results support the inference that *A. stapfiana* is an obligate, lichenicolous species, in so far as the adjacent, free-living *Acarosporae* were distinct from the lichenicolous one. But one surprising result was that the ITS sequence of *A. stafiana* was identical with a free-living specimen of *Acarospora hilaris* collected from Spain. If this finding is supported by additional investigations, then the assumption of independent evolution of the lichenicolous growth-form for this species is not supported and it may be that *Acarospora stapfiana* and *A. hilaris* should be considered conspecific.

735 - Phylogenetic studies in the asexual lichen genus *Lepraria*

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Lepraria, a genus of lichenized fungi, is known to reproduce solely by asexual propagules. Widespread in distribution, members of *Lepraria* are often found growing on moist, shaded rocks and trees, frequently among mosses. Morphologically, *Lepraria* appears quite simple and bears little similarity to other genera, thus preventing its classification at a rank higher than genus. We investigate the phylogenetic position of selected *Lepraria* species in relation to other lichenized fungi using rDNA SSU and ITS sequence data. We also present preliminary data testing the hypothesis that in the absence of sexual reproduction, co-cladogenesis between symbionts occurs using rDNA ITS sequence data from both fungi and algae. Future studies will include testing for cryptic recombination within the species *L. lobificans*.

736 - Molecular studies of the *Hyphoderma setigerum* complex

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Hyphoderma setigerum (Basidiomycotina, Homobasidiomycetes) is a corticioid fungus associated with white rot and is primarily found on dead wood of deciduous trees. Morphological variation and intersterility tests within the taxonomical unit formerly known as *H. setigerum* have lead to the recognition of a species complex. The present study uses DNA sequence information from the ITS region of 50 specimens from Europe, N America, and Asia to address this variation in a phylogenetic context. A considerable amount of variation was found in the DNA sequences; based on DNA sequence analysis and interfertility tests, several hitherto undescribed species are shown to exist inside the species complex. The relation of *H. setigerum* to *H. subsetigerum* and *H. nudicephalum* is discussed.

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737 - On the presence of ectomycorrhizae in the genus *Sistotrema*

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The genus *Sistotrema* makes up a large part of the cantharelloid clade of Homobasidiomycete evolution. While ectomycorrhiza is in strong evidence for other genera of the cantharelloid clade (like *Cantharellus*, *Hydnum*, and *Clavulina*), it has hitherto not been recorded in *Sistotrema*. Using a combination of fruit-body / mycelium co-occurrence and molecular analysis (ITS, nLSU), the present study proves *Sistotrema* to contain at least two species capable of engaging in ectomycorrhizal relationships. Since the cantharelloid clade is a basal evolutionary lineage in the Homobasidiomycetes, the presence of ectomycorrhiza forming species in the clade may provide important clues to the nature of evolution in the Homobasidiomycetes. Some possible implications to phylogenetic analysis in the Homobasidiomycetes are discussed.

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738 - The Flora agaricina neerlandica project: the importance of morphological studies

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The Flora agaricina neerlandica is a critical flora, covering the agarics and boleti occurring in the Netherlands and adjacent regions. It is composed of contributions from various authors, edited by Noordeloos, Kuyper and Vellinga. It provides keys, extensive descriptions and illustrations of all taxa, and data on ecology and distribution. The flora is essentially based on own observations of the authors on fresh material and herbarium specimens collected by Dutch mycologists over more than 50 years. The research for the Flora is carried out according to standard methods and it uses in principal a morphological species concept, though the results of molecular and other studies are incorporated if available. As such the flora is a standard work and can serve as a base for further research. The importance of thorough, standardized morphological studies is nowadays often underestimated by molecular mycologists. The project has been accelerated by substantial financial support from the Kits van Waveren Foundation. Five volumes have been published; the sixth, containing revisions of the Bolbitiaceae and *Coprinus*, is currently in print. Volumes 7-11 will follow with an interval of 2-3 years. Current projects: M. Noordeloos is revising the Boletes; M. Nauta concentrates on *Psathyrella*, *Cystoderma* and *Lyophyllum*; M. Noordeloos, A. Verbeken and J. Wisman work on Russulaceae; various Cortinariaceae genera are under revision by E. Arnolds, N. Dam and Th. Kuyper.

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739 - Anamorph form in the Xylariaceae

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Modern classification of the Xylariaceae relies more heavily on anamorph form than normally applied in traditional systematics. The form genera involved can be highly teleomorph genus specific e.g. *Camillea* and *Xylocladium*, *Astrocystis* and *Acanthodochium*, *Poronia*, *Podosordaria* and *Lindquistia*. In other genera the anamorph is not genus specific and, for example *Nodulisporium* occurs in *Hypoxylon*, *Daldinia*, *Entonaema*, *Thamnomyces* and other genera. Similarly *Geniculosporium* is associated with *Nemania*, *Euepixylon*, *Anthostomella*, *Rosellinia* and several other genera. The distinction between *Nodulisporium*, *Periconiella* and even *Geniculosporium* is not always clear although the occurrence of these form genera is seen as important in genus recognition. The relationship between anamorph and teleomorph and differences between a member of the form genera are presented based on light and Scanning Electron Microscope studies. The reliability of anamorph form is also discussed.

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740 - Morphological and molecular study of *Lactarius* section *Deliciosi* in Europe

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Representatives of the section *Deliciosi* are easily distinguished from other members of the genus *Lactarius* by their orange, red or blue latex and the remarkable colour changes of the context. There is however no consensus on the species concept. Microscopic features are rather similar in almost all taxa and the taxonomic value of the striking field characters is still discussed. Carefully observed macro- and micromorphological characters are combined here with molecular data. ITS rDNA sequences are provided for 68 specimens collected all over Europe. The resulting cladogram is shown and the monophyly of the section and species relationships are discussed. A try-out of AFLP has shown that this technique is suitable to generate very detailed fingerprints. Eight European species are clearly supported from these data.

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741 - Characterization of *Lactarius tesquorum* ectomycorrhizae on *Cistus* sp. and molecular phylogeny of related European *Lactarius* taxa

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Lactarius is one of the larger genera of ectomycorrhizal Basidiomycota, with about 400 species recognized worldwide. Members of the genus have been reported in association with numerous trees and shrubs, and their important ecological role as late-stage root colonizers in a range of ectotrophic plant communities is largely appreciated [1]. We have started a study aimed at extending our knowledge of the distribution, phylogeny, and ectomycorrhizal biology of *Lactarius* species occurring in selected ecosystems [2]. In the frame of this research, we have identified the ectomycorrhizae formed by *Lactarius tesquorum* on *Cistus* sp., one of the most common and ecologically relevant shrubs present in the semi-arid regions in the Mediterranean basin, and described them in terms of morphological, anatomical, and molecular features. In addition, an ITS rDNA sequence-based phylogenetic analysis of the related European *Lactarius* taxa currently classified together with *L. tesquorum*, subgenus *Piperites* section *Piperites*, was carried out in order to clarify species-level relationships. [1] Hutchison, L.J. (1999) Lactarius. In: Ectomycorrhizal Fungi: Key Genera in Profile. Cairney, J.W.G. and Chambers, S.M. (eds.), 269-285. Springer-Verlag, Berlin Heidelberg [2] Eberhardt, U. et al. (2000) Mycologia 92, 860-873.

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742 - A molecular phylogeny of coprophilous fungi of the Delitschiaceae and Sporormiaceae sensu Barr (Pleosporales, Ascomycota)

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Preliminary results are presented from an ongoing study of the phylogeny of Pleosporales utilising nLSU-sequences, with special focus on the families Delitschiaceae and Sporormiaceae sensu Barr. The main aims of the study are to test the circumscription of these families, and to trace the evolution of coprophilous taxa.

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743 - Karyological and molecular phylogenetic evaluation on *Wallemia sebi*

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Wallemia sebi (Fr.) Arx is a unique osmophilic fungus with some mysterious aspects. Its phylogenetic relationship and accurate life cycle have not yet been revealed. It can be treated as an anamorphic ascomycete (http://www.ncbi.nlm.nih.gov/; Kirk et al., 2001) or a teleomorphic basidiomycete (Moore, 1986). Pioneer researches on nuclear behavior in sporogenesis and septal pore structure were carried out by Hashmi and Morgan-Jones (1973) and Terracina (1974), respectively. In this study, we tried to evaluate *W. sebi* with the focus on molecular phylogeny based on nuclear rDNA sequences and karyology especially in the very unique sporogenesis using some authentic strains (e.g., JCM 8329, 11230, 11231; http://www.jcm.riken.go.jp/). Based on nSSU/nLSU rDNA sequence analyses (this study) and dolipore-like septum (Terracina, 1974; Moore, 1980), it became clear that *W. sebi* has basidiomycetous affinities. Our molecular phylogenetic researches showed that this fungus presumably locates at the base in the Ustilaginomycetes as a new clade. Moreover, our karyological research focusing on the number and size of nuclei in sporogenesis of *W. sebi* by using fluorescence microscopy and nuclear staining method agreed fairly well with the hypothesis proposed by Moore (1986). As a conclusion of the moment, we believe that *W. sebi* is very possibly a teleomorphic basidiomycete.

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744 - A revision of *Gymnopilus* in New Zealand

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Gymnopilus P. Karsten is a genus of wood-rotting basidiomycetes with over 150 species distributed worldwide. *Gymnopilus* produces a rusty-brown spore print and has rough spore ornamentation, commonly without a plage. The taxonomy of *Gymnopilus* in New Zealand is revised using numerical analysis. Ten species are recorded and described, including one new species, six new records for New Zealand (*G. ferruginosus*, *G. allantopus*, *G. tyallus*, *G. austropicreus*, *G. norfolkensis* and *G. mullaunius*) and three that were previously known (*G. junonius*, *G. crociphyllus* and *G. mesosporus*). This revision indicates a close relationship between New Zealand and Australian *Gymnopilus* and it is suggested that studies of related genera may reveal similar close associations of species.

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745 - Characterization of *Laetiporus sulphureus* complex in Japan using phylogenetic analyses, morphological study and incompatibility tests

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Phylogenetic analyses, morphological studies and incompatibility tests were conducted to define intra-generic taxa of Japanese *Laetiporus sulphureus* complex. Phylogenetic analyses based on the nucleotide sequences of the ITS regions of the ribosomal DNA and beta-tubulin gene suggest that Japanese *Laetiporus* spp. are divided into four distinct groups (groups A-D). European population of *L. sulphureus* is clearly distinct from all Japanese groups. Each group shares similar morphological characteristics and host range (conifers vs. hardwoods). Group A has a white pore layer, pinkish orange pileus surface, and imbricated pilei. This group is distributed in the cool-temperate area and associated with hardwoods. Group B has lemon yellow pore layer, pinkish orange pileus surface, and imbricated pilei. This group is associated with conifers. Group C has a lemon yellow pore layer, yellow pileus surface and non-imbricated pilei. Sometimes it produces semi-globose basidiocarps with abundant chlamydospores in the context. Group C is distributed in the warm-temperate area of central parts of Japan. Group D has a lemon yellow pore layer and pinkish orange to yellow pileus surface. This group also produces semi-globose form. This group is distributed in the warm-temperate to subtropical areas of southern part of Japan. Single spore isolates from groups A, B and C were incompatible each other, which revealed that *L. sulphureus* complex in Japan is composed of at least three species.

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746 - *Penicillium tulipae* and *P. carotae*, two new species belonging to the series *Corymbifera*

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Taxa of the *Penicillium* series *Corymbifera* are known for their synnemata and association with the rhizosphere of vegetables, most commonly with bulbs. The known species *P. allii*, causing a storage rot of garlic, *P. hordei*, associated with cereals, and *P. hirsutum* associated with a variety of vegetables can easily be separated based on colony morphology. The concepts of *P. albocoremium* and *P. venetum*, reported to cause a severe bulb rot on a variety of commercially grown flower and Allium species, later evolved from *P. hirustum* senso lato as a response to apparent differences in secondary metabolism. Further secondary metabolite investigations involving *P. albocoremium* indicated the potential presence of two or more taxa. Described here using micromorphology, colony characteristics on various media and chemotaxonomic profiling are *P. albocoremium* sensu stricto and two new species, *P. tulipae* and *P. carotae*, which were resolved during a taxonomic survey of *P. albocoremium* isolates contained within the IBT culture collection. Although these novel taxa are micromorphologically quite similar, their unique secondary metabolite profiles individually distinguish them from isolates of *P. albocoremium*. Moreover, these bulb associated species produce the following mycotoxins: citrinin, penicillic acid and terrestric acid by *P. carotae* and penitrem A and terrestric acid by *P. tulipae*.

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747 - Cladistic biogeography of *Lignincola laevis* (Halosphaeriales, Ascomycota)

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Partial large subunit and the ITS regions of the ribosomal DNA of ten *Lignincola laevis* strains, which were isolated from different geographical locations, substrata and habitat, were sequenced and subjected to phylogenetic analysis to determine their genetic difference. These strains do not form distinct geographical groupings. No correlation was discovered between strains and habitat as freshwater and marine water isolates of *L. laevis* form a coherent and well-supported group. Strains isolated from mangroves form a robust clade while isolates from other substrata constitute others. Different genes infer different phylogeny for two *L. laevis* strains. These results suggest that ascospores (asci) of *L. laevis* may be able to disperse over long distances. More strains from wider geographical locations, different habitats (freshwater, brackish water and marine water) and different substrata should be explored to explain the current distribution pattern and evolution of aquatic fungi.

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748 - Phylogenetic relationships of *Hydnodon* Banker

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The monospecific genus *Hydnodon* was described by Banker (1913), based on *Hydnum thelephorum* (Léveillé, 1844) because of the salmon-pinkish spore print and shape and ornamentation of the spores. This genus has been placed in different families of Aphyllophoralles such as Hydnaceae (Banker, 1913; Miller, 1933; Harrison, 1973), Thelephoraceae (Donk, 1964), and Hydnodontaceae (Jülich, 1981) using different morphological characters. *Hydnodon* has also been related with *Scytinopogon* because the presence of cyanophilic spores in both genera (Maas Geesteranus, 1963; Jülich, 1981). This research attempts to evaluate the phylogenetic affinities of *Hydnodon* based on recent phylogenetic hypothesis for the Homobasidiomycetes (Hibbett, 1997, Hibbett & Thorn, 2001). DNA sequences of the nuc-SSU and mt-SSU of *H. thelephorus* were added to a data matrix which included representatives of each major clade of the Homobasidiomycetes. The results corroborated a relationship between *Hydnodon* and *Scytinopogon*, supported by high bootstrap and decay values. This hypothesis agree with Maas Geestaranus (1963) and Jülich (1981) proposals, and is congruent with the presence of inflated and gloeoplerous-like hyphae and a strong cyanophilic reaction of the spore ornamentation in both genera. Also, the position of *Hydnodon* and *Scytinopogon* in the general phylogeny of the Homobasidiomycetes suggests the presence of an additional clade, however, it is necessary to include more evidence to clarify this problem.

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749 - Molecular systematics of *Ascochyta* spp. infecting legumes

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Host-specific forms of *Ascochyta* spp. were sampled from chickpea (*Cicer arietinum*), faba bean (*Vicia faba*), lentil (*Lens culinaris*), pea (*Pisum sativum*) and hairy vetch (*Vicia villosa*) from various parts of the world and used to estimate a phylogeny. Previous results from laboratory crosses indicated that the *Ascochyta* spp. infecting faba bean, lentil and chickpea should be considered distinct biological species. We tested the hypothesis that these biological species were also phylogenetic species and extended the phylogeny to include isolates sampled from other legumes. We sequenced the nuclear ribosomal internal transcribed spacer (*ITS*) and a glyceraldehyde-3-phosphate dehydrogenase gene (*G3PD*) and estimated a phylogeny for each dataset. Phylogenies estimated from the two genomic regions were highly congruent with *G3PD* revealing significantly more variation. The *G3PD* phylogeny revealed 4 clades, each with high bootstrap support. Clade 1 contained isolates of *A. rabiei* from chickpeas, Clade 2 isolates of *Phoma medicaginis* and *Mycosphaerella pinodes* from pea, Clade 3 isolates of *A. pisi* from pea and *A. fabae* from faba bean, and Clade 4 isolates of *A. lentis* from lentil and *Ascochyta* sp. from *V. villosa*. These data provide strong support for *A. rabiei*, *A. fabae* and *A. lentis* as phylogenetic and biological species. The analysis also revealed that *A. lentis* is most closely related the host specific form on *V. villosa* and that *A. fabae* is most closely related to *A. pisi* on pea.

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750 - The phylogenetic history of *Cyttaria*, an obligate parasite of *Nothofagus*

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The eleven species of the fungal genus *Cyttaria* (Cyttariales, Ascomycota) are obligately parasitic on several species of the Gondwanan angiosperm *Nothofagus* (Nothofagaceae, Hamamelididae). Seven *Cyttaria* species are endemic to southern South America (Chile and Argentina), while the other four are endemic to Australasia (Australia, Tasmania, and New Zealand). The goals of our present investigation are to: 1) identify the closest relatives of *Cyttaria*; 2) infer the phylogenetic relationships between *Cyttaria* species; and 3) infer the phylogenetic relationships within *Cyttaria* species. Thus, we have begun to construct molecular sequence data sets from the following markers: nuclear ribosomal SSU, LSU, and ITS DNA; mitochondrial ribosomal SSU and LSU DNA; and the nuclear protein coding gene RPB2. Preliminary results suggest a close relationship between *Cyttaria* and the Helotiales. They also suggest two major lineages of *Cyttaria*: one composed entirely of South American species and another composed of South American and Australasian species. There also seems to be significant intraspecific variation within certain of the more widespread species. Ultimately, we will have a robust means with which to test the widely accepted hypothesis that the species of *Cyttaria* and *Nothofagus* have cospeciated. We will also be able to examine the extent to which vicariance versus dispersal can be invoked to explain the modern distributions of *Cyttaria* species and their hosts.

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751 - *Diaporthe foeniculacea* and its *Phomopsis* anamorph in Portugal

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Niessl described *Diaporthe foeniculacea* on *Foeniculum officinale* from a collection by Moller at Coimbra, Portugal. Wehmeyer considered it to be a species of *Diaporthopsis* and transferred it to *Diaporthopsis angelicae*. He did not mention the anamorph. Saccardo described *Phoma foeniculina* on *F. officinale*. Camara transferred the name to *Phomopsis foeniculina* based on a collection from Sacavém, Portugal. Neither Saccardo nor Camara mentioned the teleomorph. In August 2001, a fungus with the general characteristics of a *Diaporthe* sp. was found on a plant of *F. vulgare* on the island of

Madeira. A similar fungus was later found at two separate localities near Lisbon. Two morphologically distinct *Phomopsis* species were found on the stems. The *Diaporthe* species were compared with specimens in the herbaria of Padova (PAD) and Coimbra (COI). The specimen in PAD had unicellular ascospores and appeared to be immature. A specimen in COI (285) collected by Moller is a typical *Diaporthe* and was regarded as *D. foeniculacea*. The specimens collected from Madeira and near Lisbon correlated morphologically with COI 285 and it is recommended that the name *Diaporthe foeniculacea* should be retained. One of the *Phomopsis* spp. on fennel stems correlated with Camaras description of *P. foeniculina*, but it is not the anamorph of *D. foeniculacea*. The teleomorph of *P. foeniculina* has not yet been found. *Diaporthe foeniculacea* is self-fertile and readily produces perithecia in culture.

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752 - Characterization of *Pisolithus* in Thailand

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A detailed study of *Pisolithus*, *Scleroderma* and *Astraeus* from a variety of hosts and geographic areas of Thailand is currently being undertaken to assess their potential for enhancement of growth in inoculated seedlings of *Pinus kesiya* and *Eucalyptus camaldulensis*. The results demonstrated that basidiospores from most *Pisolithus* isolates are ornamented with well-developed spines. At least 2 groups of spore types occur. Most basidiospores collected from *Eucalyptus* are characterized by numerous echinulate, blunt spines whilst basidiospores with coalesced spines joined at their tip have been found mainly in isolates collected from pine forest. This corresponds with a recent study of the internal transcribed spacer (ITS) DNA sequence. Sequences for 14 *Pisolithus* isolates associated with native Mediterranean hosts, *Eucalyptus camaldulensis* and *Pinus kesiya* were compared with those in GenBank. The phylogenetic analyses grouped Thai *Pisolithus* isolates into 2 distinct groups. Group 1 contained *Pisolithus* isolates collected from Pine forest and Group 2 contained those collected from *Eucalyptus* plantation. This present study indicates that basidiospore ornamentation, together with ITS sequences, can distinguish two distinctive *Pisolithus* groups in Thailand which also exhibit clear host association.

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753 - *Cercospora* species from Venezuela described by C. Chupp and A.S. Muller

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The species *Cercospora caracasensis* (on *Annona purpurea*, Annonaceae), *C. centrosematis* (on *Centrosema virginianum*, Leguminosae-Papilionoideae), *C. cestri* (on *Cestrum latifolium*, Solanaceae), *C. chirguensis* (on *Curatella americana*, Dilleniaceae), *C. conocarpi* (on *Conocarpus erectus*, Combretaceae) and *C. cyclantherae* (on *Cyclanthera pedata*, Cucurbitaceae), described by C. Chupp and A.S. Muller, were revised. Based on the morphological characteristics of reproductive structures exhibited by the type specimens and, on the correlation of these criteria with available molecular data, the following new species are proposed: *Passalora centrosematis*, *Stenella cestri*, *Pseudocercospora chirguensis* and *P. conocarpi*. *Cercospora caracasensis* is reduced to synonymy with *Pseudocercospora annonae-squamosae*, and *C. cyclantherae* is considered a synonym of *C. apii* s.lat. Descriptions and illustrations of the species treated are also provided.

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754 - Morphological characters of *Lentinellus* cultures

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The taxonomy of the genus *Lentinellus* has recently been reevaluated by Dr. R.H. Petersen and his Lab (The University of Tennessee, USA) using classical morphology of type specimens, mating study and molecular analysis. Cultural study of *Lentinellus* strains received from Dr. Petersen's Lab have been undertaken to evaluate the diagnostic potential of mycelial characters in the genus. Dikaryon cultures from different intersterility groups belonging to *L. ursinus*, *L. castoreus*, *L vulpinus*, *L. montanus*, *L. flabelliformis* and *L. micheneri* were used. The strains were grown on MEA and PDA media. Indexes of growth, macro- and micromorphology were described using traditional terminology. Mycelial structure was studied from scanning electron microscope images. Laccase and tyrosinase activities were studied by plate diffusion method (Bavendamm reaction) and spot tests (substrates: guaiacol, syringaldazine and l-tyrosine). The strains' capacity for fructification was evaluated on sawdust/bran substrate. It was shown that different species were distinguishable by differences in growth indexes, texture and color of mycelial mats and in fruitage ability. Some of the *Lentinellus* hyphal structures like clamps, gloeocystidiaes, chlamydospores, conidias, hyphal swellings, rings and incrustations were species-specific. Strains of all studied species could produce laccase, whereas tyrosinase was determined only for *L. micheneri*, *L. ursinus* and *L. castoreus*.

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755 - Group I intron evolution as mobile and non-mobile elements within the lichen-forming fungal family Acarosporaceae

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Autocatalytic group I introns in the nucleus are found only in ribosomal DNA genes. These genetic elements are most commonly inherited vertically although their widespread and often sporadic distribution suggests their ability to integrate in novel rDNA sites. Two models exist for group I intron spread: 1) intron homing facilitated by an endonuclease encoded in the intron, and 2) reverse-splicing. It seems that many organellar group I introns rely primarily on homing for spread, whereas virtually none of the rDNA introns contain endonucleases. To date only eight endonucleases have been reported in algae, non-lichenized ascomycetes, and amoeboflagellates. In this paper, we study the evolution of group I introns within the lichenized ascomycete family Acarosporaceae. Group I introns are well known in the ascomycetes and are particularly abundant in the Acarosporaceae (128 in 2.5 Kb of rDNA in 35 taxa). The family Acarosporaceae appears to be one of the most basal divergences within the lichenized ascomycetes, therefore, the study of its introns is of particular interest to better understand the evolution of lichen introns. We provide evidence for both relatively ancient and recently acquired group I intron lineages within the Acarosporaceae. Furthermore, we report for the first time in lichenized ascomycetes, the presence of a homing endonuclease in members of the Acarosporaceae. These endonucleases may facilitate the lateral transfer of some ascomycete group I introns.

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756 - The rust genus *Hemileia* - Monograph and collection of the characteristics in a DeltaAccess database

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The genus *Hemileia* is phytoparasitic mainly on hosts belonging to the families Rubiaceae, Apocynaceae and Asclepiadaceae. The species are distributed tropically to subtropically, probably originally in Africa and Asia. By the cultivation of coffee, however, members of the genus occur nowadays pantropically. Regarding the life cycle, so far only those for uredinia and telia are known. Hitherto, 53 species of *Hemileia* have been described. Some of these species were described upon the uredinial stage and require nomenclatural revision. The type species *Hemileia vastatrix* represents one of the most important pathogens world-wide in the cultivation of coffee and thus has great economical importance. Although there have been numerous studies on the morphology and the parasitism of several *Hemileia* species, no monograph of the genus exists up to now. Therefore, an investigation of all *Hemileia* species is carried out by means of light and electron microscopy. The work is based on extensive herbarium material. The morphological data gathered are entered into a database. This database was developed within the framework of the GLOPP-project and is designed to register rust fungus biodiversity, morphological features and to allow the identification of the pathogens. Our final aim is to make this database available on the internet.

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757 - Molecular identification of *Taphrina* species parasitic on *Prunus*

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The dimorphic ascomycete genus *Taphrina* comprises ca. 100 species recognised by their filamentous parasitic stage on several vascular plants. Infection symptoms may develop on leaves, fruits, shoots and flowers. Most species parasitise a single host and/or cause a specific infection symptom. The best known species is *Taphrina deformans*, causal agent of peach leaf curl, a disease with a pronounced impact on peach growing areas worldwide. Other economically important species of the genus *Prunus* are also potential *Taphrina* hosts, such as almond, cherry and plum trees. The present work focuses on the characterisation of *Taphrina* spp. on *Prunus* using molecular techniques: PCR-fingerprinting (MSP-PCR), sequencing of rDNA (ITS and D1/D2 regions) and DNA-DNA hybridizations. MSP-PCR proved to be a quick and reproducible method for species differentiation, allowing the selection of strains for sequencing. The D1/D2 region was too conserved to differentiate the *Taphrina* studied. The ITS regions were more useful for this purpose, and phylogenetic clustering of *Taphrina* spp. correlated with the host, geographic origin and/or the type of infection symptom. Intraspecific variability in the ITS regions was evaluated by comparison with the results of MSP-PCR and hybridization experiments. The conventional identification of the *Taphrina* species studied was re-evaluated in the light of the results of the molecular techniques employed.

758 - Morphotaxonomy and phylogeny of *Lentinus* and *Panus* from the neotropics

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A combined molecular and morpho-taxonomic approach was used to delineate taxa and infer main evolutionary lineages in members of *Lentinus* Fr. and *Panus* Fr. from Costa Rica and the neotropics. Previous morpho-taxonomic studies have resulted in unresolved delineation of (evolutionary) groups and conflicting classifications in this diverse and ecologically important group of wood decaying fungi of the tropics. ITS nrDNA sequences and the combined use of morphology and ITS sequences always placed *Lentinus* and *Panus* in two distinct clades. Two evolutionary lineages of *Lentinus*, and two strongly supported subclades within the *Panus* clade, were detected. The inferred phylogeny suggets that *Lentinus* is more closely related to *Polyporus* than to *Panus*. Three species assigned to the *L. crinitus*-complex (*L. crinitus*, *L. bertieri*, and *L. swartzii*), e. g. characterized by a continuum of morphotypes and not easily assigned to a specific taxon, were particularly focused. Sequences from 7 specimens of this complex indicated a relatively high level of genetic variation, distributed among mostly unresolved groups. In some *Lentinus* and *Panus* species parts of sequences were consistently double-banded, indicating intraindividual ITS polymorphism. The ITS phylogeny indicates that lamellate hymenophores and similarity in some ultrastructural features are not homologous structures in *Lentinus* and *Panus*. The two divergent groups of taxa justify the recognition of two distinct genera, *Lentinus* and *Panus*.

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759 - Identification of selected *Bipolaris*, *Curvularia* and *Exserohilum* species using cultural features, FOIL and Decision Tree(DT) classification tools

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Many closely similar species have been described within *Bipolaris*, *Curvularia* and *Exserohilum*. Identification of species is further complicated by the variability of some morphological features of the group. In other anamorphic genera, investigation of cultural and biochemical features as taxonomic criteria to supplement morphology, received attention for the same reason. The object of this study was to investigate the potential of simple measurements on solid media as identification tools in some helminthosporic fungi. Authentic and representative strains of selected *Bipolaris*, *Curvularia* and *Exserohilum* species, including the type species of each genus, were obtained form various collections. The strains were selected to vary in geographical origin and host or substrate or origin. Colony diameters of strains were measured on a variety of media testing presumptive enzymatic activities, influence of inhibitory substrates and utilization of different carbon sources. Data accumulated were processed with two data-mining techniques FOIL and DT. The value of culture based tests as diagnostic aid was demonstrated. Using FOIL algorithms and 5 of 25 tests, 79% of the strains investigated could be placed into the correct species. Further refinements to some tests could result in an identification tool or the development of selective media for some of these species.

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760 - Phylogenetic analysis of cetrarioid lichens with globose ascospores

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The group of cetrarioid lichens (Parmeliaceae, Ascomycota) with globose ascospores includes 37 species from eight genera (*Ahtiana*, *Allocetraria*, *Dactylina*, *Esslingeriana*, *Nephromopsis*, *Tuckneraria*, *Tuckermannopsis*, *Vulpicida*). Phylogenetic analysis was carried out on two different data sets - morphological (incl. anatomical and chemical) and molecular characters (ITS1, 5.8S and ITS2 rDNA sequences) - to verify the correspondence of current taxonomy to the probable evolution of the taxa involved. For 17 species both morphological and molecular characters were studied while 14 other species were checked for morphology only. The paraphyletic nature of the genus *Allocetraria* in regard to two species of *Dactylina* is assumed on morphological characters. Still, the separation of these genera is strongly supported by the analysis of molecular data. The genus *Tuckermannopsis* should be considered paraphyletic in regard to *Esslingeriana idahoensis* and *Nephromopsis weii* according to the analysis based on morphological characters. No doubt that *Nephromopsis wei* does not belong to the genus *Nephromopsis* and a new combination for this taxon is needed. The analysis of molecular characters reveals the monophyletic origin of all three *Tuckneraria* species included in the analysis. Monophyly of the genus *Ahtiana* is not supported by the analysis of morphological data while the analysis based on ITS sequences indicates a close relationship between the genera *Ahtiana* and *Tuckermannopsis*.

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761 - Systematic approach to pyrenomycete taxonomy

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Considerable progress has been made in the elucidation and stabilization of the concepts of Families, Orders and Classes of Pyenomycetes as reflected in Systema Ascomycetum and The Dictionary of the Fungi. From Systema Ascomycetum 2001, the Class Dothidiomycetes encompasses 7 Orders: Capnodiales (4 Families), Dothideales (4), Hysteriales (2), Myriangiales (2), Patellariales (1) and Pleosporales (19). These are ultimately based on the type species of 32 genera. The Class Chaetothyriomycetes has one recognized Order, Chaetothyriales, with two families plus Rhynchostomataceae without an assigned Order and *Glyphium* not formally assigned to either Family or Order. These ultimately are based on 4 species. Forty-three Families are provisionally assigned to Dothidiomycetes / Chaetothyriomycetes based on 43 generotype species. For 79 genera, details are provided on the choice of the type species and the protologue of the type specimen in the hope that future work will focus first on these fundamental species rather than those chosen based on easy availability from culture collections.

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762 - Basidiome development as a genus level character in some species of Dacrymycetales from Mexico

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The order Dacrymycetales was established by Lindau (1897), grouping genera included in the family Dacrymycetaceae by Brefeld (1888) such as *Calocera*, *Dacrymyces*, *Dacryomitra* and *Guepinia*. Subsequently, other classifications have been proposed, those of Coker (1920), Neuhoff (1936), Brasfield (1938), Kobayasi (1939), Martin (1952), and more recently, Lowy (1971), McNabb in the 60's, Reid (1974) and Oberwinkler (1994). One of the commonly debated topic among these works is the number of recognized genera, since some authors accept 11 genera, whereas others recognize only 2. Jülich (1981) established the monotypic family Cerinomycetaceae for *Cerinomyces*. In Mexico this group has been partially studied (Lowy, 1968; 1971; 1983; Sierra, 1992; 1993; 1995; 2000), for this reason the main objectives in the undergoing research of the senior author is to monograph the order at the national level and to apply new characters to its systematic. In order to contribute to clarify genera delimitation basidiome development was studied in 9 species belonging to 5 genera. The methodology proposed by Kennedy (1972) was used with modifications in the fixation (FAA), staining (PAS, saffranin-fast green, toluidin blue, etc.) and infiltrating material (Paraplast).

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763 - Two new phytoparasitic forms of hyphomycete genus *Mycovellosiella* causing foliar spots from India

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The foliicolous hyphomycete genus *Mycovellosiella* is now well established and defined genus of over 150 species till date. Many species of the genus were earlier placed under other genera like *Cercospora*, *Cercosporella*, *Cladosporium*, *Phaeoramularia*, *Ramularia* and *Stenella* etc. *Mycovellosiella* can be characterised by its superficial secondry mycelium forming prostrate and procumbent hyphae and forming rope-like structures often ascending the leaf hairs, and micronematous to semimacronematous conidiophores. Thus fungus produces phragmosporic and catenate conidia in simple or branched chains with prominent hila. *Mycovellosiella* produces leaf-spot diseases ranging from discolouration to necrosis of the leaf tissues. In continuation of the earlier additions from this region, the present communication is devoted to the description and illustrations of two new species of *Mycovellosiella* viz., *M. aegli* sp. nov. and *M. terminalae* sp. nov. occuring on leaves of *Aegle marmelos* (Rutaceae) and *Terminalea arjuna* (Combritaceae) respectively. The concerned materials have been deposited in H.C.I.O., New Delhi, India. These species have been compared with their allied or similar taxa to justify its separate identity. The scrap-mounts have been prepared in lactofuchsin and glycerine separately to observe these structures, under different magnifications of compound microscope.

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764 - *Fusarium commune*, a new species within the *Gibberella* clade identified by morphological and molecular phylogenetic data

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Fusarium commune sp. nov. was isolated from soil and *Pisum sativum* in Denmark and several geographically widespread locations within the northern hemisphere from diverse substrates including white pine, Douglas fir, carnation, corn, carrot, barley and soil. *Fusarium commune* is characterised by and distinguished from its putative sister taxon, the *F. oxysporum* complex, in having long, slender monophialides and polyphialides when cultured in complete darkness. Based on the combined DNA sequence data from translation elongation factor and the mitochondrial small subunit ribosomal DNA, the fifteen isolates of *F. commune* analysed formed a strongly supported clade closely related to but independent of the *F. oxysporum* and the *Gibberella fujikuroi* species complexes.

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765 - Identifying species in the genus *Botryosphaeria*

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Botryosphaeria species are common endophytes and opportunistic pathogens of woody hosts, world-wide. Approximately 150 *Botryosphaeria* species have been described, but their taxonomy is often confused due to limited morphological variation and the wide host range of some species. Recent studies have successfully combined rDNA sequence and morphological data to define and describe species. These characters, however, need to be evaluated for their use in defining species boundaries between closely related species and species complexes. In this study, we combined sequence and PCR-RFLP data from the ITS rDNA, β-tubulin and elongation factor-1-α, with traditional morphological and ecological criteria, to delimit various *Botryosphaeria* spp. The ITS region was sufficiently variable to distinguish all species groups. However, some closely related species such as *B. ribis* and *B. parva* could not be separated. Combined data sets of the three sequenced regions, however, clearly separated the different species. Morphological characters were found to be variable in nature. But under controlled laboratory conditions, conidial and cultural morphology could be used to recognise most species. Ecological data were also useful in defining taxa, as many species are restricted to a particular host or environment. The combination of morphology, habitat data and DNA sequences produced a reliable basis for the characterisation of *Botryosphaeria* spp., both at the phylogenetic and diagnostic levels.

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766 - Phylogenetic position of the *Caloplaca aurantia* group

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The *Caloplaca aurantia* group, consisting of the species *C. aurantia*, *C. flavescens* and *C. thallincola*, was formerly included in the subgenus *Gasparrinia*, but is distinguished from most other *Gasparrinia*'s by having more or less citriform spores. Based on molecular data a phylogenetic hypothesis is presented, which places the *Caloplaca aurantia* group apart from most species in *Caloplaca* subgenus *Gasparrinia*, but close to the *Caloplaca velana* group.

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767 - Characterization of *Fusarium proliferatum* (Matsushima) Nirenberg, the causal agent of bakanae disease of rice

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Sixty-four samples of bakanae disease of rice were collected from different locations in Thailand during the years 1997-2000. Cultural and morphological characteristics of 38 isolates were studied and examined scrutinizedly using Nelson et al. (1983)'s methods. The result revealed that both cultural and morphological appearances of all isolates were identical and identified as *F. proliferatum* (Matsushima) Nirenberg, when considering the production of polyphialides. Colonies on PDA floccose, white when young, and became pinkish orange to reddish or bluish purple when old (7-14 days). Culture were often tinged with light blue; reverse pale orange to light or dark blue. Some isolates showed blue spots of sclerotia. On CLA, microconidia were abundant, formed in chains of varying length and in false heads on monophialides and polyphialides which often appeared in 'V' shape. They are primary single cells, oval to club-shaped with flattened base, 6.5 - 11.6 × 2.1 - 3.4 µm. Macroconidia were abundant, produced from monophialides on branched conidiophores in sporodochia, hyaline, slightly sickle-shaped to almost straight with basal and ventral surfaces parallel. The walls were thin and the basal cells were foot-

shaped, 3-5 septate, 26.0 - 64.8 × 1.8 - 3.1 µm. Chlamydospores absent. Pathogenicity test was conducted on rice seeds (RD6). It was found that the inoculated rice seedlings were elongated in double of the control treatment after 6 days of inoculation.

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768 - The nature of the lichen symbiosis: Evidence for mutualism

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In 1869 the German botanist Simon Schwendener put forth his dual hypothesis proposing for the first time the symbiotic nature of lichenized fungi. The structural and metabolic complexity of many lichens suggests a long-term, synergistic interaction between the symbionts. Certainly, the lichenized condition, at least in its most basic form, has been in place since the advent of the symbiotic partners. Traditionally, lichens have been held up as the ultimate example of symbiotic mutualism; however, many lichenologists characterize the association as one of controlled parasitism or helotism where the mycobiont exploits or enslaves the photobiont, extracting organic carbon and in some cases organic nitrogen. This interpretation has received widespread support; and clearly applies to more primitive (poorly organized) lichen species. However, more highly evolved species demonstrate a complex array of mycobiont adaptations that facilitate and accommodate the photobiont and promote the dispersal and propagation of the lichen. By definition mutualism requires that both symbionts are benefited by the relationship, with each partner evolving discernable structural and/or metabolic adaptations that enhance the performance of the other symbiont. Clearly, many lichen associations meet these criteria, and therefore, should be considered mutualistic.

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769 - Morphological and ultrastructural characteristics of *Daldinia* species

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In our investigation of *Daldinia* Ces & De Not. (Xylariaceae), morphological characters of anamorphs as defined in the last world monograph by Ju et al. (Mycotaxon 61: 243f., 1997) proved valuable for segregation of species. Moreover, SEM was employed to study the surface structures and other features of ascospores (Mycolog. Progress 1: 31f., 2002). Aforementioned characters were employed to validate the results obtained by recently evolved PCR methodology and chemotaxonomy based on HPLC-UV/visual and HPLC-MS metabolite patterns (Mycolog. Res. 105, 1191f., 2001). The results of our recent studies of specimens similar to the pantropical *D. eschscholzii* (Ehrenb.: Fr.) Rehm from Sicily, the Channel Islands and the Canary Islands suggest that the variability within the genus may actually be much higher than we had suspected some time ago. The present contribution is intended to illustrate characteristic features of some well-known and some recently described *Daldinia* spp. Morphological and ultrastructural data also provide evidence that a polyphasic taxonomical approach is feasible to detect cryptic species and evaluate the biological diversity of *Daldinia*.

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770 - The ITS phylogeny highlights the evolution of morphological characters and ecological traits in *Cordyceps*

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The phylogenetic relationships of 46 *Cordyceps* spp. and 24 anamorphic taxa with known or suspected affinity to *Cordyceps* were inferred from ITS nrDNA sequence data. The ITS phylogeny recognized four evolutionary lineages and some strongly supported subclades when representatives of the four clades were subjected to new alignments and analysed separately. Morphological characters traditionally used to define subgenera of *Cordyceps* were found to have limited phylogenetic information if compared to the ITS phylogenetic tree. The ITS tree also questions the present systematics of several anamorphic species, e.g. the genera *Beauveria* and *Tolypocladium* are both polyphyletic. The ITS phylogeny confirms the results of some earlier nrDNA studies (SSU, LSU) that the plant pathogenic species *Claviceps purpurea* and *Epichloë typhina* are derived within *Cordyceps*, consequently making the genus *Cordyceps* paraphyletic. We discuss important non-molecular characters, e.g. perithecium morphology, host preferences and anamorphic affiliation in the light of the inferred ITS phylogeny. We conclude that the paraphyletic *Cordyceps* and its allies (anamorphs) deserve a new classification based on a combination of molecular and non-molecular evidence.

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771 - Are microsporidia really related to fungi ?

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The phylogenetic theory of Microsporidia, which are amitochondrial intracellular parasites, has been challenged by recent protein sequence-based phylogenies. These studies indicate a phylogenetic relationship of Microsporidia and Fungi, and in some analyses Microsporidia were demonstrated to have evolved from within fungal lineages. However, most of these studies did not include sequences from basal fungal phyla (Chytridiomycota and Zygomycota), and this biased taxon sampling could result in an erroneous phylogenetic relationship of Microsporidia and Fungi. To further investigate the evolutionary origin of Microsporidia, we performed molecular phylogenetic analyses with newly determined RPB1 and EF-1α sequences from basal fungi. Although the phylogenetic position of Microsporidia in the EF-1α tree still might be artificially misplaced due to the unusually high rate of sequence divergence of the microsporidian EF-1α gene, the phylogenies recovered based on these two protein sequences do not provide strong evidences for a close relationship between Microsporidia and Fungi. Moreover, we have identified within EF-1α genes a characteristic two amino acid deletion which is conserved in all fungal sequences currently available, whereas this deletion is absent in microsporidian sequences. These results argue against the current view of Microsporidia as highly degenerate fungi, suggesting that it still remains unresolved whether Microsporidia and Fungi are sister taxa.

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772 - The genus *Physalacria* in Japan

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The genus *Physalacria* (Agaricales) forms elliptical to subconical and hollow pileus on a central stipe. Approximately 30 species have been recognized as members of this genus. Berthier (1985) presented a worldwide monograph of this genus and three taxa were recognized based on their original descriptions from Japan. However, their type specimen could not be found according to Berthier's monograph. During my investigation on minute basidiomycetes in Japan, several *Physalacria* taxa were collected and cultured. The aims of the present study are to 1) reexamine the type specimens of the 3 Japanese taxa previously described, 2) examine the *Physalacria* flora in Japan and 3) document their cultural morphology and mating behavior. In the three taxa, only the type specimen of *P. orientalis*was preserved in good condition and redescribed. The other two taxa were considered to be doubtful species. In total, 11 taxa including 6, which were not previously described, were recognized in Japan. The cultural morphology of 8 taxa out of 11 were examined and supported the species delimitation in colony appearance, basidiocarp or stroma formation, and presence or absence of clamp connections. Mating behavior was detected for 5 taxa; two taxa exhibited a bifactorial mating system and the other three taxa exhibited an amphithallic mating system.

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773 - Pairing test among the strains of *Trametes versicolor* group from different populations from Central and South-eastern Europe

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Mating experiments were carried out to investigate genetic relationships of different strains of *Trametes versicolor* group. Collections of *Trametes versicolor* (L.: Fr.) Pilat, *T. hirsuta* (Fr.) Pilat and *T. ochracea* (Pers.) Gilb. et Ryvarden from different localities in the Czech Republic, Bulgaria and Montenegro (former Yugoslavia) were investigated. Monokaryotic single spore isolates were obtained from basidiocarps collected in the field, from dikaryotic cultures fruited in the laboratory or by bile-salts dedikaryotization. Monokaryons originating from 30 different dikaryons were mated each other. All pairings between different species were indicated by a line of demarcation between the paired isolates. Pairing of monokaryons belonging to the same species resulted mostly in the formation of heterokaryotic mycelium and no line of demarcation was observed in any case. No intersterility groups were found within the same species. Experiments confirmed the validity of contemporary species concept of *Trametes versicolor* group. This work has been supported by grant no. 526/02/1216 from the Grant Agency of the Czech Republic and by Institutional Research Concept no. AV0Z5020903

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774 - Species delimitation in the *Russula clelandii* complex from Australia

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In Australia, the genus *Russula* (49 listed species) is an important widespread mycorrhizal component of forest ecosystems. Fruiting bodies are highly coloured and conspicuous, and are distinctive components of the macrofungal flora that are suitable for ecological monitoring. *Russula* is a taxonomically complex genus and contains a number of undescribed taxa. The red-to purple-capped species of *Russula* recorded from southern Australia include *R. australiensis*, *Russula clelandii*, *R. lenkunya*, *R. purpureoflava* and *R. persanguinea*. Various Northern Hemisphere names have been used to describe members of this group, e.g. *R alutacea*, *R. atropurpurea*,*R. azurea*, *R.cyanoxantha*, *R. clusii*, *R. delica*, *R. emetica*, *R. expallens*, *R. fragilis*, *R. mariae*, *R. purpurea*, *R. queletii*, *R. rosacea*, *R. rubra*, and *R. sanguinea*. Species delimitation in this group on the basis of micromorphology and macromorphology was investigated using pattern analysis (classification and ordination). Nuclear large subunit rDNA region was sequenced of representatives of each morphological group to determine sequence variation in relation to morphological variation.

775 - *Fusarium langsethiae* Torp & Nirenberg ined.; a new species in section *Sporotrichiella*

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The new species *Fusarium langsethiae* Torp & Nirenberg ined. has recently been described, illustrated and discussed. The species is isolated from kernels of oat, wheat and barley in different European countries. Knowledge retrieved during surveys on the occurrence of *Fusarium* spp. in grain in Norway, led to the conclusion that the regular detection of T-2 toxin and HT-2 toxin in Norwegian cereals, especially in oat, is caused mainly by this *Fusarium* species. The combination of observed morphological characters and recorded mycotoxin profile had not been reported for any other *Fusarium* species before. The fungus has been misidentified mostly as *F. poae*, but it has also been reported as *F. sporotrichioides*. Morphologically it looks similar to *F. poae*, but it is differentiated from *F. poae* by the powdery appearance on PSA and PDA, slower growth, producing less aerial mycelium, no odour and the napiform or globose conidia are borne on often bent phialides on the agar surface and not on the aerial mycelium. No macroconidia are formed. Cardinal temperatures for the new species: minimum 7.5 - 10 °C, optimum 27.5 °C, maximum 35 °C. Based on the taxonomic systems of Wollenweber and Reinking (1935), Nelson et al. (1983) and Gerlach and Nirenberg (1982) the species is placed in the section *Sporotrichiella* for the time being.

776 - Molecular phylogenetic analyses of the symbionts of Costa Rican species of *Everniastrum* (lichenized Ascomycota, Parmeliaceae)

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The lichen genus *Everniastrum* is widespread with over 30 species inhabiting most tropical and some temperate montane regions. Higher species diversity in Andean South America and montane Mexico compared to Africa and Asia may suggest a New World center of origin and relatively recent speciation events. We have collected *Everniastrum* from cloud forest, elfin forest and paramo regions of Costa Rica, and are constructing phylogenies based on ITS rDNA sequences from both photobionts and mycobionts.To investigate hypotheses of codiversification of the symbionts we will test for evidence of co-cladogenesis. Additionally we will assess: 1) whether character-based species concepts, including chemotaxonomic designations, reflect the evolutionary history of the fungal species and 2) whether fungal asexual taxa and sexual taxa included in species pairs form monophyletic lineages. These preliminary studies will be expanded to include multiple-gene analyses of symbionts from species of *Everniastrum*, *Cetrariastrum*, and *Concamerella* representing their pantropical distribution to elucidate phylogeographic relationships of lichens.

777 - Detection of an anamorph of *Tuber dryophilum*: molecular and immunological evidence

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We present molecular and immunological evidence that *Tuber dryophilum*, a true truffle belonging to the species group of minor white truffles, produces an anamorph of the *Verticillium* type. Nuclear ribosomal sequences were amplified from field collected anamorph material with the primer pair ITS1F/ITS4. One sequence was nearly identical (similarity >99%) to a reference sequence of *Tuber dryophilum*. The identification of this anamorph was supported by indirect immunofluorescence using an antibody raised in rabbits against *Tuber melanosporum* antigene which has been demonstrated to be valuable for the detection of various stages in the life cycle of several *Tuber* species. According to our knowledge, this is the first report of an anamorph in the Tuberaceae. The potential ecological and biotechnological significance of anamorphs in the propagation of ectomycorrhizal fungi is not yet fully acknowledged. Seedlings inoculated with noble truffle species are often infected with less valuable white truffle species (e. g. *Tuber dryophilum*), either in the nursery or in the field, sometimes without plausible explanation for the way of infection. The existence of an alternative way of propagation in the genus *Tuber* indicates that additional precaution against cross contamination in inoculation experiments is required.

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778 - Differentiation of *Leptographium* spp. found in Canada

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Leptographium is one of the most commonly found genera of fungi causing bluestain in conifers. Some species are known to be tree pathogens. Some *Leptographium* spp are linked to specific wood infesting insects, especially bark beetles, however their biology, including their potential to grow in fresh woody substrates and to cause stain, is not fully known. The taxonomy of *Leptographium* spp is complex, and identification to species is often difficult when based on the existing taxonomy keys. Molecular biology tools are being developed but are not yet available. We looked at number of reference *Leptographium* isolates from existing culture collections and we included those collected in Canada over the last decade. We investigated additional attributes that could assist species delineation. This included growth rates on 2% malt extract agar at different temperatures and colony morphology at different stages of growth. We also compared the ability of some of the isolates to colonize freshly cut lodgepole pine phloem and to stain the sapwood. The results of these experiments and our observations are reported here.

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779 - *Lactarius* well-represented in West-Africa? Benin explored!

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The up to now 80 *Lactarius* species described from tropical Africa are particularly well-represented in the Guineo-Congolian rain forests (Congo-Kinshasa) and the Zambezian miombo woodlands (Zimbabwe, Zambia, Burundi). In these ecosystems the genus Lactarius is one of the most important ectomycorrhiza formers. The Sudanian woodlands cover large parts of West-Africa and are, compared to the Zambezian woodlands, poorly explored. They are often dominated by the ectotrophs Isoberlinia and Uapaca, while they lack the typical Zambezian trees Brachystegia and Julbernardia. Therefore, it seemed logic that the species diversity in the Sudanian woodlands would be less important than in East-Africa. Some scattered records of miombo woodland species in West-Africa already suggested an overlap in the *Lactarius* mycoflora, but more studies were needed. Intensive collecting in the Isoberlinia and Uapaca savanna woodlands in Benin, resulted in at least 24 *Lactarius* species, including some undescribed taxa. In spite of the differences in dominant ectotrophs, most *Lactarius* species from the studied area are also known from Zambezian woodlands. This study is the first to reveal a marked overlap of the Zambezian and Sudanian ectomycorrhizal mycoflora. It also indicates the absence of the Sudanian species in the Central African rain forests (Guineo-Congolian) and consequently suggests a disjunct distribution of the savanna woodland species of *Lactarius* throughout Africa.

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780 - Taxonomy and phylogeny of *Septoria*

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In the anamorph genus *Septoria* Sacc. over 2000 names have been described. Most taxa cause leaf spot on the host plant. Known teleomorphs belong in *Mycosphaerella* Johanson (Dothideales, Ascomycota), a cosmopolitan genus with over 20 reported anamorph genera. Crous et al. (2001, Mycologia 93: 1081-1101) showed that *Mycosphaerella* is monophyletic, based on ITS rDNA sequences. Both strains of *S. aceris* (teleom. *M. latebrosa*) studied clustered in a clade with *Cercospora*, separate from *S. tritici* (teleom. *M. graminicola*), indicating that *Septoria* anamorphs may have evolved in several lineages. We further investigated the phylogenetic position of *Septoria* anamorphs using ITS and partial 28S rDNA sequences of a larger set of taxa, some with known teleomorphs, isolated from a wide array of plant families. An additional eight anamorph genera linked to *Mycosphaerella* were also represented in the analyses. The results show that *Septoria* spp. all evolved within *Mycosphaerella*, but not as a result of a single event. The data support a clade comprising *S. tritici* and *S. passerini* from grasses, close to *Ramularia* spp. *Septoria* spp. from several deciduous trees represent relatively distant lineages. In contrast, many of the *Septoria* spp. from various herbaceous plant families have almost identical ITS sequences, and also often very similar morphologies in vitro and in planta, indicating that they are closely related. They are grouped with *Cercospora* spp. in a large, unresolved cluster.

781 - Morphological variation of spore ornamentation within the genus *Gomphus*

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Traditionaly the morphology of the spores has been an important source of characters to classify species in *Gomphus*. Neverthless, the understanding of morphological variation patterns in spore ornamentation within species is misleading. The goal of this research was to evaluate ornamentation similarity among differents species described as *Gomphus* and, to identify discrete states to use them in systematics. We analyzed the ornamentation in sixteen taxa using scanning electron microscopy (SEM). Three differents patterns of ornamentation shape and two of ornamentation distribution were differentiated. Reticulate-lacunose ornamentation was only found in *G. retisporus*, verrucose ornamentation was pretty common and it was found in *G. clavatus*, *G. floccosus*, and *G. subclavaeformis*; various degrees of verrucae fusion were observed. Ornamentation distribution is very characteristic in *G. grandis* and *G. retisporus*, with a distinct zone without ornamentation near hilar appendix. A preliminary phylogenetic evaluation, including other available micro and macromorphological characters, indicates that spore ornamentation provides significant evolutionary information within the genus.

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782 - The relevance of anamorphic states for a natural classification of Agaricales: results from morphological and molecular phylogenetic studies

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Single-spore cultures of more than 180 species of Agaricales were studied morphologically and by molecular phylogenetic methods. The morphological investigations revealed anamorphic states in more than 110 species, which are often specific at the genus or family level. Phylogenetic analysis of the 5' terminal domain of the nuclear gene coding for the ribosomal large subunit resulted in several well-supported groups that are consistent with anamorph morphology. Our results indicate that the anamorphic state provides valuable characters for a natural classification in the Agaricales.

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783 - Molecular variation among species of *Verticillium*

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The genus *Verticillium* within fungi imperfecti contains a heterogeneous group of asexual species. Many of these species are important plant pathogens or entomopathogens. The classification is based on morphology of the vegetative reproductive organs namely conidiophores, conidia and resting structures. Some analyses have been done on *Verticillium* using immunochemical and RFLPs methods. We analyzed the variation of the nuclear ribosomal internal transcribed spaces (ITS) of the following species: *V. albo-atrum*, *V. dahliae*, *V. longisporum*, *V. lamenicola*, *V. fungicola*, *V. catenulatum*, *V. tricopus*, *V. nigrescens*, *V. chlamydosporium*, *V. chlamydosporium*, *V. theobromeae*, and *V. psalliotae*. The DNA was duplicated using PCR. The DNA sequences were determined using a Perkin Elmer ABI PRISM sequencing apparatus. The relationship of the different species to each other was determined. A concensus tree was determined for the isolates of *V. dahliae*.

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784 - The evolution of cyanobacterial lichens inferred by phylogenetic analyses of combined mtSSU rDNA and nLSU rDNA sequence data

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Fungi associating with cyanobacteria have been found among the oldest verified fungal fossils of the Lower Devonian Rhynie chert. The extant cyanobacterial lichen associations, particularly of the Lecanorales suborder Peltigerineae, are also often claimed to include the most primitive extant lichens. It is, however, unclear if the association with cyanobacteria has arisen once or several times, if the suborder Peltigerineae as currently circumscribed is monophyletic, or if it is the sistergroup to the rest of the Lecanorales. Here, we will present preliminary results from phylogenetic analyses of two independent gene loci, including representatives of most families in Lecanorales suborder Peltigerineae, and several representatives of other Lecanoromycetes. These analyses are utilised to test several evolutionary hypotheses regarding the evolution and phylogeny of cyanobacterial lichen associations.

785 - Phylogeny of the *Gymnopus/Marasmiellus* group based on 28s nrDNA sequence

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Nuclear ribosomal large subunit sequence was collected for 89 taxa representing members of the *Gymnopus*/*Marasmiellus* group, referred to as the *Lentinula* clade in Moncalvo et al. (2002). Tissue was extracted from ^70 specimens via PCR and cycle sequencing reactions. The remaining sequences were obtained from GenBank or the fore-mentioned authors. Sequence was aligned and phylogenetic analyses were performed using PAUP. Several methods of phylogenetic analysis were utilized including weighted parsimony, maximum likelihood and neighbor joining. Bootstrap values were used to indicate structural soundness of groups represented in the tree. The aim of this study is to gather molecular evidence that will help define groups within the *Gymnopus*/*Marasmiellus* cluster that have been traditionally recognized using morphological taxonomy.

786 - Developmental similarities in *Echinosporangium transversale* and *Mortierella multidivaricata*. Early maturity hypothesis

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Mortierella multidivaricata and *Echinosporangium transversale* are related genera, and there are classified in the family Mortierellaceae, ordo Mortierellales (Voigt, Wostemeyer 2001). There are two basic features common for both genera and other species of the family Mortierellaceae -the presence of anastomoses between hyphae and the lack of the columellae. The main substantial difference concerns the morphology of the sporangia. *Echinosporangium* has non-typical sporangium of sausagous shape with spiny ends while sporangia of *Mortierella* multidivaricata are globose. Phylogenetical study (my PhD. thesis, 2000, Voigt and Wostemeyer 2001) confirm close affinity of both genera. *Echinosporangium* is located in phylogenetical tree within genus *Mortierella*. Are there any in vivo observations which can help us in understanding on one hand the phylogenetic affinity resulting from molecular methods and on the other morphological dissimilarities between sporangia? Close similarities in early ontogenetical development of asexual structures in *Mortierella multidivaricata* and *Echinosporangium transversale* were observed. The differences appear at the moment of the spores formation. Taking into account these observations and the results of molecular studies, we may propose the following hypothesis: Small numbers of mutations placed in genes for regulation of sporangium development could result in apparent morphological changes in asexual structures of *Echinosporangium*.

787 - *Peniophora* in Taiwan with simple-septate hyphae

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In this survey of Taiwanian *Peniophora* species with simple-septate hyphae five species are presented: *P. bicornis*, *P. borbonica*, *P. maliensis*, *P. reidii*, and *P. taiwanensis*. The former four species are newly recorded from Taiwan, and the last one is new to science. Description and line drawing are given for each species. Cultural and cytological studies are also provided for the five species.

788 - Diversity of *Sporidesmium* and related fungi in China

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The *Sporidesmium-Endophragmiella* complex includes dematiaceous hyphomycetes which holoblastically produce phragmosporae. This group of fungi is mostly found on dead branches or rotting wood. Only one species from literatures was known in China. However, this study showed that they widely distribute in China and a total of 147 species in 23 genera have been identified, including 37 new species and 14 new combinations. All species have been fully described and illustrated based on Chinese specimens.

789 - Revision of the genus *Septoriella* Oudem. (Coelomycetes)

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A revision of the coelomycete genus *Septoriella* Oudem. is made and 14 species are accepted. Among them, 5 are new species, *S. fusiformis* sp. nov., *S. hendersoniae* sp. nov., *S. intermedia* sp. nov., *S. latispora* sp. nov., and *S. petrakii* sp. nov.; and two are new combinations, *S. mastigospora* (Petr.) comb. nov. (syn. *Urohendersoniella mastigospora*

Petr.), and *Septoriella panicicola* (Petr.) comb. nov. (syn. *Hendersonia panicicola* Petr.). Other accepted species include *S. canadensis* Nag Raj, *S. caroliniana* Nag Raj, *S. junci* (Desm.) B. Sutton, *S. phragmitis* Oudem., *S. rockiana* (Petr.) Nag Raj and *S. thalassica* (Speg.) Nag Raj. All other species names described in *Septoriella* have been excluded from the genus and reidentified. This results in 1 new combination, *Phaeoseptaria philippinensis* (Sacc.) comb. nov. (*S. philippinensis* Sacc.). *Septoriella atrata* Sacc. is regarded as a synonym of *Septoria heterochora* Desm. In addition, one new species of the genus *Linochorella* Syd. & P. Syd., *L. cylindrospora* sp. nov., is also described. Related taxonomic problems are also discussed. A key to all accepted species in the genera *Septoriella* and *Linochorella* is provided.

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790 - DNA sequence and IGS RFLP analyses and molecular markers for identification of *Fusarium poae, F. langsethiae, F. sporotrichioides* and *F. kyushuense*

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Fusarium langsethiae was recently described to accommodate 'powdery' isolates of *F. poae*, which morphologically resemble *F. poae*, but whose metabolite profile (including toxins) is similar to that of *F. sporotrichioides*. Sequence analysis of ITS1 and 2 separated *F. langsethiae* from *F. poae*, but was unable to separate all *F. sporotrichioides* strains from *F. langsethiae* strains. Sequence analysis of ß-tubulin distinguished all four species but did not resolve them phylogenetically. The high intraspecific variability of the IGS sequences were found useful to group isolates according to their geographic origin. Analysis of all combined sequences reliably separated all species studied. Our results are in accordance with the results of the UP-PCR hybridization analysis. IGS-RFLP analysis also allowed differentiation between the closely related species. According to the phylogenetic analysis of IGS-RFLP patterns *F. langsethiae*, *F. sporotrichioides*, *F. poae* and *F. kyushuense* strains formed four well-supported clades with high bootstrap values. Based on the sequence differences in the IGS region, species-specific primers were designed for the *F. langsethiae*/*F. sporotrichioides* group and for *F. poae*. The two phylogenetic subgroups of *F. langsethiae*, found by IGS sequencing analysis, were clearly separated on the basis of size differences of the amplification products with primers CNL12/PulvIGSr (specific for the *F. langsethiae*/*F. sporotrichioides* group).

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791 - Molecular, morphological and phylogenetic analysis of *Fusarium avenaceum* / *F. arthrosporioides* / *F. tricinctum* species complex

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The great variation in the IGS region offers more potential to resolve intraspecific phylogenetic relationships, while beta-tubulin gives more reliable phylogenetic information at species level. The European *F. avenaceum* strains of main group II had identical beta-tubulin sequences with one American *F. avenaceum* strain and four European *F. arthrosporioides* strains, while *F. avenaceum* strains of main group I were closely related to two European *F. arthrosporioides* strains and to one Japanese *F. anguoides* strain. According to the combined beta-tubulin/IGS/ITS sequence tree, European *F. arthrosporioides* strains were divided into four groups. *F. tricinctum* strains formed a well-supported cluster, in which two European clusters were separated from one African isolate. In the IGS sequence tree two European *F. acuminatum* strains together with one American *F. acuminatum* strain formed a cluster, which was separate from another American *F. acuminatum* strain. The *F. acuminatum* cluster was nested within the large *F. tricinctum* cluster together with one *F. reticulatum* strain in the combined IGS/beta-tubulin tree. Several strains may be intermediate between the *F. avenaceum* / *F. arthrosporioides* / *F. anguoides* and *F. tricinctum* clusters and represent their own species. These results are partially supported by the results of UP-PCR hybridization analysis. Thus the molecular results may be helpful in future revision in the taxonomy of these species.

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792 - Clarification of the taxonomic relationhips among onion neck rot *Botrytis* spp.: *B. allii* is an interspecific hybrid of *B. aclada* and *B. byssoidea*

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Three species of *Botrytis* are recognized as associated with neck rot of onions. Due to the difficulty of distinguishing them by morphological criteria and lack of type material associated with two of the names, several synonomies have been proposed. Species may be differentiated by conidial size and shape, but the character is subtle, variable and there is some overlap. Both the smallest spored group and the largest spored group have 16 mitotic chromosomes, while the intermediate group has 32. Based on significant differences in Nei's coefficient of genetic differentiation derived from universally primed PCR (UP-PCR) fingerprints it was possible to recognize distinctions among the three nomengroups and *B. cinerea* and *B. squamosa*. Primers, designed from a sequence characterized UP-PCR fragment, were used for direct sequencing of DNA from isolates of the 16 chromosome nomengroups. Because of apparent ambiguities in the UP-PCR fragment from the 32-chromosome group, it was cloned and sequenced. Clustering show identity with the small-spored *B. aclada* and the large-spored *B byssoidea* for the two cloned molecules from *B. allii*. Further, the internally transcribed spacer rDNA (ITS) amplicons of *B. aclada* has 2 SphI restriction sites; that of *B. allii* has 1 SphI site. The cumulative data suggest that the three groups are genetically distinct from each other and that isolates *B. aclada* and *B. byssoidea* were the ancestors of *B. allii*.

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793 - Morphological and molecular characterization of small-spored *Alternaria* species

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The importance and diversity of the genus *Alternaria* highlights the need for accurate identification of species. However, many small-spored *Alternaria* isolates have been misidentified due to the use of spore size as the only identifying character. In this study eighty one isolates of small-spored *Alternaria* were segregated into morphological and pathogenic groups or species as *A. gaisen*, *A. mali*, *A. tenuissima*, *A. longipes*, *A. citri*, *A. arborescens*, *A. infectoria*, and *A. alternata*. Molecular characteristics of these isolates were determined using universal rice primer (URP)-PCR analysis, sequence analyses of nuclear internal transcribed spacer (ITS) and mitochondrial small subunit (SSU) ribosomal DNA (rDNA). Based on cluster analysis of URP-PCR fragment patterns, the eighty one isolates were segregated into distinct groups that are morphologically similar but identifiable as *A. gaisen*, *A. mali*, *A. tenuissima*, *A. longipes*, *A. citri*, *A. arborescens*, *A. infectoria*, and *A. alternata*. Based on analyses of ITS and mitochondrial SSU rDNA sequence data, there was no variability in ITS and mt SSU rDNA sequences for species among *A. gaisen*, *A. mali*, *A. tenuissima*, *A. longipes*, *A. citri*, *A. arborescens*, and *A. alternata*. However, *A. infectoria* was differ from other species at 21 and 16 nucleotides in ITS and mt SSU rDNA, respectively.

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794 - A contribution to the identification of *Trichoderma* species in Iran

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The aim of the present study is to identify *Trichoderma* species isolated from Iran. Soil samples were collected from different parts of Iran focusing on the agricultural fields. *Trichoderma* selective media and malt extract agar (MEA) were used to isolate *Trichoderma* species from the soil samples and Petri plates were incubated at 25 °C in the dark for the first 24 hours and then 12/12 (dark/ flourescent light) with the same temperature. All the cultures were purified on 2% water agar by hyphal tip method prior to morphological examination. Morphological observations were carried out on the cultures grown on 2% MEA at 20 °C under ambient laboratory conditions. Microscopic features of conidiophores and shape and size of conidia were studied and recorded 3-5 days after inoculation. Out of 350 obtained isolates, using morphological features ten species (*T. citrinoviride*, *T. longibrachiatum*, *T. saturnisporum*, *T. hamatum*, *T. harzianum*, *T. inhamatum*, *T. tomentosum*, *T. virens*, *T. asperellum*, *T. koningii*) were identified. Among the species *T. harzianum* with 150 and *T. virens* with 57 isolates were the most frequent species.

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795 - An integrated approach to the taxonomy of plant-associated *Verticillium* species

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Molecular approaches were used to re-evaluate the morphological criteria used to identify plant-associated species of *Verticillium* Nees. ITS-RFLPs divided the 31 studied strains of seven *Verticillium* species, (including the type species) into four clusters. Cluster one comprised strains of the type species, *V. luteo-album* (Link: Fries) Subramanian, cluster two *V. albo-atrum* Reinke & Berthold, *V. dahliae* Klebahn, *V. nubilum* Pethybridge and *V. tricorpus* Isaac; cluster three comprised strains of *V. theobromae* (Turconi) E. Mason & S. Hughes, and cluster four comprised strains of *V. nigrescens* Pethybridge. Beta-tubulin gene RFLPs offered a higher degree of resolution, distinguishing all seven species from each other. The highest degree of resolution was obtained from mitochondrial DNA-RFLPs that divided strains of *V. theobromae* and *V. nigrescens* into infraspecific groups. The beta-tubulin gene digested by Hae III offers a reliable way to separate the two economically important and controversial species *V. albo-atrum* and *V. dahliae*.

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796 - Species of the genus *Macentina* Vezda (Ascomycota, Verrucariaceae) as tertiary relicts of the lichen flora Ukraine

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Natural ranges of the lichens of the genus *Macentina* are concentrated in two distribution centers. The first center is located in tropical forests of Africa, where the folicolous *Macentina* species are growing. The second center is located in Europe and includes corticolous species. *M. dictyospora* is known from Sweden, Spain, Switzerland, the Czech Republic, Slovakia, and Ukraine. *M. abscondita* was registered in Great Britain, Austria, and Ukraine. *M. stigonemoides* is reported from Great Britain, France, Spain, Belgium, and Sweden. The natural habitats of corticolous species of the genus *Macentina* are connected with suboceanic and mountane regions of Europe. It gives evidence that *Macentina* species prefer humid habitats. In Ukraine, species of the genus *Macentina* are known from the Dniper and Podolia heights. These geomorphological structures have never been covered by glaciers during the Quarternary, and thus acted as refugia for species of forest vegetation, and the Tertiary flora as well. European species of the genus *Macentina* are corticolous lichens, they probably were widespread in broad-leaved forests during the Neogene. In our opinion, species of the genus *Macentina* are Tertiary relicts of the lichen flora of Ukraine. This conclusion is based on the analysis of their geographic distribution, ecology of *Macentina* species, and data of geological and palaeobotanical studies.

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797 - *Gibberella konza,* a new species from prairie grasses

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The *Gibberella fujikuroi* species complex (*Fusarium* section *Liseola* and allied taxa) is composed of an increasingly large number of morphological, biological, and phylogenetic species. Most of the known species in this group have been isolated from agricultural ecosystems, or have been described from only a small number of isolates. We have been sampling *Fusarium* communities from native prairie grasses in Kansas and have recovered a large number of isolates that superficially resemble *F. anthophilum*. We have used a combination of morphological, biological, and molecular characters to describe a new biological species, *Gibberella konza (Gibberella fujikuroi* mating population I [MP-I]), from native prairie grasses in Kansas. Although female fertility for field isolates of this species appears to be low, *G. konza* is heterothallic, and we have generated reliably female fertile mating population tester strains for this biological species. The *F. konzum* anamorph is differentiated from *F. anthophilum* and from other *Fusarium* species in section Liseola by mating compatibility, morphology, AFLP fingerprint profile, and the DNA sequence of the ß-tubulin gene.

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798 - Floristic composition of rust fungi in Tibetian Everest Himalaya

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The rust flora of Tibetan Everest Himalaya mainly consists of temperate genera and species. Among the 22 known genera (excluding form genera), *Puccinia*, *Uromyces* and *Phragmidium* are cosmopolitan; *Chrysomyxa*, *Coleosporium*, *Cronartium*, *Gymnosporangium*, *Hyalopsora*, *Melampsora*, *Melampsoridium*, *Pucciniastrum*, *Thekopsora* and *Uredinopsis* are mainly north temperate; *Blastospora*, *Miyagia* and *Pucciniostele* are typically eastern Asian; *Endophyllum*, *Phakopsora*, *Physopella*, *Pileolaria*, *Ravenelia* and *Trochodium* are tropical. Of the 155 known species, 20 (sharing 13% of the total) are endemic in Himalaya. Apart from cosmopolitan species (12%) and species restricted to China (3%) and Himalaya, the predominant floral elements are eastern Asian (27%), northern temperate (20%) and Old World temperate (8%). Nine species (6%) are disjunctive between eastern Asia and North America. No typical Central Asian species is found. The arid and frigid plateau steppe and desert in northern Tibet impede intermigration of rusts, resulting in the separation of the rust flora from that of Central Asia. Only about 10% of the total known species are tropical in affinities with the majority of these (8%) identifiable with species of general distribution in Indo-Malaysia. The lower percentage of tropical species suggests that the connexion between the rust flora and that of the tropics seems weak.

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799 - Monitoring of patulin production of *Penicillium expansum* using Fungi static 2-Deoxy D-Glucose

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Three isolates of *Penicillium expansum* which known as patulin producer were treated with three concentrations of 2-deoxy-D-Glucose. The 2-deoxy-D-Glucose which used as a post harvest disease control of *P. expansum*. The High Performance Liquid Chromatography (HPLC) data revealed that the 2-Deoxy-D-Glucose reduced patulin production on the pure culture but in the main time enhanced the other metabolites production like citrinin and ochratoxin A.

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800 - Bacteriological and mycological study of external otitis in patients referred to ENT cilinic in sari in 1999

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We decided to study the etiological agents of external otitis in summer in 78 sampling were collected from the patients who were diagnosed clinically as external otitis. The samples was performed by two sterile swabs wetted in sterile saline and transferred to laboratory as soon as possible to culture in media. The sample was inoculated on blood agar and Macconky agar incobated for 24 hours and was followed by the biochmical tests to identify the species of bacteria. Meanwhile the same sample was inoculated on saborauds dextrose agar incubated at for 48 hours to identify fungi. A wet mount with 10% KOH for morphological observation. In this study out of 101 (59 females and 42 males) cases suspected to clinical external otitis 39 (38.6%) and 23 (22.8%) cases were of bacterial and fungal respectively and 16 (15.2%) cases were of both. x test showed significant relationship between the occurrence of otomycosis and gender of the patients ($p<00.5$) which there was not significant bacterial otitis and gender of patients, in 22.7% of cases no organism was isolated but in same patients the normal flora of skin (staphyloccous epidermidis, corynebacteria and non haemolytic streptoccous) were isolated. The most common isolated bacteria was *Pseudomonas aerogenosa* in 37 (36.6%) cases and the most common isolated fungi were as following: *Aspergillus niger* in 10 (9.9%) cases yeast species in 9 (8.9%) cases and *Aspergillus fomigatus* in 5 (5%) cases.

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801 - Biological control Of aflatoxins, ochratoxin A and/or fumonisin B1 production by *Trichoderma harzianum* On corn

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The effect of *Trichoderma harzianum* (as a biological agents) on aflatoxins, ochratoxin A (OA) and/or fumonisin B1 production by *Aspergillus parasiticus*, *Aspergillus ochraceus* and/or *Fusarium moniliforme* was studied. The results indicated that the reduction of aflatoxins or ochratoxin A production by *T. harzianum* 73.48 and 93.31% respectively, while the reduction of toxins with combination of cultures were 80.54 and 97.20%; in the same order. After one week incubation of *A. parasiticus* or *A. ochraceus*, the reduction of aflatoxin B1 and ochratoxin A was 9.78 and 44.57% by *T. harzianum*, while they were 45.49% and 91.09% in the combination of both *A. parasiticus* and *A. ochraceus* incubated in the same time. The data also showed that the inhibition production of aflatoxins and fumonisin B1 by *T. harzianum* was 46.85% and 91.41%, respectively, while reduction of toxins with the combination of cultures were 87.44% and 98.04%, in the same order. Inoculation of *T. harzianum* after one week of *A. parasiticus* and *F. moniliforme* incubation was found to reduce toxins production by 22.41% and 33.23% respectively. while the reduction was 46.51% and 91.41% in the combination of both *A. parasiticus* and *A. ochraceus* incubated in the same time.

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802 - Mold development in Houston Texas following hurricane Allison

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Tropical Storm Allison transformed buildings and homes in Houston Texas into a habitat that favored mold development. High humidity brought about by the hurricane coupled with the warm temperature in Houston led to colonization of many buildings and home. Many materials inside buildings and homes were colonized by molds. Tests have shown that the quality of air in those buildings and homes was compromised. Consequently attention is being given to fungi in indoor environments. Debate is ongoing amongst mycologists, attorneys, real estate agents and insurance companies. This paper presents case and laboratory studies conducted in Houston, Texas, U.S.A.

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803 - Black spores Aspergilli in Turkish raisin

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In this study, black spores *Aspergillus* strains isolated from fresh grapes and raisins, yielded in 1999, in 20 different vineyards which contain 55 different stations in Izmir and Manisa province, were given in their detailed descriptions. These are follows: *A. aculeatus*, *A. awamori*, *A. carbonarius*, *A. ficuum*, *A. foetidus*, *A. foetidus* var. *acidus*, *A. foetidus* var. *pallidus*, *A. heteromorphus*, *A. japonicus*, *A. niger*, *A. olivaceo-fuscus*, *A. phoenicis*, *A. pulverulentus* and *A. tubingensis*.

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804 - Computer tomograph for the living trees internal inspection

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There was been realised the computer tomograph (CT) construction at the Technical Univerity in Zvolen which allows to inspect the internal structure of trees. This construction allows inspect the trees up to 18 cm in diameter but the tomograph going to the 70 cm in diameter is under construction. The CT is working in the fan beam geometry and the radioisotope source of radiation is used. The parallel beam geometry is also possible under the special conditions. The detector system is based on the CdZnTe semiconductor detector arrays. The picture of internal structure can be obtained up to five minutes. According to the state authorities approval the CT is not ecologically dangerous. The CT is portable (meanwhile 120 kg of weight) and it is working under 24 volts of DC. The reconstruction algorithms are of our own realisation and the communication software is very user friendly. With our CT we can inspect the internal structures of the living trees and we are able to detect the internal cracks, red decay, rot, root of knots and so on. The future of our construction we see in the possibilities to detect the healthy conditions of the internal structures of the tree far before their consequences are visible in outside. Next it is possible to inspect the wood quality before cutting and to be able to take special care of the high quality wood material.

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805 - A chemical comparison of two types of antifungal defence: reaction zones and branch protective zones in *Eucalyptus globulus*

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While reaction zones are actively formed in response to fungal infection of sapwood, protective zones form passively during the process of branch death. In *Eucalyptus globulus*, the protective zone appears to form across the whole branch and is rarely surpassed by invading decay fungi after branches are pruned. When living branches are pruned before the protective zone forms, invading decay often spreads into the sapwood, where it is constricted by a reaction zone. Samples of branch wood, protective zones, stem sapwood near the branch and reaction zones were prepared from 19 living or dead branches (from 5 different trees) and extracted with 70% acetone. Total phenol levels of reactions zones were almost double that of the protective zones, but both were significantly increased from the stem sapwood and branch sapwood. Phenolic compounds were identified by liquid chromatography - mass spectrometry and included hydrolyzable tannins (pedunculagin, casuarinin, methyl-di-galloylglucose, tri- and tetra-galloylglucose), epicatechin-3-*O*- gallate and a flavonone glycoside. This poster will present the trends in semi-quantitative levels of these compounds between the four tissues.

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806 - *Acacia mangium* heartrot: preliminary studies of a poor defence

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Acacia mangium is the major plantation tree species in Indonesia. Recent surveys in Indonesia (Irianto et al., unpublished) have shown that heartrot is of high incidence in areas of Sumatra and Java. Heartrot has been previously reported in other SE Asian countries as a major problem for solid-wood production. Previous studies of wounded *A. mangium* branches indicated that defence responses were poorly developed - for example no tyloses were formed. To investigate this we have studied both the anatomy and phenol chemistry of various *A. mangium* tissues. We have studied samples of heartwood (decayed, discoloured and sound) and sapwood from naturally-infected harvest-age *A.*

mangium from two sites in Sumatra. Total phenol levels were highest in inner heartwood and discoloured wood. Analysis by liquid chromatography - mass spectrometry revealed that condensed tannins and a range of flavonoids (e.g. tetrahydroxyflavonone) were mainly responsible for this increase. Studies of the antifungal nature of these extracts in bioassays will be presented as well as anatomical investigations.

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807 - Study of of onychomycosis in Tehran

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Fungal infection of the nail, also known as onychomycosis, is a worldwide problem. It is estimated that onychomycosis constitutes 15-22% of all nail disorders. It is also known that 2-5% of the adult population in Europe have onychomycosis. This problem is quite common in the dermatology clinic of the countries in the Midle East, India and the Far East. The infection rates and types of fungi involved in onychomycosis vary with conditions such as age, sex, occupation, hygiene, foot wear and several enviromental and climatic factors. This study reports the causative agents of onychomycosis in the presented patients in the section of Medical Mycology, Pasteur Institute of Iran. The 1985 patients were examined for onychomycosis. Diagnosis was confirmed by demonstration of fungi in direct (KOH 20%) and cultured examination. Onychomycosis was proven in 601 patients. 350 patients was femailes (59%) and ranging in age from 3 mounths to 84 years old. In the present study, onychomycosis was observed to be more common in females than in male. In this study Dermatophytes was 51%, Candida 46% and other molds was 3%.

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808 - Effects of selected *Penicillium* mycotoxins in food waste on pig lymphocytes

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The six *Penicillium* mycotoxins citrinin, cyclopiazonic acid, ochratoxin A, patulin, penicillic acid, and roquefortine C may be present in mouldy food and are known or suspected to be immunomodulators. The aims of our study were to determine the effect on lymphocyte proliferation of individual toxins and their combinations. We used an in vitro method that expresses the direct mycotoxin effect on T-lymphocytes and implies a simplification of the immunotoxic effect elicited from in vivo exposure. Blood samples were collected from clinically healthy pigs of 3-4 months age. The mitogen concanavallin A and pure mycotoxins were added to isolated lymphocytes and incubated, before further incubation with tritiated thymidine. Cell proliferation was measured as radioactivity ingested by the cells. Ochratoxin A and patulin were the most potent inhibitors of lymphocyte proliferation. Based on molar concentrations, these toxins were 15, 30, 40 and 65 times more potent inhibitors than penicillic acid, citrinin, cyclopiazonic acid, and roquefortine C, respectively. Examination of the cellular response of toxin combinations revealed that ochratoxin A and citrinin elicited synergistic effect. Other pair combinations showed additive, independent or antagonistic effects. As occurring mycotoxins may elicited very different kinds of interactive effects, the sum effect of mouldy food may be difficult to predict.

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809 - Occurrence of fine root pathogens in native scots pine forests of North Scotland

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Soil samples were removed from around Scots pine trees in five semi-natural and plantation forests in the north of Scotland. Pathogens were isolated by apple baiting from the soil samples and identified to the genus level using microscopy. Genera identified included species of *Pythium*, *Phytophthora*, *Fusarium* (*Nectria*) and *Verticillium*. Further characterization of isolates using ITS PCR and sequencing identified several potential pathogens, including *Pythium ultimum* var. *ultimum*, *Pythium undulatum*, *Phytophthora cinnamomi* var. *cinnamomi*, *Nectria gliocladiodes*, *Nectria galligena* and *Verticillium rexianum*. Different pathogen species were not present in all forest areas sampled. In tests of virulence on Scots pine seedlings, isolates had varying effects on root system development in both in vitro and potted plants. The apparently widespread occurrence of fine root pathogens in native Scots pine forest areas suggests that these organisms may have a role in fine root turnover dynamics in forest ecosystems and could impact on overall tree health and regeneration potential in different microclimates.

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810 - Acute toxicity of two toxigenic strains of fungi isolated from poultry feed on locally produced saudi arabian fertilized eggs and chicks

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Acute toxicity assays were performed for two toxigenic strains of fungi, *Aspergillus parasiticus* and *Penicillium viridicatum* isolated from poultry feed (sorghum, millet) on fertilized eggs and chicks. Two to three day old chicken embryos were inoculated with either standard toxin or spore suspensions of the toxic fungi. The most frequent malformations were observed after administrating the spore suspension. These malformations were characterized by vascular fragility, hemorrhage, and defects of the limbs. Results demonstrate the possibility of toxigenic fungi growth inside the egg, and consequent production of mycotoxins at incubation temperature. Heavy fungal growth took place in the air sac area, spreading to the shell membrane, and, to a lesser extent, the surrounding albumen. In CHEST assays, four-week-old chicks were given feed contaminated with *A. parasiticus*, or water containing a fungus filtrate. Chicks receiving a 1:1 ratio of contaminated to normal feed had the highest mortality rate (100%). Postmortem examination showed that all individuals who died suffered hemorrhages of the liver and liver enlargement.

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811 - The distribution of trichothecenes in shell and kernel of oats

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Twenty-nine samples of oats were collected from a mill in Moss, Norway (Regal Mølle) from the harvest of 2000. This mill produces mainly products for the food industry. All the samples were divided into shells and kernels. The grinded samples (25 g) were extracted with acetonitrile / water (84:16, v/v). After filtration, the extracts were cleaned on a Mycosep #225 column (Romer Labs), evaporated and derivatized with pentafluoropropionic acid (PFPA) before they were analysed on GC-MS. In the shells, HT-2 toxin was found in 90% and T-2 toxin in 66% of the samples. The median concentrations of HT-2 and T-2 toxin were 181 and 56 µg/kg, respectively. The highest concentrations of HT-2 and T-2 toxin were 711 and 196 µg/kg, respectively. Deoxynivalenol (DON) was detected in a few samples, with the highest level 220 µg/kg. In the kernel samples, no trichothecenes were detected (detection levels for all trichothecenes in the trial was 20 µg/kg). The results indicate that the main amounts of trichothecenes are connected to the shell part of the oat. As oat shell is of minimal use, the problem with mycotoxin contamination in oats seems to be a smaller problem than first assumed. The investigation will be continued.

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812 - Exploitation of the mycoparasitic fungus *Trichoderma harzianum* against plant pathogens

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Trichoderma harzianum is a worldwide soilborne fungus belonging between anamorphic fungi. It is a facultative parasite of a wide spectrum of fungi, but it is able to live as saprophyte too. The manifestation of its antagonism display as support competition, parasitism and antibiosis. Biofungicide Supresivit conteining konidia of *T. harzianum* was registrated in the Czech republic in 1994. It is licenced for protection of ornamental plants, ornamental and forest trees and peas. It could be applicated on seeds before sawing, on roots of sedlings or as watering or spraying. Commercial prepared mixture the biopreparation and granulated mineral fertilizer presents the new way of its introduction to plant enviroment. This method of application had the positive effect on 5% rising of yields of significant field crops (winter wheat, spring barley, winter, maize, and potatoes). The qualitative parametres of crop yield was slightly better (gluten conteins of winter wheat, oiliness of winter, starchiness of potatoes) or coincident (oily acid conteins of winter cole-seed, protein conteins of spring barley, gluten index of winter wheat) in comparison with yields on check parcels. The important result is, that occurrence of mycotoxines is decreased. The Supresivit suppress grey mould too. The regulal spraying of strawberries increased weight of healthy fruits and decreased the attack of grey mould even by strong infection press in 2001.

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813 - Impact of essential oils on growth and ochratoxin production by *Aspergillus ochraceus* and *Penicillium verrucosum* on a wheat-based substrate

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The effect of water activity (a_w; 0.901-0.999) on growth of the ochratoxigenic species *Aspergillus ochraceus* and *Penicillium verrucosum* (3 strains) was evaluated on a wheat-based substrate. Water relation profiles were similar for all strains and species. Optimum growth was observed at sub-optimal a_w levels (0.95-0.98). An in-vitro initial screen of a range of 24 essential oils on inhibition of spore germination and mycelial growth was made for all strains at 25 °C and 0.995 a_w. Of the essential oils screened, bay leaf (west Indian), cinnamon leaf, clove bud, clove leaf (cleaned), lemongrass and thyme were found to be the most effective. A detailed screen of the most effective essential oils was made using a 2% wheat agar modified with glycerol to a range of water activities (0.901-0.999).

Essential oils were incorporated at different concentrations (0; 50; 100; 250; 500; 1000 ppm). Three isolates of each species were inoculated and colony growth was periodically measured for 21 days at two temperatures. At sub-optimal concentrations, some essential oils were found to stimulate growth. Ochratoxin production was modified by the different treatments. Acknowledgement: This work is part of the EU project 'Prevention of ochratoxin A in cereals' (FAIR QLK1-CT-1999-00433).

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814 - Degradation of lignocellulose by acellulolytic strains (c-) of *Pleurotus ostreatus*

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The main obstacle for bioutilization of lingocellulose by-products is lignin but It can be degraded by white-rot fungi like the edible mushroom *Pleurotus ostreatus*. This fungus is cultivated on agricultural and forestry wastes but wild strains produce a simultaneous degradation of polysaccharides and lignin. Acellulolytic strains (C-) of *Pleurotus ostreatus* were bred from acellulolytic mutants to promote a selective degradation of lignin. Commercial and acellulolytic *Pleurotus* sp. strains were cultivated on a commercial substrate prepared with fermented straw. Changes in substrate composition were followed during the whole production cycle. Lignin, glucan, xylan and ash content were determined at spawning (t=0), at the end of the incubation period (t=1) and after the first flush of mushrooms (t=2) Ash content in substrate increased with all strains when substrate was fully invaded and after the first crop of mushrooms indicating substrate degradation. Highly significant differences in lignin, glucan and xylan degradation were observed. Acellulolytic strains (C-) produced a larger degradation than commercial strains. However, acellulolytic strain 71x512(C-) showed identical lignin and xylan degradation as commercial strain K8501 and its glucan degradation was also identical to that of another commercial strain, K1508. Remarkably, it was observed with all strains that production of mushrooms increases as xylan degradation decreases.

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815 - Virulence of soil borne pathogenic fungi isolated from forest areas and nurseries in north of Scotland on scots pine and common alder

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Soil borne fungi isolated from forest areas and nurseries in North east of Scotland using baiting techniques, were identified using classical taxonomy and molecular methods (PCR amplification of ribosomal ITS regions; restriction digestion; sequencing of PCR products) as *Fusarium lateritium*, *Fusarium tricinctum*, *Phytophthora cinnamomi*, *Pythium ultimum* var. *ultimum* and *Rhizoctonia* binucleate (*Ceratobasidium* sp.). Virulence was tested *in vitro* on young seedlings of *Pinus sylvestris* and *Alnus glutinosa*, and Koch's postulates fulfilled through reisolation of the pathogens and confirmation of fungal penetration into host tissues. Root growth was measured using the Winrhizo program, and dry weights recorded. Symptoms on aerial parts were assessed 8, 12 and 48 days after inoculation using a categorical scale from 0 (healthy) to 5 (damage > 76%). *Fusarium* spp. caused significantly different ($P< 0.01$) symptom intensity on both host plants. However, no significant difference in root growth was found between treatments and control ($P < 0.05$). Highest mean root length, numbers of root tips and forks, volume and surface area on *Alnus glutinosa* occurred in plants inoculated with *P. cinnamomi* and *Pythium ultimum*.

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816 - Antifungal activity of natural antimicrobial compounds incorporated in to cyclodextrin for slow release in food packaging systems

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The antifungal effect of spices has been known for centuries. Examples are the use of hobs in beer making and the use of myrrh and thyme in mummification. Recently attention on using essential oils from spices as antimicrobial agent in food has grown. The effects of essential oils are strongly depended on application method, storage conditions, source and age of the oils and of course on the microorganisms likely to colonise the food product. In this research project we are investigating if antimicrobial compounds from spices and herbs can be used in food packaging systems either as a surface coat or integrated into a film. These compounds are often too volatile and insufficiently heat stable to be used directly. Incorporation of these active compounds into cyclodextrin (CD) can radically improve the heat stability of these compounds and to some degree control their release into the packaging atmosphere. We will here report our findings from studies of antifungal effect of selected active agent from essential oils incorporated into cyclodextrin, as compared to the effect of the corresponding pure essential oils. The active agents that were used were allyl-isothiocyanate (AITC) from mustard oil, citral from lemongrass oil and thymol from thyme oil. The moulds, *Penicillium commune*, *P. roqueforti* and *P. nalgiovense*, all associated with cheese, were used as test organisms.

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817 - Dimmeric naphtoquinone metabolite aurofusarin - new mycotoxin produced by *Fusarium graminearum*

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Fusarium species of fungi produce a range of mycotoxins (trichothecene, zeralenone and fumonisins). There is a group of pigments which are produced by *Fusarium* species and they have been ignored until recently. A comprehensive work conducted by Kotyk (1999) with such a pigment, aurofusarin, clearly has shown that this compound could be included into the list of important *Fusarium* mycotoxins. A series of our experiments on effect of aurofusarin on quails showed that there was no toxicity signs when the compound was fed at a level of 26.4 mg/kg. Egg production was decreased, but body weight egg weight and yolk/albumin proportions did not change. There were negative changes in immune system of quails (titre antibody to Newcastle disease significantly decreased and the spleen was reduced in size). Antioxidant system of the adult quails was compromised as a result of aurofusarin consumption. Histochemical studies revealed degenerative changes in kidney (degeneration and resorption of the glomeruli). The ovary was brown in colour with a decreased weight and number of follicles, and liver was enlarged with haemorrhages. The egg yolk colour was changed from yellow-orange to brown-greenish. Aurofusarin decreased DHA proportion in egg yolk lipids and increased linoleic acid concentration. Concentrations of vitamin E, carotenoids and vitamin A in the egg yolk also decreased as a result of aurofusarin supplementation. Fertility and hatchability of aurofusarin enriched eggs was decreased.

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818 - Traumatic oil glands induced by pruning in the wound-associated phloem of *Eucalyptus globulus*: chemistry and histology

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The natural occurrence of oil glands in various organs such as bark and leaves is well established as a characteristic of Myrtaceae, but this is the first reported case of traumatic oil glands induced in response to wounding. The new phloem enveloping the wound, which was formed in the two years following pruning in 5-year-old *Eucalyptus globulus*, was morphologically distinct from healthy stem phloem. Histological examinations revealed this wound-associated phloem to be largely composed of secretory cavities similar in appearance to oil glands. Subsequent analysis of the wound-associated phloem extracts by GC-MS confirmed the presence of volatile terpenes and phenols. The total oil content determined for wound-associated phloem extracts was significantly higher (>50 times) than for healthy stem phloem extracts. The identities of twenty-six components of the oil extracts and their approximate relative abundances are presented. Implications of the role of terpenes as an inducible secondary metabolite in tree wound responses are discussed.

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819 - Host response to natural infection by a stem canker (*Cytonaema* sp.) in the aerial bark of *Eucalyptus globulus*: a preliminary report

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The chemical and anatomical host responses to natural infection by a *Cytonaema* sp. in the aerial bark of 3-year-old *E. globulus* plantation trees were examined for the first time. The lesion margin (LM) of the canker-infected bark was characterized by the formation of a layer of dark extractives visible to the naked eye. Chemical analysis of the LM by GC-MS, HPLC-UV and HPLC-MS using negative ion electrospray ionisation indicated the presence of a range of compounds including hydrolyzable tannins, proanthocyanidins, flavonone glycoside, formlyated phloroglucinol compounds and volatile terpenes. These compounds were either undetectable in healthy tissue or else present at significantly lower concentrations than in the LM. The LM of the canker-infected bark was morphologically distinct from healthy phloem, its characteristics varying depending on severity of canker infection. In superficial infections (in which only the phloem was affected), three types of LMs were observed i). a continuous ligno-suberized boundary zone of multiple layers positioned between polyphenolic parenchyma cells ii). a boundary zone comprising of only polyphenolic parenchyma cells derived from the de-differentiation of pre-existing parenchyma cells iii). a combination of i and ii. In cases of severe canker infections in which the vascular cambium had been killed, the new phloem formed subsequently contained traumatic oil glands in addition to the responses observed for superficial canker infections.

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820 - Breeding bread wheat with multiple resistance and high yield for Ethiopia

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Yield of bread wheat (*Triticum aestivum*) in Ethiopia is poor. Five leaf diseases of fungal origin are most harmful: stripe rust (*Puccinia striiformis*), stem rust (*P. graminis*), leaf rust (*P. dispersa*), *Septoria tritici* leaf blotch, and tan spot (*Pyrenophora tritici-repentis*). 'Bread for the World' subsidized a project for the development of new genotypes with better resistance, higher yield and regional adaptation. *Aegilops tauschii*, a natural diploid progenitor of hexaploid bread wheat, is employed as alien source for new resistances against the different diseases. *A. tauschii* derived resistances are exploited by bridge crosses with tetraploid *T. durum*, embryo rescue culture and several bread wheat crosses. - For multiple resistance, a single plant selection until F7 is indispensable. Further development is based on a shuttle between greenhouse work (resistance selection and further crossing) and field tests. Top lines are multiple resistant, and yield is increased by 20 to 30% (average from 5-6 replicated experiment station trials). Regional differences in yield are striking. Two new varieties were already released.

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821 - Saxicolous lichens and rock-inhabiting fungi on different medieval monuments in Aosta Valley (Italy)

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Microorganisms colonize all types of cultural artefacts causing biodeterioration, but little is known about the species involved. Among these, it seems nowadays that dematiaceous meristematic fungi are more widespread than previously thought. Saxicolous lichens and rock-inhabiting fungi on three different Italian medieval monuments have been investigated. The collecting sites are located in Aosta Valley (Western Alps), an area with a somewhat continental climate, at an altitude of about 1000m above sea-level. The monuments were built in XI-XIV century, with local different stones belonging to magmatic and metamorfic rocks, weathered and deformed to different extent. The stones, with the organisms to sample, were observed directly. Specific cultural medium for meristematic fungi was used. Statistical analysis has been performed. Six different lichens were identified, two of them rare for this mountain area. All of them grow on subneutral or acid substrata, in sunny, rather xerophytic, sites with weak eutrophication. A total of 10 micromycetes taxa was identified; besides white yeasts, mycelium with clamps, dematiaceous fungi with meristematic growth and mycelia sterilia were present. Eleven different types of stone were recognized. This preliminary data would suggest that lithic substrate is not selective for fungal communities living on the rocks. At the moment an association between lichen and fungal population does not seem to be significant.

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822 - Does the plant defensin-like peptide SPI1 from the gymnosperm Norway spruce protect seeds and seedlings against pathogenic fungi?

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Plant defensins are thought to protect germinating seeds, seedlings and plants against pathogenic fungi. However, the detection, developmental accumulation and cellular location of the corresponding protein in a gymnosperm have not been reported before. On this background and based on the predicted SPI1 amino acid sequence, antibodies were raised to detect and localize the putative plant defensin protein in the conifer Norway spruce [*Picea abies* (L.) Karst.]. The purpose was to detect and localize the 5kD SPI1 protein in seeds and during development, and to study the local and systemic effect on its accumulation after infection with three different pathogens, in young Norway spruce seedlings. The pathogenic fungi used for the infection studies were *Heterobasidion parviporum*, *Ceratocystis polonica* and the pathogenic oomycete *Pythium dimorphum*. The accumulation of the SPI1 protein during root development and after infection is described using immunoblotting. We also report that the putative plant defensin SPI1 is detected by antibodies on *Pythium dimorphum* hyphae invading the root cortex, suggesting a specific interaction between the SPI1 protein and hyphae.

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823 - Morphological and pathological variations of the Indonesian *Cochliobolus heterostrophus*

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Morphological variations of *Cochliobolus heterostrophus* (Drechsler) Drechsler collected from different places of Indonesia including corn areas in Bali, Java, Kalimantan, Sulawesi, and Sumatera were investigated microscopically. This mainly included observation on colony colour, conidial size and septa, and ascal size although variations in hyphae and conidiophores were also examined. All of the fungal isolates were grown in Petri dishes containing complete medium. Pathogenicity tests of the isolates were subsequently established. The experiments that involved different corn varieties, both local and introduced, were conducted by exposing all corn varieties to each of the different isolates of *C. heterostrophus*. Disease symptoms were recorded on a daily basis. Data collected from the present study indicated that morphological variations, including number of septa and size of conidia, among Indonesian isolates of *C. heterostrophus* existed, an indication that Indonesian *C. heterostrophus* might have originated from different ancestors. In contrast, pathogenicity of each isolate measured by disease incidence on several corn varieties was not significantly different. Disease severity of the corn varieties inoculated with each isolate was also relatively the same. The results imply that the corn varieties tested possess potential risks of southern corn leaf blight outbreaks.

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824 - Diagnosis of cryptic microbes based on ITS2 rRNA secondary structure information

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Conventional techniques of isolating and identifying fungi or other cryptic microbes are time-consuming and expensive, limiting their utility for rapid and widespread surveys. We invented a novel method for detection and identification of microbes including those never before isolated or identified. This approach relies on analyses of the internal transcribed spacer 2 (ITS2) rDNA sequences - highly variable regions flanked by the 5.8S and 28S ribosomal subunits within nuclear DNA. After determining secondary structures of ITS2 rRNAs we identify nucleotide motifs between 20 and 50 nt in length within each primary sequence. These signature sequences, readily defined and present in high copy number, allow taxonomic diagnoses of known and unknown microbes from various substrates including agricultural, medical and ecological samples. Such sequences are readily adapted for use with probing techniques including PCR, microarray assays or other molecular detection methods.

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825 - First report of *Coniothyrium* stem canker on *E. camaldulensis* in Ethiopia

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During a survey of *Eucalyptus* diseases in Ethiopia, a serious stem canker disease was discovered on *E. camaldulensis* trees at several localities in the South and South Western parts of the country. The disease is characterised by the presence of discrete necrotic lesions, stem cankers, cracking of the stems, production of kino pockets in the wood, as well as malformation of stems. These symptoms are similar to those caused by *Coniothyrium zuluense* in South Africa. The aim of this study was to positively identify the causal agent of the disease in Ethiopia. This was achieved by sequencing the ITS region of the rRNA operon for a representative set of isolates. Sequences for the Ethiopian isolates were compared with those from authenticated isolates collected in South Africa, Thailand and Mexico. Based on these data, the Ethiopian isolates were shown to group more closely with those from South Africa, than with those from other areas. This study represents the first report of *C. zuluense* and its associated disease in Ethiopia. Currently the disease is causing considerable losses in yield and quality of timber and it also impacts negatively on the lives of subsistence farmers. We are currently studying additional isolates of the fungus and utilising sequences of different genes to study the phylogenetic relationships between *C. zuluense*, from a wide range of countries.

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826 - Enzyme mediated infection of host *Pinus* short lateral roots by *Tricholoma matsutake*

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Tricholoma matsutake (Ito et Imai) Sing. (Matsutake) is an edible mycorrhizal basidiomycete highly prized in Japan for its culinary and medicinal properties and historical social significance. However, the natural supply of Matsutake in Japan is in decline and domestic demand exceeds supply. Consequently, the development of a viable commercial artificial cultivation system for this mushroom is of prime concern. In both natural conditions and artificial culture, Matsutake forms morphologically atypical ectomycorrhizas on host *Pinus* short lateral roots. Furthermore, aspects of the mushroom's nutrition are unusual and it is this atypical interaction which must be understood in order to devise an artificial cultivation system. Here, we present evidence of host infection and the parallel ability of *T. matsutake* to form a range of plant cell wall degrading hydrolytic enzymes in artificial culture. The hydrolytic enzymes tested were detected over a range of activities but the majority peaked after 2 or 3 weeks of incubation. Conversely, xylosidase and glucosidase enzymes were detected in comparatively high activities which did not peak but rather increased in activity over the duration of the assay. The significance of the hydrolytic enzymes produced in terms of Matsutake ectomycorrhizal establishment, development and observed morphology is discussed.

827 - Molecular probe development for tree root associated *Rhizoctonia* sp. and *Suillus bovinus* and detection by Southern dot blot and liquid hybridization

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Specific rDNA ITS (internal transcribed spacer) targeted probes for detection of different conifer root associated *Rhizoctonia* strains and *Suillus bovinus* were developed by comparing two different Southern (dot blot and liquid) hybridization methods. Probes (124-151bp) specific to six different *Rhizoctonia* strains (uninucleate strain 263, binucleate strains 268, AV-2, 251, 266, and 269 and multinucleate *R. solani*) and one specific to *Suillus bovinus* and a positive control probe were developed using pure culture DNA. Shorter (20-25 bp) oligonucleotide probes specific to strains 263, 268 and *Suillus bovinus* were developed in order to obtain more specificity and for used in liquid hybridizations. Total fungal DNA was used as a target with longer DIG (digoxigenin) labeled probes, but in biotin labeled oligonucleotide probes the target DNA was PCR amplified ITS (ITS1, 5.8S, ITS2). The optimal hybridization temperatures obtained from dot blots also gave the strongest signals in liquid hybridization protocol, although low temperature washing (30-35 °C) ocassionally affected to the specificity. We found the liquid hybridization protocol more useful when probing unknown target DNA, as it resolves targets using combined probe hybridization and ITS length polymorphism by resolving fragments differing by 5 bp in a sequencing gel (ALF express).

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828 - Ethnomycological knowledge and ecology of *Phlebopus sudanicus* in Burkina Faso

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The use and the ecology of *Phlebopus* cf *sudanicus* is examined in this study undertaken in the Reserve of Hippopotamus pound of Bala (Burkina Faso) and its vicinities. Fixed questions regarding ethnomycological knowledge were submitted to 180 native informants from 6 villages. Collecting field works toward the forest were organised to recognise the mean habitats of this fungus. It appears that a total of 98% of the informants recognise this species as being edible. Two kinds of habitats of *Phlebopus sudanicus* have been found: under *Piliostigma thonningii* (Caesalpiniaceae) and *Mitragina inermis* (Rubiaceae) during the fieldwork.

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829 - Genetic diversity of fungi associated with seeds of tropical forest trees (*Podocarpus falcatus* and *Prunus africana*)

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The association and interactions between plants and fungi have long been observed and studied. However, the knowledge about the diversity of the fungal flora and the nature of their interactions with their hosts has been very scarce in the area of tropical forest tree seeds. In this study, matured fruits of *Podocarpus falcatus* and *Prunus africana* were collected directly from the crowns and from the ground just under the mother trees in March 2000 and May - June 2001. Seed collection was carried out at different forest sites in Ethiopia. Fungi were isolated from fresh fruits, and from seeds with and without seed coat. In all cases, surface sterilised and unsterilised seeds were plated on standard growth media. All in all 150 fungal isolates belonging to 25 genera have been identified by morphological and molecular methods (internal transcribed spacer- ITS sequence analysis) at least to the genus level. Most of the isolates were ascomycetes and a few were basidiomycetes. Some of these fungi are putative seedborne pathogens that will be studied further.

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830 - Biocontrol of the Canada thistle (*Cirsium arvense*) with fungal pathogens

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In order to develop a mycoherbicide for biocontrol of *Cirsium arvense* (L.) Scop. (Canada thistle), fungal pathogens were tested, both singly and in combination, in semi-field pot experiments in the course of two vegetation periods. Isolates were selected from fungi previously isolated both as pathogens and endophytes from *C. arvense* and subsequently tested for their virulence. The fungi inoculated singly were *Phoma destructiva*, *Phoma hedericola*, *Phoma nebulosa*, M. sterila isolate 1.4a-4 and the rust, *Puccinia punctiformis*. Disease severity was evaluated according to disease symptoms (degree of chloroses, necroses, macerations) and various parameters of growth and development, i.e. length and death rates of main and secondary shoots, inflorescences, seed production and germination, wet and dry weights of shoots and roots after harvest. With the exception of *Puccinia punctiformis* (local infections) all of the isolates applied singly, negatively influenced all measured parameters, those of wet and dry weights of roots and shoots as well as the number of inflorescences, significantly. A combined inoculum of *Puccinia punctiformis* and *P. hedericola* was less effective than *P. hedericola* inoculated singly. The most effective inoculum was an inoculum mixture of the four perthotrophs. Further experiments should concentrate on formulation of the pathogens and application in field experiments.

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831 - Real-time PCR procedure to reliably detect and quantitate the pathogen *Heterobasidion parviporum* in infected spruce tissue culture clones

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One of our main interests is to learn about the molecular basis of host defense responses, using the coniferous host Norway spruce infected with the pathogen *Heterobasidion parviporum* as the experimental system. This basidiomycete and the closely related pathogen *H. annosum* are the major root rot causing pathogens in conifers. To screen host material for differential resistance towards *H. parviporum*, it is a necessity to quantify the fungal colonization of the host tissues. Therefore, we aimed to develop and compare the sensitivity of a real-time PCR to an ergosterol based method for determining the rate of colonization. We developed a quantitative multiplex real-time PCR procedure that reliably detecting down to 1pg *H. parviporum* DNA and 1ng host DNA. There was a very high correlation between the fungal-biomass/total-biomass and fungal-DNA/total-DNA rankings obtained with ergosterol and real-time PCR, strengthening the credibility of both methods. The results indicate that this real-time procedure can be a useful method to screen different spruce material for their relative resistance to the pathogen *H. parviporum*.

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832 - A TaqMan^{TM} real-time PCR assay for detection and quantification of T-2/HT-2 toxin producing *Fusarium* species in cereal matrices

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Fusarium sporotrichiodes and *Fusarium langsethiae* Torp & Nirenberg ined. are known producers of T-2/HT-2 toxin. However, these taxa may easily be confused with other taxa of *Fusarium*, e.g. *F. poae*, that very rarely have been reported to produce the toxin. The toxin is one of the most important mycotoxins in cereal products in the Norhern part of Europe, and it is therefore considered to be important to detect and quantify the presence of producers of the toxin. Based on partial translation elongation factor 1á gene sequences and phylogenetic analyses, we have identified diagnostic sequence motifs and successively developed a quantitative real-time PCR assay for detection of these two taxa in cereal matrices, using TaqMan^{TM} chemistry. The assay and its performance will be presented.

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833 - A multi-target environmental approach to control growth and mycotoxin production by *Fusarium* species using essential oils and antioxidants

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This study investigated how *Fusarium culmorum* and *F. graminearum* isolates from the UK are affected by a_w, temperature, and fungicidal agents. The combined effects of these different variables were also investigated. In addition to how the morphology and ecology of the species is affected by the environmental conditions, the effect on mycotoxin production (in particular deoxynivalenol) was also determined. Essential oils and antioxidants were screened for antifungal properties in vitro using clearing zone assays and temporal growth studies. The five most effective agents identified by the in vitro screen were tested further in situ on irradiated wheat grain under different environmental conditions and a_w levels. Growth rates of *F. culmorum* and *F. graminearum* were significantly effected by temperature,a_w and antifungal agent. Efficacy of essential oils and antioxidants agents at inhibiting fungal growth was dose and type dependent. At least 500 ppm was required for significant inhibition of growth. Cinnamon and clove essential oils were the most effective growth inhibitors of the five agents across all the conditions. Of antioxidants, PP and BHA were most effective. The effect on DON and Nivalenol mycotoxins was variable with effects better at 500 ppm of essential oils than 100 regradless of a_w level. The potential for using these alternative food grade preservatives in stored cereals will be discussed.

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834 - A study of the trypsin genes in *Metarhizium*

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Metarhizium anisopliae var. *anisopliae* produces 3 trypsins among a variety of other cuticle degrading enzymes. As well as animals, the trypsin family of *Metarhizium* has homologs in streptomycetes and four related pathogenic pyrenomycetous ascomycetes. The limited distribution of trypsins in a small group of related fungi is consistent with

their being derived from an animal or streptomycete source via horizontal gene transfer. To investigate trypsin distribution, fragments from genomic DNA of 43 representative fungal species were amplified using degenerate primers. The fragments contain a highly conserved and diagnostic area for trypsins that includes the active site His and Ser residues. Trypsin-like genes were absent from most ascomycetes and had a very patchy distribution in zygomycete and basidiomycete fungi. The results were analyzed using sequence composition, maximum parsimony, maximum likelihood and distance methods. They show that there are several lineages of trypsins in fungi and that multiple gain and loss events happened during the evolution of this gene family.

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835 - Detect and diagnosis *Monosporascus cannonballus*, the causal agent of muskmelon root rot/vein decline, by PCR method

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In Taiwan, root rot and vine decline became a growth-limited factor of muskmelon and watermelon since 1994. The causal agent was *Monosporascus cannonballus*, a soilborne pathogen. This species is notoriously difficult to identify, it lacks a conidial stage, perithecia form only after 3 to 4 weeks in culture, and the ascospores do not germinate under standard laboratory conditions. The isolates often fail to produce perithecia in culture, making their identification impossible. We detected the pathogen using a PCR-based method, which developed by Lovic et al. A 430-bp DNA product was amplified from *M. cannonballus* isolates of Taiwan, and the product was obtained from symptomatic muskmelon and other cucurbitaceous plants collected from fields. No PCR product was obtained from the plants without symptoms. The fungus could be detected during the growing seasons. The method is being used to detect and monitor the pathogen on different cucurbitaceous plants and varieties for the selection of the disease resistance/tolerance rootstock.

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836 - The influence of the biopreparation Supresivit (*Trichoderma harzianum*) in the mixture with inorganic fertilizers on cereals

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The influence of the treatment with the biopreparation Supresivit (*Trichoderma harzianum*) in the mixture with mineral fertilizers (NPK, LAV - ammonium nitrate with limestone) was studied. The optimal dose of the biopreparate was 0.5 g per 1 kg of the fertilizer. The infestation of leaf and ear pathogen of winter wheat (septorioses and fusarioses) and on spring barley (rhynchosporioses and helminthosporioses) decreased. The yield was also increased (approximately about 5-8%). It was due to the decrease of leaf and ear spot fungal diseases.

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837 - Mycotoxines of the genus *Fusarium* on spring barley and the protection against them

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Spring barley (varieties: Akcent, Amulet, Kompakt, Nordus, Olbram and Tolar) was cultivated after pre-crops: sugar-beet, cereals and Maize. We infected artificially named varieties with the isolate of *Fusarium culmorum* which produced deoxynivalenol and other trichothecene mycotoxines. After artificial infection we studied the spectrum of the genus *Fusarium*. Introduced *Fusarium culmorum* was isolated only in little amount. The genus of *Fusarium tricinctum* prevailed, less occurrence had the genus of *Fusarium poae*. Some strains of *Fusarium culmorum* differed in the production of mycotoxines in the mycelium and in young plants (age 5 years). Maximal yields were evaluated after pre-crop of sugar beet (6.2 t/ha) the second one was the maize (5.25 t/ha) and the last there were cereals (4.39 t/ha). The treatment with the fungicide Folicur BT increased the yield about 25.7%, other one with Charisma increased the yield about 19.8 (application in the phase of flowering). The lowest occurrence of contaminated grain after pre-crop of sugar beet (20.5%), the highest one was after pre-crop maize (34%).

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838 - Diversity of *Fusarium* spp. isolates from the wheat and rice growing areas of rice-wheat cropping system of Punjab, Pakistan: Isolation, pathogenicity and RAPDs analysis

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Surveys were conducted for the assessment of leaf and root diseases of wheat and rice crops in main rice-wheat cropping areas, namely Gujranwala, Sheikhupura, Sialkot

and Narowal districts of Punjab. Assessment of prevalence and disease intensity of leaf and root diseases was done. Leaf and root fungi were isolated and identified. From leaf and root samples *Fusarium* spp. were isolated. Pathogenicity experiments were conducted on the two commercial varieties of rice and wheat. *Fusarium* spp. were pathogenic on wheat and rice varieties. Spore suspension method was used for the evaluation of virulent isolates of *Fusarium* spp. After 30 days the diseased roots were platted and fungi were re-isolated and compared with mother culture for the confirmation of Koch Postulates. After pathogenicity the Random Amplified Polymorphic DNA (RAPD) technique was used for identification and differentiation of *Fusarium* isolates. For plant pathogenic fungi, RAPD analysis has been used to analyze genetic variations or distinguish races in *Fusarium* isolates. 100 strains were studied using a RAPD analysis with different decamer primers. All the primers produced polymorphic amplification patterns. The results were analyzed by Phylip programme. From this survey it was concluded that *Fusarium* was more prevalent fungi.

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839 - Protection of *Lepidium sativum* against phytopathogenic fungi *Pythium ultimum* by inoculation of nonpathogenic *Fusarium* isolates

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Two nonpathogenic *Fusarium* strains, Ls-F-in-4-1 and Rs-F-in-11, induced the resistance in *L. sativum* against pathogenic fungi, *Pythium ultimum*. These *Fusarium* strains caused the increase of the content of antifungal compounds and its precursor, isothiocyanate and glucosinolate, in roots of the host plants. The resistance of *L. sativum* against *P. ultimum* may be due to the increase of isothiocyanate content induced by the inoculation of *Fusarium* fungi. The increase of the content of isothiocyanate occurred regardless of the abilities of inoculated fungal strains whether they could hydrolyze glucosinolate to isothiocyanate or not, so these changes seemed to be the defense responses of host plant. Ls-F-in-4-1, a *Fusarium* isolate which showed myrosinase activity, inhibited the growth of *P. ultimum* on agar medium containing glucosinolate. This isolate would hydrolyze glucosinolate to generate isothiocyanate in tissue or rhizosphere of host plant and inhibit the growth of pathogenic fungi consequentially. This isolate may have another protection mechanism in rhizosphere of *L. sativum*, in addition to the ability to cause the increase of antifungal compounds in the roots of host plant.

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840 - Opportunistic mold development in controlled environment agriculture

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Hydroponically grown crop plants are being considered by NASA for air revitalization and food production in space. Hydroponic cultures can be infected by opportunistic molds, which proliferate due to the humid environment and warm temperature inside the growth chambers. This paper reports mold development in different hydroponic cultures. Fungal isolates include *Pythium aphanidermatum*, *Pythium dissotocum*, *Alternaria* species, *Cladosporium herbarium*, *Penicillium* species, *Aspergillus* species, *Rhizoctonia* species and *Fusarium* species. The results are discussed in terms of impact of pathogenic and opportunictic molds on plant growth.

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841 - Inhibition of sexual mating of *Ustilago scitaminea* by bacterial extracts

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Ustilago scitaminea is an important pathogenic fungus of sugarcane which causes culmicolous smut disease and results in severe losses every year. Pathogenic infection of the fungus is caused by dikaryotic mycelium which is produced after mating of non-pathogenic, yeast-like sporidia that derived from germination of teliospore on the germinating nodes of sugarcane. Sexual mating is a short period that separates the yeast-like sporidia and the dikaryotic mycelium growth phases and is a potential target for developing new approaches for disease control. To explore the possibility, we isolated teliospores from the infected sugarcane and confirmed that the sporidia of *U. scitaminae* can be classified into two mating types. We have screened more than 1400 bacterial isolates for mating-inhibition phenotype. Among them 16 were found to secrete substances which inhibit mating or hyphae growth. Preliminary taxonomic analysis suggests that they belong to 10 genera, including *Bacillus* sp., *Chryseobacterium* sp. and *Filibacter* sp. The inhibitors from the three species have been extracted and showed to be heat-resistant and are of low molecular weight, suggesting the non-enzymatic nature of the inhibitors. Conventional and electron microscopy indicate that the inhibitors have no effect on morphology of both mating types of *U. scitaminae* sporidia, except on hyphae development. Studies are in progress to understand the chemical nature of these inhibitors and their molecular targets.

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842 - Gliotoxin production by *Aspergillus fumigatus*

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It is well known that various moulds can grow on animal feed and produce a range of toxic metabolites (mycotoxins). To study production of mycotoxins, *Aspergillus fumigatus*, that is one of the most common fungi in animal feed, was chosen. *A. fumigatus* is known to produce, among other toxins, gliotoxin, a highly toxic mycotoxin. The purpose of the study is to develop a screening *in vitro* method to identify toxicological components in feed, which may help to minimise application of animal tests also for other purposes. *A. fumigatus* CCUG 17460 were grown on Czapek-Dox Broth with 30% glucose (37 °C; 10 samples). The Czapek-Dox Broth was extracted and purified by a solid-phase purification method. The extracts were run at HPLC, and gliotoxin was detected in 9/10. The maximum concentration of gliotoxin was produced after 87 hours of inoculation. The purified extracts were applied to the human neuroblastoma SH-SY5 cells, and the general cytotoxicity was determined. The extracts showed to be cytotoxic to the SH-SY5 cells. This study proves that it is possible to produce gliotoxin *in vitro*by means of inoculating Czapek-Dox Broth with *A. fumigatus*. The effects of the *in vitro* produced gliotoxin agree with the effects of pure gliotoxin.

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843 - Expressed sequences in the basidiomycetaus tree pathogen *Heterobasidion annosum* during early infection of Scots pine

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The basidiomycete fungus *Heterobasidion annosum* is the causal agent of annosum root rot and is economically the most important disease of coniferous forests in northern temperate regions. *H. annosum* is a wood-decaying pathogen which can utilize a variety of carbon sources. The purpose of this research project is to investigate the molecular mechanisms behind the pathogenicity of *H. annosum* and to identify key factors, which enables the fungus to infect and cause disease. A cDNA library was constructed from mycelia that had been challenged with pine seedling roots. Fragment lengths are spanning from 300 bp to 3000 bp with an average of 850 bp. Individual cDNA fragments were sequenced once from the 5' end to generate expressed sequence tags (ESTs) which were used for similarity searches against already available sequence data. In this way we can get an idea about the putuative function of the corresponding genes. So far 1150 fragments have been sequenced, corresponding to 337 different contigs from which 228 have significant similarity to other genes. The largest fuctional groups are those involved in information pathways (including protein synthesis) and basic metabolism but genes involved in electron transport and transmembrane transport are also common. In order to pinpoint the genes that are important for the pathogenicity of the fungus the cDNA fragments are screened for differential expression during different stages of the infection process.

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844 - Fungal infection for the recipients of living donor liver transplantation

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(Introduction) Fungal infection is potentially fatal complication in patients with liver transplantation. The aim of this study was to review the single-center experience with fungal infections in living donor liver transplantation and to report its management for the early diagnosis and treatment. (Methods) From July 1991 to December 2001, 52 cases of LDLTx were performed in our institution. Immunosuppression consisted of tacrolimus or cyclosporine, corticosteroids and in some cases azathioprine or mycophenolate mofetil. The diagnosis of fungal infection was based on clinical findings, culture and serological examinations such as β-D glucan, *Candida* Ag, *Aspergillus* Ag and *Cryptococcus* Ag. For the prophylaxis of the fungal infection, administration of fluconazole and gargle by amphotericin B were performed before LDLTx. Active fungal infection was treated by fluconazole, amphotericin B and flucytosine. (Results) Mycoses were cultured for 41 cases (78.8%) and 23 cases(44.2%) were detected before LDLTx. *C. albicans* was accounted for more than 60% of the recipients and the greatest number of fungal isolates were obtained from pharynx and stool. There was no recipient who died directly because of invasive fungal infection. Detection of β-D glucan was effective for the prevention from severe invasive fungal infection. (Conclusion) For the management of fungal infection in LDLTx, it is important to notify the role of prophylaxis, empiric and pre-emptive therapy.

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845 - *Phyllachora* epidemic on common ragweed (*Ambrosia artemisiifolia*): a unique natural control phenomenon in Hungary in 1999

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Since the early 1990s, common ragweed (*Ambrosia artemisiifolia*) has become the most widespread and most important allergenic weed in Hungary. Our annual surveys of fungal diseases of ragweed revealed that this plant, introduced to Europe from North America, is one of the healthiest weeds in the Carpathian basin. Only nine species of fungal pathogens were found on ragweed in Hungary that caused only minor infections in the field between 1995-1999. However, in summer 1999, a serious epidemic developed on ragweed caused by *Phyllachora ambrosiae*. This holobiotrophic pathogen has not been reported from Europe prior to 1999 [1]. The identification of the pathogen was based on both morphological and molecular data. From mid-September, all plants examined in all regions of the country were infected and exhibited dead leaves and inflorescences. Thus, *P. ambrosiae* reduced the fitness of ragweed and also the period of production of the allergenic pollen in Hungary in 1999. According to the data of the pollen monitoring service in Budapest, much less ragweed pollen occurred in the air in September and October 1999 than in the previous ten years. This natural epidemic clearly demonstrated the capacity of a pathogenic fungus to reduce the harmful effects of a noxious weed. Its main lesson is that biological control using fungal pathogens could be a possibility to suppress ragweed populations in Hungary. [1] Vajna, L., Bohar, Gy. Kiss, L.: Plant Dis 84, 489 (2000).

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846 - AFLP of cheese contaminating *Penicillium* strain

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Amplified fragment length polymorphism (AFLP) analysis was performed on isolates of *Penicillium commune* and *P. palitans* originating from cheese and indoor environment in four cheese factories. The AFLP method was found to be a useful tool for identification of *P. commune* and *P. palitans* on, as well as below, species level. Specific *P. commune* and *P. palitans* strains were found in the same factories over a period of more than a year and showed that the cheese factories have contaminating strains that are well established. Several of cheese contaminating *Penicillium* strains could be related to air in the wrapping room, which must be considered to be a critical point for contamination of cheese.

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847 - The telomeric repeat sequence of *Aspergillus oryzae* consists of unique dodeca-nucleotides

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The telomeres of the chromosomes of *Aspergillus oryzae* NFRI1599 were cloned and identified. They were sensitive to BAL31 exonuclease digestion, compared with the chromosome internal control, 18S rDNA, and thus were proved to be located at the most terminal region of the chromosomes. The telomeric repeat sequence of *A. oryzae* consisted of unique dodeca-nucleotides, TTAGGGTCAACA. The former six nucleotides TTAGGG in the repeat sequence was conserved as telomeric repeat sequence in several filamentous fungi, including *Aspergillus nidulans* (Bhattacharyya and Blackburn 1997). The remaining sequence TCAACA in the repeat is not reported as telomeric sequence in any other organisms. Therefore the newly identified *A. oryzae* telomere is unique in this organism. Length of the telomeric repeat region was 114-136 bp. Considering that the length of the telomere was similar to that of *A. nidulans*, the rigid regulation of the telomere length might be conserved among *Aspergillus* species.

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848 - Assessment of air-borne fungi present in a biomedical plastics factory

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Fungi can be found in high concentrations in the bioaerosol of northeast Mexico, where we have a subtropical climate and agriculture is the main activity. Because our city is in the border with the United States, there are hundreds factories. One of them, dedicated to injecting biomedical application plastics, had a fungal contamination problem. We were asked to evaluate the issue, and accordingly the production zone and warehouse were carefully sampled. Media used were: Potato-Dextrose Agar, 8-Vegetables Agar and Lennox Broth supplemented with agar. By the pattern of distribution of genera, we found that the entrance of fungi was the ventilation system, and that this income of bioaerosol was accummulating on horizontal high zones and then being slowly distributed, specially over the packing area. We found 20 genera of fungi, being the most important in terms of frequency: *Cladosporium* (3 species), *Aspergillus niger*, *Penicillium* (four species), *Rhizopus* and *Alternaria* (2 species). A close examination of the cleaning policies and ventilation system of the factory showed that, althought being efficient for the environment where they were designed, they were not the best options for a region like ours, because its high average annual temperature (25 centigrades) and relative humidity (50% or more). Among suggestions were a much more frequent filter change, sealing of all porous surfaces and a tight control of relative humidity inside the production zone.

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849 - A new *Phytophthora infestans* molecular marker

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Short interspersed elements (SINEs) or short retroposons are 80-400 bp repetitive DNA sequences that proliferate in eukaryotic genomes via transcription followed by reverse trancription. Recently SINEs have been used as molecular markers for intraspecies differentiation. Most SINEs have an internal promoter for RNA polymerase III composed of two boxes (A and B) spaced by 30-35 bp similar to tRNA genes. To detect and clone novel tRNA-derived SINEs in *Phytophthora infestans* genome we have used PCR-amplified total genomic DNA as a template and primers specific to boxes A and B for PCR amplification. This reaction, designated A-B PCR, amplifies the region between boxes. The resulting DNA fragments have been cloned and sequenced. The sequences obtained of 8 fragments (45-51 bp) are similar but not identical suggesting that they may have been amplified from individual copies of a single SINE famile. All 8 fragments have 24 bp conservative sequence. On the basis of this sequence we constructed one primer to use it as a marker for *P. infestans* intraspecies differentiation. This marker is more reliable than RAPD because it is specific sequence and it gives the same results in the several experiment repeatings. Acknowledgements: This work was supported by the grant from the International Science and Technology Center No. 1640.

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850 - Plant and microorganisms interactions mediated by *Pythium oligandrum*

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Over the past few years, experiments performed by our group have demonstrated that the role played by antagonist fungus, *Pythium oligandrum*, on plant development and protection involves a tripartite interaction between the biocontrol microorganism, the pathogen agent and the plant. Such a complex process includes indirect effects through the control of pathogens in the rhizosphere and/or direct effects mediated by plant-induced resistance. Interaction of *P. oligandrum* with soil-borne pathogens to control their development in the rhizosphere and even *in planta* consists in a series of events involving mainly mycoparasitism and/or antibiosis. However, antagonism is a multifaceted process dependent on the involved target host; for example, by forming a thicken wall barrier *Rhizoctonia solani* and *Phytophthora cinnamomi* limit *P. oligandrum* antagonistic activity. We also showed hyperparasitism on various sclerotia. Plant-induced resistance mediated by *P. oligandrum* is a key event in this kind of fungus-plant interaction. Evidence of plant sensitisation by *P. oligandrum* to respond more rapidly and efficiently to pathogen attacks was also provided. It can protect plants from *B. cinerea*, *Fusarium oxysporum* f.sp. *radicis lycopersici*, *Phytophthora parasitica*, *P. ultimum* attacks by either inducing local protection or triggering systemic resistance.

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851 - Selection of baiting materials for *Phytophthora palmivora* (Butler) Butler., the causal agent of coconut nut falling disease

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Coconut nut falling disease is caused by *Phytophthora palmivora* (Butler) Butler. A serious outbreak of this disease was found on 'Yellow Dwarf' cultivar of coconut grown at Surat Thani Rubber Research Center. Since *P. palmivora* is a soil-borne fungus, to isolate the fungus needs to apply baiting techniques to trap the fungus from the soil. Leaves of nine crop plant species, namely pararubber, cacao, citrus, durian, coffee. Black pepper, oil palm, and coconut cvs. 'yellow Dwarf' and F1 Hybrid Sawee I. The leaves were immersed in the zoospore suspension for 1, 3, 6 and 24 hours. It was found the leaves of all tested plant species excluding pararubber could trap up to 95% of the fungal spores when immersed in the zoospore suspension for 24 hr. An experiment was conducted to study on the optimum duration of fungal existiance in the soil to be best baited by using cacao leaves. Among 1, 15, 30, 45, and 60 days of the durations, it was found that 90% of the fungus could be trapped from the soil at 60-days duration. Suitable methods in using leaves of nine plant species to bait *P. palmivora* from the soil was also performed. The three methods include placing the leaves on the soil surface, placing the leaves one inch below the soil surface, and immersing the leaves in the infected soil suspension. The results revealed that immersing the leaves in the infected soil suspension was best in baiting the fungus from the soil.

852 - Inhibition of microconidia of *F. oxysporum* var. *vasinfectum* with antibiotic substances from *Bacillus subtilis* strains

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Previously obtained results from comparative physiological, biochemical and antimicrobal investigation of endophyte and soil borne cultures of *Bacillus subtilis* N and 23 had allowed to suggest that both strains produce the same antibiotic substances active against cotton phytopathogens. To additionally prove the hypothesis, we carried out phase-contrast microscopy of phytopathogens' cells in the inhibition zone. The use of phase-contrast microscopy had shown inhibitory effect of antibiotic substances from 23 and N strains of *B. subtilis* on growth of microconidia which was different depending on the diffusion of antibiotic substances from the 0.5 cm well on agar in the centre with antibiotic filtrates from cultural liquids of *B. subtilis* strains towards edges of Petri dishes. Basing on the analysis of comparative influence of filtrates of 23 and N strains in the following dilutions: 1:1, 1:2, 1:4, 1:9 and 1:99, we had systemized investigated microconidia of *F. vasifectum* by the character of inhibition to four conventional groups. Obtained results allowed to determine the unit of biological activity of filtrates of 23 and N strains which was equal to 85 mg of extracted antibiotic substances, whereas 1 ml of filtrates of each strain contained 11.8 units of antibiotic activity. Further experiments will be directed on studies of mechanisms of biological action of antibiotics produced by 23 and N strains on cells of different groups of fungal and bacterial microorganisms.

853 - Bee pollen as a substrate for fungal development and mycotoxin production

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Pollen is a basic food for bee larvae development due to its high content of proteins, which contain all the amino acids. Pollen also contains minerals, vitamins, enzymes, growth regulators, fatty and organic acids and flavonoids. It is considered by the FAO a source of essential nutrients for the daily intake. Pollen consumption has increased in the last years as a diet complement in cases of fatigue, undernourishment and in vegetarian diets. Bees gather pollen in flowers, mix it with honey and nectar to make pellets and carry it to the beehive to serve as a food for larvae. Beekeepers catch pollen in traps put at the hive entry. Pollen remains in traps for some time, then it is taken and carried to stores where it is cleaned, fumigated, stored and marketed. During this stage pollen can be contaminated by several fungal species, among them those that are mycotoxin producers. We have evaluated for the first time the capacity of bee pollen to serve as a substrate for the development of *Fusarium* and *Aspergillus* species that are potential producers of zearalenone, deoxynivalenol, fumonisins and aflatoxins. The levels of the mycotoxins produced in pollen have been determined and a comparative study between pollen and other especially susceptible substrates, like cereal grains has been made. The results obtained point out that the toxin levels found in pollen are significantly higher than those produced by the same isolates in cereal grains under the same incubation conditions.

854 - Analysis of gene expression in targeted infection structures of obligate parasites, *Blumeria graminis* f. sp. *hordai* by intracellular RT-PCR

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To detect specific mRNAs in postinfectional differentiation of obligate fungal parasites *Blumeria graminis* f. sp. *hordai*, we applied an intracellular RT-PCR to conidia inoculated onto barley coleoptile epidermis. The RT-PCR primers for target genes were constructed using a EST library of powdery-mildewed Italian ryeglass leaves. In the present study, the chitin synthase gene (*chs1*) was used as a target gene expressed specifically during the differentiation of fungal infection structures. The primers were constructed on the base of nucleotide sequences between translation and 3'-untranslation regions. The first strand cDNA was conducted in the fungal structures (conidia, appressoria and secondary hyphae) by microinjecting the reaction mixture (primers, reverse transcriptase, rTaq polymerase and dNTPs) and subsequently amplified by PCR using the primers amplifying the internal region of the *chs1*. The nucleotide sequence of the amplified DNA was determined and confirmed to be consistent with the *chs1* gene of *B. graminis* f. sp. *hordai*. Thus, the present work provides a new molecular tool for analyzing gene expression during the differentiation process of infection structures of obligate fungal pathogens, especially emphasizing that cell-specific gene expression in individual infection structures of the pathogen can be monitored with respect to host and parasite interactions.

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855 - Evaluation of the occurrence of toxigenic fungi in barley and malt in Spain

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The presence of some fungal species in malt produced from contaminated barley may pose health risks due to the production of mycotoxins. The predominant mycobiota can vary due to several factors, including barley growing location or climatic conditions. Heavy fungal contamination can affect the malting process, the malt quality and final beer attributes, but the presence of some fungal species requires especial attention due to the unacceptable risk of mycotoxin contamination in barley and malt. In Spain there is little information about the occurrence of mycotoxin-producing species in barley and malt destined to breweries. The potential mycotoxin-producing mycobiota occurring in barley and malt taken in Spanish breweries and stores is studied in this work. The nature and extent of fungal contamination were performed using direct plating techniques with different culture media. After the incubation period, fungal colonies were examined, identified and transferred to appropriate culture media for mycotoxin production. The results show that both barley and malt were contaminated at different levels with *Penicillium*, *Aspergillus* and *Fusarium*. Other fungi such as *Alternaria* and *Cladosporium* were found but their frequencies were significantly lower than those reported in other countries. Various fungal species involved in the production of ochratoxins, deoxynivalenol and aflatoxins were found whereas species implicated in production of other mycotoxins showed low occurrence level.

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856 - Fungi of indoor environs of occupational importance in India: Are they significant in causing allergy

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Occupational environs such as cobbler's shops, ginneries, flour and sawmills were surveyed for their fungi suspended in the air. Forty fungal forms were recovered from cobbler shops. The flora of the cobbler shops was dominated by Aspergilli. Some fungal forms tested for their allergenic behaviour showed positive reactions among the patients in varying degrees. From the ginneries only 17 fungi were isolated but their load in the environment was very high. Almost 50% of the workers of the ginneries interviewed had allergenic and respiratory disorders. But no testing could be done for the fungi from the ginneries. Forty-one fungi were isolated from flourmills while fifty from sawmills. No significant variation was noted between the indoor and outdoor fungal flora of flour and sawmills. Spores of various fungi were also observed through the samplers in varying frequencies. In these environs too Aspergilli dominated the flora. Thus, at all the places surveyed, species of the genus *Aspergillus* were the dominant ones. *Aspergillus flavus*, however, was greater in occurrence in flourmills than saw mills. Distribution and dominance of fungal forms were correlated with ecological factors and the nature of occupation. Most of the fungal forms were found to have allergenic potentialites.

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857 - Fungi, paper materials and preservation conditions

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In several libraries and museums of Genoa, in which the thermohygrometric conditions were not optimum to the preservation, were found on books and maps mouldy spots. The determinations pointed out some species widespread in Genoa and moulds correlated to the micro-environmental conditions of the surrounding. In fact on eleven species, that were analysed in the library inside the Botanical Garden, ten were soil fungi whereas smaller amount was found in a book-warehouse located in a built-up area. The fungal species could be introduced by frequenters of near gardens and this species could grow with suitable conditions. Therefore this suggest limit the entry of people or at least to avoid they to introduce spores. In addition some books and maps showed rusty-red spots of foxing: a typical paper infection. Twenty paper samples of different centuries were examined to devise a no destructive method for taking samples from spots of museum's maps. The stains were examined by fluorescence and was checked that colonies no develop from areas without fluorescence therefore samples were taken using swabs from spots fluorescent. Moreover was proved that on the spots not clearly visible by naked eyes defined 'discoloring', but fluorescent, develop fungi if placed in humidity chamber. We should recommend the early identification of 'discoloring' by fluorescence in order to arrange suitable thermohygrometric conditions for preventing development of infection.

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858 - Specific toxigenic, biological and molecular traits of a *Gibberella moniliformis* (*Fusarium verticillioides*) population from banana

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Gibberella fujikuroi includes at least 8 mating populations (MP), corresponding to biological species. Each MP generally possess a specific toxicological profile and a preferential host. *Fusarium verticillioides* and *F. thapsinum*, anamorphs of MPs A (*G. moniliformis*) and F (*G. thapsina*), shared the identical morphological traits, but they have a different preferential host (maize and sorghum) and only members of MP A can produce fumonisins. Strains morphologically identified as *F. verticillioides* were isolated from banana fruits, analysed for fumonisin production, none being a producer, and crossed by tester strains of MPs A and F. Although they produced fertile perithecia of MP A, time for obtaining perithecia and size of them differed significantly from usual fertile crosses among strains from maize. Pathogenicity tests with both banana and maize strains were also performed on banana fruits showing higher ability of strains isolated from banana to cause infection. Finally, banana and maize strains were compared by using AFLP and sequence of IGS, ITSs and calmodulin regions, allowing to split in two different clusters. Finally, strains of MP A from banana showed peculiar traits in toxin production, pathogenicity and molecular profiles. This could reflect important differences in ecology and natural history and triggers further investigations on the mechanisms of toxin production and pathogenicity in the same MP. Support of EU Fifth Framework (Detox fungi QLK1-CT-1999-001380).

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859 - *Gnomonia fragariae* - a causal agent of strawberry root and crown rot in Latvia

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In a survey on possible causes for an observed ongoing decreased productivity of strawberry plantations in Latvia about 200 severely stunted and wilted strawberry plants from 16 plantations were assessed for fungal root rot pathogens. An often encountered, and also the most clearly pathogenic fungus isolated was identified as *Gnomonia fragariae*. Agar plating on PDA and PCA were used for isolation and identification, based on morphological characters. Isolates of this pathogen have so far been obtained from five fields in three locations. It was isolated from roots and crowns of stunted plants, showing wilt, reddish color in the older leaves and crown necrosis. Pathogenicity was confirmed using a detached leaf assay where wounded and unwounded leaves of the strawberry cultivar 'Honeoye' were inoculated by agar plugs with fungal colonies. Clear necrotic lesions were observed around agar plugs 2-3 days after inoculation on wounded leaves, and after 3-4 days on unwounded leaves. After few weeks the fungus also formed perithecia with long necks on infected leaves. The fungus was re-isolated from surface sterilized infected tissues. Based on the results obtained we conclude that *Gnomonia fragariae* is an important pathogen in strawberry root rot complex in Latvia and this respect also is of great economic importance.

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860 - Population dynamics and mycotoxin production of filamentous fungi in winter wheat grown in different tillage systems in eastern Germany

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The effect of different wheat cultivation systems on mycoflora in wheat plants during the vegetation period from April to the harvest in August was investigated in long time field trials. Systems with silage maize or potatoes as preciding crops and conservation soil tillage with cultivator or conventional soil tillage with plough were compared concerning the population dynamics of filamentous fungi and the mycotoxin accumulation in plants or in harvested kernels. Special attention was given to the widely distributed genera *Fusarium* and *Alternaria*. Many species of them are plant-pathogens and able to synthesize toxic metabolites: trichothecenes and zearalenone by *Fusarium* species and alternariols and tenuazonic acid by *Alternaria* spp. *Alternaria* toxins were determined by reversed phase HPLC with fluorescence or DAD detection. The *Fusarium* toxins were analysed by means of ELISA test kits. The four different cultivation variants showed similar quantitative fungal colonization with an increase of the total fungal population during vegetation period. At each sampling date, the plants grown in conservation soil tillage with maize as preceding crop had the greater population of the genera *Fusarium* and *Alternaria*. In some cases *Fusarium* toxins were already analysed in wheat ears six weeks before the harvest independent of the tillage system. *Alternaria* toxins were only rarely detected in all variants. The influence of abiotic driving factors (temperature, rain fall) is discussed.

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861 - Monitoring of compost-derived volatiles in the surrounding of composting facilities

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Composting plants are known to emit malodorous compounds due to microbial activity. The objective of this study was to evaluate the emission of (M)VOC and their occurrence in the surrounding of composting plants. Data on the amount of windblown losses of malodorous compounds should be obtained. The spectrum of (M)VOC was compared with those of fungal cultures on compost as substrate in laboratory experiments. Samples were analysed by thermal desorption and GC/MS. Sampling locations were set up in downwind direction in distances of 50 m up to 800 m from the composting plants, and within these. Three compost plants varying in the annual turnover and type of process engineering were investigated. Laboratory experiments were carried out to characterise the species-specific production of (M)VOC by microfungi. (M)VOC were detected in distances up to 800 m, but the amount did not seem to be correlated with the type of process engineering, but rather with the activity within the facilities. The occurrence of (M)VOC coincided with the sensory perception of typical compost-odour on the sampling locations and increased numbers of microfungi in the air. Emission of (M)VOCs during composting cannot be avoided even if more sophisticated techniques of process engineering are used. It turned out that detection of certain VOC may be used as indicator for the occurrence of plant-related emissions of bioaerosols including microfungi in the surrounding of composting plants.

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862 - Fungal diseases of cultivated mushrooms in Armenia

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Nowadays only two species - *Agaricus bisporus* and *Pleurotus ostreatus* - are cultivated in special cultivated areas in Armenia. In this report we are providing summarized information about cultivated areas of Armenia, data about inspection of some cultivated areas, and information concerning detection and occurrence of mushroom diseases in these areas. We have collected all possible data from various governmental and private organizations about mushrooms cultivated areas in different regions of Armenia. We have interviewed the local population concerning approaches to the cultivation, for example the carpophores of *Agaricus bisporus* were infected mostly by Wet Bubble Disease (*Mycogone perniciosa*), which has been recorded in very severe form. The percentage of infected areas was very high, approximately 80-90%. The preliminary examination has shown that now the small scale mushrooms' farms are attacked by several species of moulds and bacteria: *Trichoderma*, *Penicillium*, *Aspergillus*; *Papulospora byssina*; *Chaetomium olivaceum*; agaricoid fungi from genera *Coprinus*; *Pseudomonas tolaasii*, *Dactylium dendroides*. The results of investigation show that these diseases are observed often in the farms with insufficient ventilation, increased air and compost humidity, infringement of compost preparation technology, overuse of nitrogen fertilizers, wrong pasterization. New approaches to the investigation and further theoretical study in this field are required.

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863 - Anti-fungal activity of natural preservatives from plant extracts depends on the application method

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Antimicrobials from species and herbs are interesting alternatives to traditional preservatives, as they may act through the gas phase and have complementary antimicrobial spectra. Traditionally antimicrobial compounds are either added to the product or applied on the surface. For foods like bread and cheese addition to the packaging gas may be more effective. Ten plant essential oils were investigated by addition either 1) directly to a rye bread media or 2) to the surface or 3) as volatiles to the packaging atmosphere. Oils were from: bay, cinnamon leaf, clove, lemongrass, mustard, orange, sage, thyme and rosemary (two formulations). Test organisms were the common bread spoilage fungi *Penicillium roqueforti*, *P. corylophilum*, *Erotium repens*, *Aspergillus flavus* and *Endomyces fibuliger*. Smaller volatile compounds, such as allyl isothiocyanate, limonene, (and citral), were most efficient when applied through the gas-phase, whereas larger phenolic compounds, such as thymol and eugenol, worked better in direct contact, e.g.100 times more allyl isothiocyanate had to be added to the media to get the same effect as observed through gas phase addition. The optimal mixture of essential oils on the surface varied by the total amount added. A combination of cinnamon bark and lemon grass gave the best results. These results showed that application method is crucial and that, for active packaging applications, small highly volatile compounds are most interesting.

864 - Simultaneous detection of GFP- and GUS-marked fungi of different formae speciales of *Fusarium oxysporum* on plant roots

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In our attempt to visualize infection behavior of the fungal wilt pathogens inoculated onto plant roots, the fungi were genetically marked with two reporter genes. *F. oxysporum* f. sp. *lycopersici* (FOL) and *F. o.* f. sp. *melonis* (FOM) were transformed with the green fluorescence protein gene (GFP) and the β-glucuronidase gene (GUS), respectively. In the present study, we focused mainly on the attachment and subsequent hyphal elongation by microconidia inoculated onto roots of tomato and melon seedling. In addition, we attempted to directly distinguish different formae speciales of *F. oxysporum* onto the same plant roots by expression of different marker genes. Microconidia of GFP-marked FOL (KFOL-001) and GUS-marked FOM (KFOM-002) were inoculated onto roots of cotyledonal seedlings, and inoculated roots were first observed under a fluorescence microscope to detect KFOL-001 and then stained with X-gluc (substrate for GUS assay) to detect KFOM-002 under a light microscope. Consequently, both transformed pathogens could be clearly distinguished at the same site of inoculation. These results suggest that dual transformation of *F. oxysporum* is useful for analyzing behavior of nonpathogenic *F. oxysporum* challenge-inoculated with pathogenic *F. oxysporum*.

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865 - *Biscogniauxia* and *Daldinia*; latent pathogens of deciduous trees

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Biscogniauxia Kuntze and *Daldinia* Ces. and De Not. are two wood inhabiting xylariaceous genera. *Biscogniauxia* species are frequently linked with canker diseases in stressed hosts e.g. *B. mediterranea* causes coal canker in *Quercus suber* (cork oak) and *B. nummularia* canker in *Fagus* (beech) while *D. concentrica* (Bolt. ex. Fr.) causes calico wood in *Fraxinus* (ash). Studies on ascospore germination and development of the anamorphs in culture in response to host extracts is presented. *Biscogniauxia nummularia* and *Daldinia concentrica*. have been isolated from their respective host leaves and branches and there are frequency of isolation maybe linked to ascospore production. The presence of *Daldinia* in leaves and in wood has been investigated microscopically, chemically and by molecular techniques, in addition to traditional isolation techniques following surface sterilisation. The presence of latent pathogens and their relationship to stress of the host is presented. Experimentation on conditions leading to the latent invasion and subsequent development of teliomorphs are being undertaken in both field and laboratory.

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866 - Establishment of the first Karnal Bunt testing laboratory in South Africa

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The fungal wheat disease *Tilletia indica*, commonly known as Karnal Bunt (KB), was detected in a limited area of one of South Africa's wheat producing regions during 1999. Aimed at managing *T. indica*, a national survey to determine the occurrence of the disease was initiated by the Directorate Plant, Health and Quality, National Department of Agriculture. To this end, the Mycology Unit, ARC-Plant Protection Research Institute, Pretoria was tasked with setting up a laboratory for the analyses of seed and grain samples. Due consideration was given to the geographical distance of the laboratory from the main wheat producing areas of the country. The KB protocol, as recommended by the USDA/APHIS, was followed with some adaptations. Analyses were conducted for two consecutive years, providing valuable experience in managing a quarantine analytical facility of this nature. Protocols and procedures representing different phases of the process were devised for each workstation. These phases included: reception and registering of samples, sub-sampling, washing and sieving, centrifuging and preparing of microscope slides, detection of *T. indica*, data processing and reporting, and waste management. Laboratory procedures, problems encountered and the development of novel techniques, as well as the management and maintenance of the quarantine facility, are discussed.

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867 - Aflatoxins in the weaning food of Kenyan children

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Cereal grains run a high risk of mycotoxin contamination yet they form the basis of gruels used in weaning children in Kenya. To these grains (maize, sorghum, millet) supplements such as cassava, groundnuts, beans, and fish are added and ground together, depending on the means and education of the parents. Among the mycotoxins, aflatoxins have been implicated in human diseases, including kwashiorkor. Sampling for aflatoxin contamination was done in Kisumu District, Kenya, an area with high relative humidity and temperatures, high incidence of kwashiorkor and the highest prevalence of absolute poverty, 63%, in the country. A total of 180 samples of the weaning flour were collected from Winam Division of Kisumu District, Kenya. These samples were analysed for aflatoxins in order to find out whether there exists a relationship between the high incidence of kwashiorkor and aflatoxin contamination. The samples were analysed by thin layer chromatography. Fifty two samples (29%) were positive for aflatoxins (concentration range 2-82 µg/kg), some exceeding the advisory limit. This indicates that aflatoxin is likely to present a major health hazard to weaned children in this part of Kenya.

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868 - Prevention of ochratoxin A in cereals - an EU project within Quality of Life and Management of Living Resources

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Ochratoxin A (OTA) is a mycotoxin of considerable concern for human health and is classified as a possible human carcinogen. Cereals normally account for 50-80% of average consumer intake of OTA. Consequently, prevention of OTA formation by specific moulds in cereals would have a significant impact on levels of human exposure. The aim of this multidisciplinary project is to identify the key elements in an effective HACCP programme for OTA in cereals, and provide tools for preventative and control procedures. The project includes the whole food chain from field to the final processed product. The project objectives are divided into 4 different tasks, all important steps in a HACCP managing programme for ochratoxin A in cereals. Task 1: Identification of the critical control points (CCP). The ochratoxin producing species are being identified and characterised in order to identify the sources of contamination. Task 2: Establishment of critical limits for the CCP's. New knowledge concerning the microbial ecology of the ochratoxin producing species is provided and critical limits are being specified. Task 3: Developing rapid monitoring methods. Rapid detection methods for ochratoxin A and for the producing fungi are being developed. Task 4: Establish corrective actions. Reduction during cereal processing (milling etc) and during malting and brewing, is being investigated.

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869 - Biological efficacy of *Trichoderma harzianum* against wilt disease of sugarcane

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Biocontrol efficacy of *Trichoderma harzianum* isolate Th1 and Th2, against sugarcane wilt disease caused by *Fusarium subglutinans* and *Cephalosporium sacchari* were determined. Result from pot experiment show that both isolates of *T. harzianum* were able to significantly reduced wilt pathogen population in soil and wilt infection percentage of sugarcane roots. Their effect on wilt disease has been further studied in field experiment. Each isolate of *T. harzianum* was grown on sorghum seeds and then mixed the infested seeds with rice polish and manure. The mixture of each isolate of *T. harzianum* was applied to wilt pathogens infested field soils. Population of wilt pathogens in soil, percentage of infected root and yield of sugarcane were determined in all treatments. Population of wilt pathogens in infested field soil were significantly reduced by both *T. harzianum* Th1 (2.1 × 103 cfu/g soil) and *T. harzianum* Th2 (2.8 × 103 cfu/g soil), as compare with control (5.5 × 103 cfu/g soil). Percentage of wilt infected root only was reduced by *T. harzianum* Th2 (20.4%). Significant difference in percentage of wilt infected root was not observed between *T. harzianum* Th1 (25.3%) and control (26.3%) treatments. Yield of sugarcane in *T. harzianum* Th2 treatment was also higher than in *T. harzianum* Th1 and control treatments. *T. harzianum* isolate Th2 has shown to be the effective biocontrol agent of wilt disease of sugarcane.

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870 - Natural flavonols fungitoxic to *formae speciales* of *Fusarium oxysporum* pathogenic to some ornamentals

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Three constitutive flavonols from as many carnation (*Dianthus caryophyllus*) cultivars, resistant to *Fusarium oxysporum* f. sp. *dianthi* pathotype 2, have been assayed, alone or in combination, against the six following *formae speciales* of *F. oxysporum* affecting ornamental plants: f. sp.*dianthi* pathotypes 2 and 8, f. sp. *asparagi*, f. sp. *ranunculi*, f. sp.*cyclaminis*, f. sp. *lilii*. The fungitoxic effect was tested both as mycelial growth and as spore germination inhibition. The results show that some constitutive flavonols of carnation are fungitoxic towards the tested fungal *formae speciales*. Besides other fungitoxic mechanisms, such as the mitochondrial oxidative phosphorylation inhibition, flavonols may be antifungal due to their ability to associate as polydentate ligands with pathogen proteins: only a low solubility ensures a strong association via phenolic groups, and the low soluble flavonol aglycones could be therefore more effective, as complexing agents, than the respective soluble glycosides. In effect, among the flavonol aglycones, the 4'-methoxylated kaempferide was particularly effective, while the glycosylated forms were less active than the corresponding aglycones. The inhibitory activity of the assayed flavonols was different depending on the fungal *forma specialis*, and a common physiochemical basis to explain the effect of the tested compounds was not found.

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871 - Broad spectrum herbal antifungal active against onychomycosis

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During antifungal evaluation of some essential oils, *Eugenia caryophyllata* Thunb. was found to be most effective antifungal. The minimum inhibitory concentrations were found 0.1 µl ml^{-1} against *E. floccosum*, *T. mentagrophytes*, *T. rubrum* and 0.4, 0.5 µl ml^{-1} against *Candida albicans*, *Scytalidium dimidiatum* respectively. The oil inhibited potency against heavy doses of inoculum at 1.0 µl ml^{-1} concentrations. The oil also have broad fungitoxic spectrum as it also killed some other fungi at the range of 0.1-1.0 µl ml^{-1} concentrations. Our tests proved that the oil did not cause any adverse effect on human nail as well as on skin upto 5 µl ml^{-1} concentration. Further, we have formulated the oil in the form of antifungal lotion and ointment (2 µl ml^{-1}) and subjected to topical testing on patients attending Out Patient Department (OPD) of M.L.N. Medical College, Allahabad. 10 patients were selected on the basis of KOH positive results and diagnosed as fungal nail infection. After 4th week of treatment, 60% patients were mycological cure (KOH negative). At the end of 10th week, all patients cured mycologically. No KOH negative cases of relapse were observed when patients were re-examined after two months following the end of 10th weeks. Thus, the oil in the form of lotion can be exploited as commercially after undergoing successful multicenter clinical trials, which is in progress.

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872 - Microwave irradiation: a tool to improve the staining with fluorochromes of *Aspergillus fumigatus* conidia

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Fungal conidia are ubiquitous in the air, where they can be the major pollutant and sources of infections, allergic reactions and toxicoses. Microscopy is the only currently available method for evaluating the total load of fungus aerosol. This kind of analysis could be made easier by the use of fluorochromes but, unfortunately, propagules of most fungi are not stainable or only a small percentage of the total number fluoresce, owing to their resistant wall. In this study we tested the use of chemical and physical treatments, respectively oxidation by the use of sodium hypochlorite and microwave irradiation, to improve the staining of *A. fumigatus* conidia with six different fluorochromes. We calculated the percentage of stained conidia respect to the total ones, comparing the percentages obtained after each treatment with those of the treatment free samples (control samples). In the control samples percentage of stained conidia was always lower than 44%; only Auramine O gave higher percentage (99%). The treatment with sodium hypochlorite gave high percentages of stained conidia (up to 99% with DAPI) but more than 30% of them were lost during the following two centrifugations required to remove the reagent. On the contrary microwave irradiation always gave percentages higher than 98% and did not require further treatment. In addition fluorescence intensity appeared enhanced, making easier the observations by epifluorescence microscopy.

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873 - Flow cytometry, a novel approach to quantitative assessment of airborne fungal propagules

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Current limitations in the methodology for enumeration of airborne fungal propagules compromise the precision and accuracy of bioaerosol exposure assessment. Flow cytometry (FCM) is a tool with great potential for use in environmental microbiology because of the quantity and quality of data it provides in a timely fashion. There have been no reports of the application of FCM to the study of airborne mycoflora. In this study, FCM was used to count fungal propagules in laboratory suspensions with concentrations ranging from 10^3 to 10^6 conidia/ml and in field samples collected by an impinger device. To verify the accuracy and the precision of this technique, fungal propagules counts made by flow cytometry were compared with counts by direct observation using epifluorescence microscopy. Flow cytometric counts of laboratory suspensions were performed by the use of forward angle light scattering, a parameter related to particle size. Field samples were stained with propidium iodide after microwave irradiation, to discriminate the biological particles, while forward angle light scattering was used for identifying and counting fungal propagules population. A close agreement was found between FCM and epifluorescence microscopy counts.

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874 - A 6 methylsalicylic acid synthase (6MSAS) homologous gene isolated *Byssochlamys nivea* is expressed during patulin production

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The *Byssochlamys nivea* NRRL 2615 strain can produce mycophenolic acid and patulin. We used degenerate PCR primers matching a ketosynthase nucleotide motif for a RACE-PCR strategy and isolated a polyketide synthase gene from *B. nivea*. The deduced amino acid sequence (1778 residues) displays 74% identity with *Penicillium patulum* 6-methylsalicylic acid synthase (6MSAS). Two 6MSAS homologous fragments located respectively on the 5 and 3 extremities were isolated from the mycophenolic acid producer *P. brevicompactum* genome. After translation, these fragments display 87 and 93% identity with *P. patulum* 6MSAS. *B. nivea* and *P. brevicompactum* cultures were monitored for PKS transcription kinetics by RT-PCR. The *B. nivea* messenger is expressed throughout the first 10 days of culture with a maximum observed level between day 2 and 5. Using the HPLC/DAD and LC/MS, the patulin precursor 6- methylsalicylic acid (day1-5), patulin and mycophenolic acid (2-10) were detected in *B. nivea* cultures. On the other hand, the mycophenolic acid was detected in *P. brevicompactum* cultures, but not patulin and 6-methylsalicylic acid. The *P. brevicompactum* messenger was not expressed during culture. The *B. nivea* amino acid sequence does not contain any methyl transferase site required in the 5-methylorsellinic acid synthesis (first precursor mycophenolic acid). The results strongly support the identification of a new 6MSAS involved in patulin and not in acid mycophenolic acid synthesis in *B. nivea*.

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875 - Comparison of genotypes and pathobiological phenotypes of environmental and commensal isolates of *Candida albicans*

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The patterns of genetic variation, production of phospholipase, and growth rate were analyzed among 129 isolates of the human pathogenic yeast, *Candida albicans*. Two populations of *C. albicans* were studied: 71 strains were collected from aquatic sources and 58 control strains were isolated from oral samples of healthy human volunteers. The phenotypes and genotypes were compared to determine if environmental isolates represent a greater risk to human health than commensal isolates. Genetic analysis, which was performed by PCR fingerprinting, revealed these two populations were genetically similar and agree with previous studies based on codominant genetic markers that the population structure of *C. albicans* is predominately clonal. There was no difference in the percent of phospholipase positive cultures between the two populations. However, among the isolates that were positive for enzyme production, the commensal isolates secreted significantly more phospholipase than the environmental isolates. Growth rate studies revealed that the environmental isolates replicated at approximately the same rate, as did commensal isolates. The DNA genotypes and the phospholipase results also were similar for the environmental and commensal isolates.

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876 - Ultrastructure of pycnidial cells and conidiogenesis of *Septoria hyperici* in dead tissues of host plant

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The techniques of light and electron microscopy were used to elucidate the details of pycnidial cells and conidium ontogeny in *Septoria hyperici* Desm., causal agent of foliage disease (leaf blotch) of common St. John's wort (*Hypericum perforatum* L.). The pycnidia were present in the intercellular spaces of host leaf tissues. The wall of subspherical pycnidium, visible under the light microscope, was composed of 1-3 layers of hyphae with normal structure. The hyphal cell protoplast was highly vacuolated, with well defined nucleus, numerous mitochondria, ribosomes, short cisternae of ER, and concentric bodies. Pycnidial wall septa had typical ascomycetous structure. The conidiophores with electron-dense cytosol, numerous mitochondria and large nucleus were found in inner layer of pycnidial wall. Conidiophores were less vacuolated, than pycnidial cells. In the apical region of conidiophore numerous vesicles were found. Ontogeny of conidia was holoblastic. Contents of the conidiophore and conidium were continuous during migration of full complement of organelles into conidial cell. Then a delimiting septum formed. Conidia were liberated schizolytically. Fusiform, septate conidia with numerous mitochondria, ribosomes and large lipid droplets were observed in the mucilage-filled central cavity of pycnidium. The conidium organelles were difficult to distinguish. Conidial wall consisted of two layers. The outer layer was moderately electron-dense wirg fibrillar surface.

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877 - Breeding of *Pleurotus* and *Lentinula* hybrids by pairings of neohaplonts for commercial cultivation

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Cultivation of *Lentinula edodes* is slow and rather inefficient and aggressive strains, able to grow on a wide range of substrates, like hybrids of *Lentinula* with *Pleurotus* is a promising option. Interspecies hybrids cannot be obtained by conventional breeding, they have been produced only by protoplasts fusion, but such hybrids are sterile and highly unstable. Dedikaryotization yields the 2 monokaryotic components of a strain and it has been used for improving strains of the same species. Therefore, 7 strains of *Lentinula* and 3 of *Pleurotus* were dedikaryotized, *Lentinula* neohaplonts were classified in 4 incompatibility groups and *Pleurotus* neohaplonts were also classified in 4 groups. Surprisingly, matings of neohaplonts of *Lentinula* and *Pleurotus* yielded 73 hybrids and only 5 negative pairings. From 19 hybrids cultivated on fermented straw, 2 produced fruit bodies with *Lentinula* morphology in the first flushes while 15 hybrids yielded *Pleurotus* morphology with BE around 160%. Parental strains of *Lentinula* and *Pleurotus* yielded fruit bodies with the typical morphology for each species; BE values were around 148 to 182% for *Pleurotus* and 24 to 261% for *Lentinula*. In the meiotic progeny from 3 hybrids, 4 mating types were present in 1:1:1:1 ratio (p 95%), confirming fertility of the hybrids and inheritance of tetrapolar heterothallic compatibility. Separation of these organisms into 2 different strains has to be reconsidered in view of these results.

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878 - Influence of environmental factors and fungicides on growth and deoxynivalenol production by *Fusarium graminearum*

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The impact of five fungicides (prochloraz, propioconazole, epoxiconazole, tebuconazole and azoxystrobyn, 0.5-50 ppm) on growth of *F. graminearum* was evaluated in relation to water activity (a_W, 0.99, 0.97, 0.95) and temperature (15 and 25 °C) on wheat-based media (*in vitro*). All fungicides reduce growth rates when compared to the control, and this reduction increased as the fungicide concentration increased. In general, none of the isolates was able to grow in the presence of any fungicide at concentrations higher than 15 ppm, regardless of the a_W/temperature regime. The same fungicides were used in a second study on wheat grain (*in situ*), in order to evaluate the effect of two concentrations (0.5 and 5 ppm), three a_W levels (0.995, 0.99 and 0.97) and two temperatures (15 and 25 °C) and their interaction on growth rate and deoxynivalenol (DON) production by *F. graminearum*. All fungicides showed inhibition of growth at both concentrations in most conditions. The fungicides tested were less effective on grain in controlling growth than in *in vitro* studies. All fungicides showed DON stimulation or reduction in at least one of the conditions assayed. Our results show that stimulation or reduction in DON production in the presence of fungicides is influenced by complex interactions between a_W, temperature, fungicide concentration and time of incubation in both strains studied. Such information is critical for effective fungicide control of *Fusarium* head blight of wheat.

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879 - Relationship between osmotic and matric potential, growth and endogenous accumulation of polyols and sugars in colonies of *Fusarium graminearum*

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The effect of ionic (NaCl) and non-ionic osmotic potential (glycerol) and matric potential (PEG 8000) in the range -0.7 to -11.2 MPa, on growth of *F. graminearum* at 15 and 25 °C on 2% wheat flour agar has been studied. The effect of stresses on endogenous accumulation of low and high wt polyols (glycerol, erythritol, arabitol and mannitol) and sugars (glucose and trehalose) in whole colonies of this fungus were also quantified. At 25 °C and high water potential growth rates on matric-modified media were higher than on osmotic-modified media. However, in the same range of water potentials on osmotically modified media, *F. graminearum* grew faster on NaCl than on glycerol-amended media. In general, the concentration of total polyols in whole colonies of *F. graminearum* increased as osmotic and matric potential was reduced to -8.4 MPa. Also marked differences in the ratios of low and high mol wt polyols in mycelial colonies were found. There was an increase in the glycerol content with a

progressive decrease in mannitol, arabitol and erythritol in colonies as osmotic potential decreased to -8.4 MPa on glycerol and NaCl amended media. However, on matrically modified media there was an increase in arabitol with a progressive decrease of glycerol and erythritol in colonies as the matric potential was decreased. There were also marked changes in the content of trehalose and glucose in colonies of *F. graminearum* in relation to osmotic and matric potential modification.

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880 - Inhibition of biosinthesis of different mycotoxins by using antioxidants alone or in association with food preservatives

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The efficacy of different antioxidant compounds both synthetic and of natural origin, alone or in combination, has been tested in order to verify their action both on the growth and on toxins production in different mycotoxigenic fungi. In particular we have tested some of the toxigenic fungi that often contaminate food and feed: *Aspergillus ochraceus* and *Penicillium verrucosum* (ochratoxin A producers), *Fusarium graminearum* (Zearalenon producer), *F. moniliforme* and *F. proliferatum* (fumonisins producers). The experiments have been performed on wheat and maize seeds moistened up to a water activity of 0.85 and 0.95 and incubated at 28 °C (*A. ochraceus* and *P. verrucosum*) or at 25 °C (*Fusaria* strains) for 30 days after the inoculum. The tested compounds were BHA, BHT 0.02% w/v, K sorbate 0.2% w/v, Na propionate 0.2% w/v, resveratrol 10-4 and 10-3 M, ellagic acid 10-4 and 10-3 M, *Lentinula edodes* 4% w/v cultural filtrates (LCF). Concerning both the ochratoxin A and fumonisin producing fungi, the best results, both on the growth and on toxin production (more than 80% inhibition) have been observed with BHA and resveratrol on maize seeds and with K sorbate or resveratrol on wheat seeds. In some cases it has been detected a very high inhibition of ochratoxin A production on wheat seeds also by using LCF Other experiments are in progress in order to obtain a long-lasting inhibiting effect. This work has been sponsored by grants PL-QLRT-1999-433 and 966 of EC.

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881 - Effect of fungicides commonly used for *Podosphaera leucotricha* control on the powdery mildew hyperparasite, *Ampelomyces quisqualis*

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Apple powdery mildew, caused by *Podosphaera leucotricha*, can significantly reduce crop yield. Severity may increase with successive seasons, resulting in stunting and distortion of leaves and general decline in tree health. Current strategy for apple powdery mildew control relies mainly on fungicide application at intervals of approximately ten days throughout the growing season. A biological control agent, using the hyperparasite *Ampelomyces quisqualis*, is one possible way to reduce this intensive chemical input. For it to be incorporated into the current management practice the hyperparasite must tolerate most commonly used fungicides. Eight isolates of *A. quisqualis*, representing each of the eight ITS groups (based on analysis by SSCP), and the commercial formulation AQ10, were tested for their tolerance to Systhane, Dorado, Nimrod, Topas, and Sulphur. The effects of fungicide on spore germination and colony growth were investigated. Dorado had no significant effect on the rate of germination of isolate DSM 2223, but otherwise all fungicides had a suppressive effect on spore germination of the isolates examined. This suppression varied between 15.28% for Systhane on isolate CBS 130.79 and 89.63% for Topas on isolate 265. Fungicide application to established colonies seemed to have little initial effect, but by 21 days post application hyphal growth had been arrested. Sulphur and Systhane had the least and the greatest effect on hyphal growth rate respectively.

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882 - The main wheat leaf spot pathogens in the Czech Republic in the last years

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Leaf spot diseases of wheat are caused by different pathogenic fungi. This study was focused on relatively new wheat leaf spot diseases in the Czech Republic especially on tan spot caused by *Pyrenophora tritici-repentis* (anam. *Drechslera tritici-repentis*). The occurrence of wheat leaf spot diseases was monitored in the Czech Republic in 2000 and 2001. The samples were collected from different localities of the Czech Republic. Fungal pathogens were determined with microscopic methods. Frequency of the pathogens varied in both years because the occurrence of fungal leaf spot pathogens depends on climatic factors especially on the temperature, the humidity and the precipitation. The most prevalent fungal leaf spot pathogens of wheat were *Pyrenophora tritici-repentis* and *Phaeosphaeria nodorum* (anam. *Stagonospora nodorum*) in 2000. *Mycosphaerella graminicola* (anam. *Septoria tritici*) was the most common fungus isolated from the wheat leaf spot samples collected in 2001. *Didymella exitialis* (anam. *Ascochyta* sp.) was one of the most important pathogens in 2001. Probably it has not been described in the Czech Republic yet. This study was supported by the Grant Agency of the Czech Republic (project No. 521/00/0226).

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883 - The grass endophyte gene for the first step in ergot alkaloid biosynthesis

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The endophytic *Neotyphodium* spp. grow in aerial tissues of grass hosts where they cause no symptoms and exhibit no signs; yet, often they help protect against insects, nematodes and vertebrate herbivores, increase drought tolerance, and improve plant growth and nutrient acquisition. Several endophyte alkaloids are variously active against insects and/or vertebrates. Among these are ergot alkaloids, including lysergic acid and its peptine derivative, ergovaline. Ergovaline is closely related to ergotamine, which is produced by the infamous ergot fungus (*Claviceps purpurea*), and is a potent mycotoxin (but a useful pharmaceutical at low doses). Ergovaline is thought to be responsible for toxicoses and reproductive problems in livestock that ingest certain endophyte-infected fescues and ryegrasses. The *dmaW* gene, encoding the probable first step in ergot alkaloid biosynthesis (dimethylallyltryptophan synthase), was cloned from *Claviceps fusiformis* and *C. purpurea*, then from the tall fescue endophyte, *N. coenophialum*, and a *Neotyphodium* sp. (Lp1) from perennial ryegrass. There were two *dmaW* genes in *N. coenophialum*, but only one in Lp1. Marker-exchange mutagenesis of *dmaW* in Lp1 eliminated production of ergovaline as well as simpler ergot alkaloids, confirming the role of the *dmaW* gene. Mutagenesis of toxin-production genes in this manner presents opportunities to develop forage grass cultivars with protective endophytes that lack anti-livestock activities.

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884 - A polyphasic study of *Fusarium* species in the section *Sporotrichiella* - different approaches for distinguishing closely related fungal species

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An integrated systematic study was carried out to clarify the taxonomic position and relationship of *Fusarium langsethiae* Torp and Nirenberg, ined. within the *Fusarium* section *Sporotrichiella*. Different isolates of this species were compared with strains of the closely related species *F. poae* and *F. sporotrichioides* using a composite data set. This set consisted of DNA sequences derived from the intergenic spacer region (IGS), the internal transcribed spacer region (ITS) and partial sequences of the b-tubulin and the elongation factor-1 alpha (EF-1a) gene, AFLP fingerprints, chromatographic data on secondary metabolites and morphology. From these combined data a consensus matrix was calculated. This was used as the basis for the construction of an UPGMA dendrogram and a multidimensional scaling which both revealed a clear separation of these three taxa. The discriminatory power of the different approaches varied significantly. The correlation of the clustering obtained by analysis of the combined data and that one obtained by an individual experiment was worst with ITS sequences, whereas AFLP appeared to reflect the composite data matrix best.

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885 - Development of SCAR-PCR primers for the detection of ochratoxinogenic Aspergilli

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The production of ochratoxin A (OTA) is described for *Penicillium verrucosum*, *P. nordicum* and a wide variety of *Aspergillus* species belonging to different sections. Besides the taxonomic inhomogenity of OTA producing fungi the ability to synthesize OTA is highly variable even within a species. AFLP (amplified fragment length polymorphism) typing revealed, that no clear correlation of the ability of a strain to produce the toxin and the taxonomic position can be found. As a consequence it was decided to develop group specific PCR primers for potential OTA producing *Aspergillus* species. The strains were screened for polymorphic DNA markers using AFLP. Bands that were considerd to be characteristic for a specific group were cut out from silverstaind polyacrylamide gels. The DNA was

eluted from the gel, reamplified and the fragments were sequenced. Based on these results SCAR-PCR primers (sequence characterized amplified region) were constructed, the PCRs were optimized and tested against DNA of different fungal strains. The results show that this approach is valuable for the rapid early detection of potential ochratoxinogenic fungi in food.

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886 - Identification of *Malassezia* species in patients with pityriasis versicolor submitted to the Razi Hospital in Tehran

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Lipophilic yeasts of the genus *Malassezia* (*Pityrosporum*) belong to the normal flora of human skin and many warm-blooded animals. These fungi can produce a diverse range of diseases that most frequent of them is pityriasis versicolor, a chronic recurrent skin disease occurring primarily in tropical regions. The genus *Malassezia* has been recently enlarged to include seven distinct species. This survey was undertaken to present a practical approach for differantiation of all *Malassezia* yeasts isolated from clinical materials of patients with pityriasis versicolor for the first time in Iran. The presence of disease was confirmed on the basis of the observation of budding yeast cells and short curved hyphae in skin specimens by direct microscopy. *Malassezia* yeasts were isolated after culturing the samples on modified Dixon (mDixon) agar. A combination of different characteristics includes yeast cell morphology, ability to growth on sabouraud dextrose agar, catalase test and ability to utilize individual Tweens (20,40,60,80) were used for identification of species. In general, 138 patients with pityriasis versicolor includes 52.2% male and 47.8% female were identified. Direct microscopy and culture results were positive in 94.4% and 63% of patients, respectively. Totally, 91 isolates of *Malassezia* belonging to four different species, *M. globosa* (66 isolates), *M. furfur* (18 isolates), *M. obtusa* (5 isolates) and *M. sympodialis* (2 isolates) were identified.

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887 - Allergic fungal sinusitis (AFS) among the candidates of functional endoscopy sinus surgery (FESS)

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This study was designed to determine the rate of AFS among the patients with chronic sinusitis who failed to respond to repeated courses of antibiotic and were candidated for FESS in Sari. Allergic mucin and Sinus lavage were collected during FESS from 100 Patients meeting diagnostic criteria for AFS and were submitted for mycology and pathology investigations. The specimens were centrifuged and the sediment was mounted in KOH and Gram stained for direct examination. Multiple fragments of mucosa were removed at surgery and stained with H&E and PAS for the pathology evaluation. In this study we report 9 proven cases of AFS (with demonstration of fungal hyphae by direct exam and culture) and 8 suspected cases (confirmed by direct exam or culture). The patients' age ranged 12 to 62 years with a mean age of 24.5 years with female predominance. All of the patients were immunocompetent. 47% of the patients had a history of atopy. Histopathologically, hyphae were not seen. The genera of the fungus identifiable in all but in one patient it was not identifiable. 53% of isolated fungus are of hyaline hyphomycete particularly *Aspergillus* and *Penicillium* and 47% of them are members of dematiaceous family particularly *Cladosporium* and *Nigrospora*. In eleven out of the seventeen, fungal hyphae were noted and in all of the seventeen, there were positive fungal culture. During 6 months follow up; we identified recurrence in 2 patients clinically without any microscopy and culture evidence.

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888 - Identification of virulence variety in ascochyta blight fungus [*Ascochyta* rabiei (Pass) Lab.] using differentials lines

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The study was conducted to distinguish the pathogenecity variety of the fungus population in the country. To achieve this goal, 26 isolates were choosing as candidates for 16 provinces that were the most important chickpea production areas. Pathogenecity test was achieved using of 16 differential lines in the greenhouse condition. It was used current methods for classification of isolates as well as a new method on the base of analysis of variance. The later method was better than other methods in classification of variety because of statistical base. In this method differential lines have been grouped in six levels of resistance and isolates have been grouped in six pathotypes. Also their distribution pattern has been identified in Iran. Results of this method are more useful for resistance selecting in national mass. As whole, the study revealed that there were too vigorous pathogenic group in west and north west of the country, that none of tested lines didn't show any resistance an this study. It seems that it is better to design breeding projects for screening of resistance line regarding to identify pathotype groups in each region.

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889 - Performance of *Pinus roxburghii* inoculated with pure culture of four indigenous ectomycorrhizal fungi

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Many ectomycorrhizal fungi were isolated from the sporocarps of different places such as (Dadheldhura, Makwanpur and Palpa district etc.) of pine forest of Nepal.Out of the isolated ectomycorrhizal fungi, four different ectomycorrhizal fungi namely *Astraeus hygrometricus*, *Boletus* sp., *Lycoperdon pyriforme* and *Pisoolithus arrijus*. These four fungi were subjected to pure culture synthesis. These fungi were selected from the pot culture experiment. Then these four indigenous pure ectomycorrhizal fungi were inoculated in nursery *Pinus roxburghii* for their performance at two nursery sites, Godavari forest nursery and Chalnakhel forest nursery sites. Their performance were observed after six, nine and twelve months of seedlings. Inoculated seedlings attained better shoot height and had better root length. more lateral roots, more mycorrhizal roots, more weight of shoot and more weight of root per plant. The inoculated transplants had higher survival rate in the field as compared to those which were not inoculated.

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890 - Effect of immunization against candidiasis after ultrasonic treatment of fungal keratoconjunctivitis

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Ocular candidiasis was diagnosed in six 12-weeks old Persian kittens after four-weeks unsuccessful treatment of chronic keratoconjunctivitis by Sofradex. Bilateral corneal erosions and conjunctival infiltrations were detected. Examination of corneal scrapings revealed oval, yeast-like cells attributable to *Candida albicans*. Examination of conjunctival fluid samples by ELISA revealed decreasing of Immunoglobulins A and G quantity with absence of specific IgA and IgG to *Candida* whole-cell antigens. Ultrasound treatment was applied on right eyes of all cats for determination of antifungal activity and immunostimulation of ultrasound in course of 10 applications daily by 10 minutes with 0.2 W/cm2 dosage. Almost full healing was occurred in all, even in contralateral eyes after treatment. *Candida* cells were absent, specific IgA to *Candida* whole-cell antigens were detected in high titers. Probably, ultrasound destroyed *Candida* cells and delivered antigens through epithelium to immune representative cells followed by local humoral immune response formation. Local immunity was stimulated by ultrasound as well. This immune response was not limited in exposed eyes, but was expanded to contralateral eyes due to specific immune cells exchange. Thus, ultrasound can be useful for some transmucous vaccine delivery systems and for specific treatment without infectious agents determination, particularly, in parasiteocenotic cases.

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891 - Role of salicylic acid in systemic resistance induced by *Pseudomonas* spp. against *Macrophomina phaseolina* in chickpea

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Pseudomonas fluorescens isolate 4-92, applied to roots, induced systemic resistance against charcoal rot disease in chickpea roots caused by *Macrophomina phaseolina* (Tassi) Goid. Salicylic acid (SA) from bacterial culture on plant tissues was quantified by high performance liquid chromatography. *P. fluorescens* produced SA in king's B broth and also induced chickpea roots to accumulate endogenous SA one day after bacterial inoculation. Using a split root system, more SA accumulated in roots treated with bacteria than in distant roots on the opposite side of the root system in the first two-three days, but this difference disappeared after 5-6 days. SA levels were significantly higher in plants treated with bacteria compared to the split control, from one to six days after bacterization. SA did not inhibit sclerotial growth of *M. phaseolina* at 100-200 µg ml^{-1}, invitro, but higher levels inhibited sclerotial growth. Exogenously applied Sa failed to induce local or systemic resistance against a challenge infection by the pathogen in planta. The results of this study show that exogenous applied SA does not induce systemic resistance against charcoal rot disease in chickpea root caused by *M. phaseolina*, but endogenous SA accumulation in chickpea roots may be involved in induced systemic resistance.

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892 - Investigations of *Phytophthora infestans* oospores in Moscow region

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Our purpose was to investigate naturally formed oospores in Moscow region. On the presence of oospores, 1099 blighted potato leaves, 123 tomato leaves, and 188 tomato fruits were checked. The frequency of oospores in potato leaves was 5.6%. In tomato fruits and leaves the oospores were found more often. Their frequencies were 22.8 and 42.0% respectively. This can make the problems for tomato cropping in Moscow region. The oospores had a quite different morphology. They varied in size (their diameter was from 18 μ to 48 μ), thickness of the wall, as well as in the presence of antheridium and the form of oogonium. According to these features, in 1999 and 2000 45 morphological types were established among detected oospores. They corresponded to different stages of oospore maturation. The oospores can be formed as a result of cross-fertilization between mycelia of A1 and A2 mating types (hybrid oospores), as well as of self-fertilization, and of parthenogenesis (non-hybrid oospores - from one mycelium). Studying the distribution of mating types and morphology of oospores it was possible to assess their origin. It was found that in 1999 and 2000 non-hybrid oospores occurred much more often than hybrid oospores. The estimated frequency of hybrid oospores was about 15%. The hybrid and non-hybrid oospores may have different role in the infection cycle of *P. infestans*.

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893 - The needle blight fungus *Delphinella abietis* attacks *Abies* species in western Norway

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During the last decade the needle blight fungus *Delphinella abietis* has been attacking various *Abies* species in western Norway. A clonal trial with subalpine fir (*Abies lasiocarpa*) was seriously attacked in 2000. It was significant differences between clones in the attack. Some clones were heavily attacked while others were free for symptoms. In a greenery trial of Nobel fir (*Abies procera*), most branches had good quality until 1994 when 31% of the branches were discarded after attack by *D. abietis*. Since then a high level of losses has been noted and 87% of the branches were discarded in 2000. It seems that frost did not initiate or maintain the disease in the stand, but generally low temperatures may have been important.

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894 - Toxicological characterisation of animal feed using *in vitro* methods

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Mould growth on animal feed is a worldwide problem that can reduce the quality of the feed. It is known that moulds can produce mycotoxins, which can be mutagenic, carcinogenic but also neurotoxic. Poisoning by mycotoxins can cause suffering for animals, massive economical losses and a possible transfer of toxins to humans via the foodstuffs. Therefore there is a need for identification of feed of bad quality that may possess hazards to animals and/or humans. *Aspergillus fumigatus*, which is known to produce, among other toxins, gliotoxin, was chosen for development of a screening method for various toxic substances in animal feed. Three matrixes - maize, commercial animal feed and wheat - were adjusted to water content 12, 21 and 32%, and inoculated with *A. fumigatus* for 3 days, 2 and 4 weeks at 37 °C. The samples were extracted and purified by solid-phase technique. HPLC and LC-MS was used to detect gliotoxin as a marker of toxic substances produced by *A. fumigatus*. No gliotoxin was detected in the samples. The purified extracts were also applied to the human neuroblastoma SH-SY5Y cells, and the general cytotoxicity was determined. The extracts showed to be cytotoxic to the SH-SY5Y cells. The results indicate that the screening method based on the SH-SY5Y cells can be used for screening of toxic metabolites of *A. fumigatus*. Further research is in progress to investigate if the method can be applicable for other toxic substances.

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895 - A nucleic acid probe detects different species of *Pestalotiopsis* spp. isolated from five fruit crops

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Fruit crops play a major role in the economy of the country. *Pestalotiopsis* species causes leaf spot, leaf blight and fruit rot diseases in many fruit crops. The reports in the literature say that the *Pestalotiopsis* species are named after the host it is isolated from. To overcome this discrepancy, in the present studies, the pathogen was isolated from the leaves of mango grapes, guava, sapota and macadamia, from the berries and canes of grapes and also from fruits of guava. The pathogen was cultured on Potato Dextrose Agar medium. Total Nucleic Acid of the fungus was isolated from each isolate. A 5.3 kb EcoRI fragment generated from genomic DNA of the mango isolate was labeled with DIG and was used as a probe. Genomic DNA of the fungus isolated from mango, sapota and grape leaf bound to the probe strongly while that from grape berries, macadamia and guava bound to the probe only at low stringency. The isolate from grape cane did not bind to two other pathogens commonly found on fruit trees namely *Greenari uvicola* and *Colletotrichum capsici*. Morphological differences in the spores and cross-pathogenicity of these isolated were also found.

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896 - Enhancement of seedling vigour and biological control of *Alternaria* leaf spot of chili (*Capsicum annum* L.) using *Trichoderma harzianum*

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Isolates of *Trichoderma harzianum* enhanced seedling vigour in Chili (*Capsicum annum*) germinated in potting soil. When chili seeds were sown in the potting soil inoculated with *Trichoderma harzianum* 25-92 or 29-92 the germination rate increased by 15 and 20%, respectively, compared with water treatment control. After 5 days of growth, the average plant height increased by 25 and 39%, respectively; the average fresh weight increased by 33 and 36%, respectively. The level of nitrogenase, peroxidase, glucanases, chitnases and average phenolic content in the roots was evaluated as key indicators of vigour. 1.5 to 3 fold increase in nitrogenase, peroxidase and chitinase was recorded, whereas, the phenolic contents increased by 18 and 17%, respectively. There was no any significant increase in glucanses in roots. Total chlorophyll in first, second and third leave was increase by 35-56%. The plants were exposed to *Alternaria solani* by spraying the conidial suspension on leaves of 3-week-old plants previously assesed for enhanced vigour. No disease was recorded on the plants indicated that pretreatment of chili seedlings at nursery level with *Trichoderma* isolates induced resistance in the plants.

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897 - Use of a microcosm system for biological screening against *Botrytis cinerea* on *Pinus sylvestris* seedlings

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Botrytis cinerea, the causal agent of grey mould, is the most common and serious pathogen in all Swedish forest nursery production. Most nurseries make use of fungicidal spraying programs to prevent grey mould infections. Biocontrol has so far not been tested in forest nurseries even if biological control agents (BCA) are used internationally in agriculture. We set up and standardised an inoculation method in a microcosm system, which would allow us to screen fungal and bacterial isolates against *B. cinerea*. In this microcosm system small *P. sylvestris* seedlings are heat stressed before inoculated with a spore solution containing pathogen spores, BCA or a combination of those. Results are scored ten days after inoculation by examining the seedlings for the presence of *B. cinerea*'s conidia. To test the reliability of this microcosm system method, the performance of three commercial biocontrol products, in suppressing *B. cinerea* on pine seedlings was tested. Those results were then compared with two other testing systems, laboratory experiments and field trials using the same commercial products. In all three testing systems we found that the commercial products Binab TF.WP™ and GlioMix™ performed better than Mycostop™ and we concluded that the similarity of the results between the different testing systems was a strong indication for the usefulness of the microcosm system in screening isolates against *B. cinerea*.

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898 - Effect of *Lactobacillus rhamnosus* and a fermented milk on the growth of *Aspergillus* and *Penicillium* species

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This study was done to investigate the effect of *Lactobacillus rhamnosus* VT1 and skim milk fermented by this strain on the growth of *Aspergillus niger* and *Penicillium commune*. Initially, live bacterial cells (0.1% v/v, 1% v/v, 10% v/v), as well as skim milk fermented by *L. rhamnosus* VT1 (0.1% v/v, 1% v/v, 10% v/v), were added to modified MRS broth, which was simultaneously inoculated (1% v/v) with *Aspergillus niger* or *Penicillium commune*. The cultures were incubated at 30 °C for 10 days. The mycelial weight and the pH of the broth were determined on days 0, 2, 4, 6, 8, and 10. All concentrations of *Lactobacillus rhamnosus* VT1 as well as the fermented skim milk were found to significantly reduce the growth rate of both *Aspergillus niger* and *Penicillium commune* in modified MRS broth. However, the *L. rhamnosus* VT1 treatment was slightly more effective than the fermented milk treatment at controlling mycelial growth of both *P. commune* and *A. niger*. *Lactobacillus rhamnosus* VT1 at 1% caused a 50% reduction in mycelial weight of *P. commune* and a 36% reduction in mycelial weight of *A. niger* at 10 days of incubation, while the fermented milk caused a 27% reduction in mycelial weight of *P. commune* and a 34% reduction in mycelial weight of *A. niger* for the same time period. These results suggest that *L. rhamnosus* VT1 has potential as a biological control agent of molds in the food industry.

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899 - Research and education in specialty gourmet and medicinal fungi in Tasmania, Australia

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The research group at the Tasmanian Institute of Agricultural Research is the main research and euducation provider for the specialty mushroom industry in Australia. Current research projects investigate the edible fungi: *Grifola frondosa* (Maitake), *Morchella* species (Morel) and *Tricholoma matsutake* (Matsutake). The edible fungus *Morchella* has been collected in Tasmania, in situations ranging from forest through to garden soil and bark chip mulch. Twenty six cultures were obtained and, on the basis of fruitbody morphological characters, placed into one of the three following species: *M. elata*, *M. deliciosa*, *M. esculenta* var. *crassipes*/*angusticeps*. Maitake has been grown, using the Japanese bag method, to assess the response of this fungus to eucalypt substrate ameliorated with different percentages of rice bran, maize meal and wheat bran. Findings indicate that only relatively small quantities of additive will be required for successful cultivation. The development of an artificial cultivation system for Matsutake (*Tricholoma matsutake*) is commercially desirable and necessary to ensure the survival of this highly prized mushroom. Interaction studies between the fungus and host *Pinus* lateral roots are being undertaken to elucidate the infection process and to understand the exact nature of this atypical symbiosis. In collaboration with the University of Tasmania, the researchers are offering on-line and residential courses on specialty gourmet and medicinal fungi.

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900 - The inmpact of *Phytophthora cinnamomi* in National Parks in New South Wales, Australia

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Although *Phytophthora cinnamomi* is regarded as having a significant impact on native vegetation in many parts of southern Australia, the pathogen has been considered benign and possibly endemic in New South Wales. The evidence for the different behaviour in NSW has included that 1) *P. cinnamomi* is extremely widespread and easy to detect in soils, 2) generally susceptible genera such as *Banksia* are unaffected when it is present (suggesting a long host/pathogen interaction), 3) multiple plant deaths associated with *P. cinnamomi* are extremely rare and 4) *P. cinnamomi* has been recovered in remote areas (suggesting that it is endemic). Recent surveys of National Parks in eastern NSW have found that although *P. cinnamomi* is widespread it cannot be detected in some areas despite extensive soil sampling. Although most *Banksia* spp. seem to be relatively resistant to symptoms of infection, other taxa (especially some *Xanthorrhoea* spp.) are very susceptible. The loss of *Xanthorrhoea* cover may adversely affect threatened animals such as the Smoky Mouse and Southern Brown Bandicoot, which use the plants for cover and nesting. Glasshouse susceptibility trials have shown that a number of rare taxa are very susceptible to infection and molecular analysis has shown that while some variation occurs in populations this variation is limited. *Phytophthora cinnamomi* may be widespread in NSW, however it is doubtful that it is endemic and is certainly not always benign.

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901 - The survey of spring barley and rye contamination by *Fusarium* mycotoxins (artificial and natural infection)

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Majority of wheat and barley varieties are susceptible to *Fusarium* spp. infection, and there is no host specificity in the species *F. graminearum* and *F. culmorum*. Using quantitative ELISA method the DON content was determined in the samples of spring barley artificially infected by *F. culmorum*. 12 varieties were tested in 2000 and 19 in 2001. The other 32 spring barley and 15 rye samples were earned from 32 districts of the Czech Republic. The DON content in artificially infected barley in 2000 varied from 5,1 to 19,1 ppm (average 9,7 ppm); in 2001 from 1,3 to 8,2 ppm. In 2000 the correlation between DON content and TGW-values was close (r = -0,89). The comparison of DON content and TGW values in 2000 and 2001 was performed. DON content in 2001 was lower than in 2000 and TGW- values were in 2001 higher than in 2000. Monitoring of DON content and mycological control in barley samples confirmed DON appearance in all samples (0,03 to 3,77; average 0,4 ppm). The limit value was exceeded in the one case. *F. graminearum* was in 2001 prevailing toxinogenic species. Its occurrence is consistent with higher levels of DON. It confirmed that mycological control of all studied samples is need. There is also evident that toxinogenic species *F. culmorum* was replaced in 2001 by *F. graminearum* which was strong producer of DON too. The DON content found in tested rye samples was very low (0,02 to 0,33 ppm) and therefore the mycological control was not done.

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902 - *Pyrenopeziza betulicola* and *Marssonina betulae* leaf spot fungi in birch: Monitoring the disease development

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In late summer and autumn birch (*Betula* sp.) leaves are full of spots caused by fungi. Often these fungi are also a reason for premature yellowing and defoliation, which may reduce growth and frost hardening of trees. In Finland common necrotic leaf spots causing species are *Pyrenopeziza betulicola* and *Marssonina betulae*. Though these fungi have been characterized and found to be common, there are very few studies of their infection biology or disease development in trees. We have studied leaf spot fungi - *Betula pendula* - interaction. Because global climatic change is assumed to increase atmospheric ozone and CO_2 concentration in the future, we have also followed the effect of these gases on the development of

leaf spot disease. For monitoring the disease development we have used both image analyses technique and visual observation. According to the results the ability of *P. betulicola* strains to infect birch leaves as also the resistance of birch clones to the disease varied. There were also differences between strain-clone -combinations in the intensity and rate of premature yellowing and falling of leaves. The ability of *M. betulae* to cause leaf spots was affected by leaf age. In the older leaves necrotic spots developed faster and the diseased leaf area was also larger. Under ozone and CO_2 fumigation treatments the gases alone didn't explain the disease development, but the disease was dependent also on birch clone, age of leaves and performance year of the experiments.

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903 - Genetic diversity in *Ampelomyces* hyperparasites inferred from SSCP analysis

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SSCP analysis (Single Strand Conformation Polymorphism) allows a rapid and sensitive detection of sequence differences between nucleic acid samples. The method relies on the different migration rates of single stranded nucleic acid fragments. It is known that even a single point mutation can cause a change in the secondary structure and thus a changed running behaviour of DNA or RNA samples in the gel. SSCP can thus be used to rapidly screen a range of individuals for intraspecific genomic variation. This method has not yet been applied widely in plant pathology, but we have recently used it to assess variation in a world-wide collection of isolates of the mycoparasitic genus *Ampelomyces*. These fungi are common intracellular hyperparasites of powdery mildews. To study their genetic diversity, 29 isolates were studied by SSCP analysis of the rDNA ITS region. Based on SSCP profiles, the isolates were included in eight different groups. These results largely supported earlier data [1, 2], but revealed additional genetic differences, too. Interestingly, a new group of isolates were delineated that consisted of all *Ampelomyces* isolates obtained from apple powdery mildew in Hungary. 1. Kiss, L.: Mycol Res 101, 1073 (1997). 2. Kiss, L., Nakasone, K.K.: Curr Genet 33, 362 (1998).

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904 - Mixed infection of *Trichosporon cutaneum* and *Candida parapsilosis* in a white piedra case from Qatar

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White piedra is a rare fungal infection of the hair shaft characterized by small, firm, irregular white-brown nodules. The infection is due to *Trichosporon cutaneum* (= *T. beigelii*). The disease occurs in tropical and subtropical areas and reported in Saudi Arabia and Kuwait. Until now, where a 28 year old female patient acquired the infection, it was not reported in Qatar. In this case the scalp was the only site affected but in a very extensive manner. The hair had yeast odour and appeared beaded with nodules. Under the microscope, the nodules were light-brown made of a compact mass of arthrospores arranged perpendicularly around the hair shaft and varying in size, measuring up to 1.5 mm. Sabouraud`s culture developed rapidly *T. cutaneum* accompanied by *Candida parapsilosis* along the infected hair shaft at room temperature and 37 °C. Colonies of *T. cutaneum* were wrinkled, tan and cycloheximide negative. Assimilation profile was consistent with the organism identification. Microscopic examination showed hyphae which fragmented into rectangular arthrospores and budding cells. The patient treated by daily application of Econazol (shampoo & cream) followed by Ketoconazole shampoo, completely cured (clinically & mycologically) after two months. White piedra infection in this patient is caused by mixed infection with *C. parapsilosis*. It is not clear if the disease was due to the synergistic action between *T. cutaneum* and *C. parapsilosis*.

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905 - Allergic *Aspergillus flavus* rhinosinusitis: A case report from Qatar

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Fungal involvement of the rhinosinusitis is classified into four major forms: mycetoma, invasive, allergic and fulminant form. It can become life-threatening if not diagnosed and treated properly. The preliminary diagnosis is usually by nasal endoscopy and computed tomography (CT) imaging but tissue biopsy and culture is of vital importance in confirming the disease and in planning treatment. We present a case of allergic fungal rhinosinusitis caused by *Aspergillus flavus*. Clinical manifestation of the disease was the presence of an extensive left nasal polyp. Allergic workup revealed systemic eosinophilia (11.7%), High serum IgE level (1201 IU ml^{-1}) and positive skin test for *Aspergillus*. CT scan showed a total opacification and expansion of the left nasal cavity and sinuses with secondary inflammatory reaction on the right side. There was no bony erosion beyond the sinus walls. The patient has been operated by endoscopic approach (polypectomy and ethmoidectomy) where an abundant amount of allergic fungal mucin and dark crusts were found filling the sinuses. Fungal hyphae and spores were evident by direct lactophenol mount and by histopathological sections of the removed polypi. Culture of the debris resulted in growth of *Aspergillus flavus*. The findings were highly suggestive of allergic fungal rhinosinusitis. The patient received a full course of systemic steroids and had no recurrence after 9 months follow up.

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906 - Aflatoxin B1 in Rice bran: Sources of contamination

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A wide survey on the prevalence of Aflatoxin B1 (AFB1) in rice bran was conducted in South India. Sixty two per cent of the samples contained AFB1 and the levels far exceeded the permissible limit of 50 ppb, with 60% of the positive samples having 50-500 ppb and 30% up to 2000 ppb. The mycoflora of bran included over 20 osmophilic species, of which *Aspergillus flavus* Link showed the highest frequency (75%) as well as abundance (37.6%). The high frequency and high levels of AFB1 showed no correlation with moisture content of the samples, climatic variations, or with the raw or parboiled status of the paddy, but with the maintenance of sanitation in the rice mills, suspected to be a major source of contamination of bran with aflatoxigenic fungi/aflatoxin. This was done by subjecting specific lots of rough rice (paddy) to parboiling in the mill followed by drying through different procedures; milling of the raw and parboiled paddy samples separated in commercial mills and in the laboratory, and comparing the mycoflora with reference to *A. flavus* - and AFB1. The mill environment did act as a source of contamination of bran, but only to enhance the toxin which had already formed earlier in the paddy. It was thus a source of secondary contamination, and its actual contribution to the prevalence of AFB1 in rice bran should be determined only by making a survey of the prevalence of AFB1 in paddy in the region and comparing it with that of bran.

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907 - Aflatoxin in conidia of airborne fungi and its relevance for occupational hygiene

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At work places in the waste-handling industry mycotoxin-producing fungi can be found in high numbers in air. In the present study, the amount of mycotoxins associated with airborne conidia was investigated. Species from the *A. flavus* group known to produce aflatoxins were tested as a model system. The production of mycotoxins was investigated for fresh isolates from a composting plant. Conidial suspensions of known aflatoxin producers (*A. flavus*, *A. parasiticus*) were investigated qualitatively and quantitatively for their aflatoxin content. Analysis was done using both HPLC-FLD and ELISA. Extracts of additional species (*A. niger*, *A. versicolor*, *A. fumigatus*, *E. nidulans*) and mycotoxin standards were tested for cross reactivities. The amount of aflatoxin correlated with the number of conidia extracted. *A. parasiticus* conidia contained significantly more aflatoxins (amounts of pg per 10,000 conidia) than those of *A. flavus*. *A. versicolor* showed a slight unspecific reactivity that may be due to sterigmatocystin, a precursor in the aflatoxin biosynthesis. The results show that aflatoxin can be detected in relatively small amounts of conidia. As species of the *A. flavus* gr. occur in numbers of >1000 cfu per cbm air in composting plants, they may be of toxicological relevance. Further investigations are necessary to evaluate the incidence of toxinogenic strains in situ.

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908 - Attraction of phorid and sciarid pest flies during cultivation of *Agaricus bisporus*

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The cultivation of *Agaricus bisporus* is a worldwide, multi-million pound industry. It is susceptible to many pests and pathogens, the dipterans *Lycoriella castanescens* (sciarid) and *Megaselia halterata* (phorid) are most serious causing losses of 7% of annual revenue in the UK. Pest control is largely by chemicals and there is a need for alternative modes of pest control. Determining the source of attraction for these pest flies could provide a method for preventing their infestation of mushroom houses. A static air olfactometer was developed to quantify the behavioural response of female sciarid and phorid flies to mushroom cultivation substrates: pasteurised compost, 4 d spawned compost, fully spawned compost and button mushrooms. Phorid response varied depending on substrate, with greatest attraction to fully spawned compost, those that best support development of this obligate mycelial feeder. Sciarids were attracted to all substrates. Phorid oviposition increased with mycelial growth through the substrate, whilst sciarids exhibited high oviposition on all substrates. Emergence of both species was substrate dependent. Sciarid emergence was highest from pasteurised compost, decreasing with mycelial growth through the substrate indicating that mycelial growth is deterimental to development. Greatest emergence of phorids was from the 4 day spawned compost. Results are consistent with the use of volatiles in detection of oviposition sites for both species.

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909 - Ochratoxin producing *Aspergillus* species in organic raisins and sultanas

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On behalf of the Norwegian National Food Control Authority, a study on potential mycotoxin producing fungi in organically grown raisins and sultanas was conducted. The 61 tested samples were bought in stores in Oslo during spring and summer 2001. The dried fruits were cut in halves and plated out on Dichloran Glycerol agar and incubated at 25 °C for seven days. Potential mycotoxin producers were isolated from all the 15 samples of sultanas, while 44 samples (95%) of raisins were affected. Fungi belonging to the *Aspergillus niger* aggregate were the most dominant, infesting all samples of sultanas and 95% of the raisins, while *Aspergillus carbonarius* was isolated from 93% of the sultanas and 2% of the raisins. The high recovery of these fungi in raisins and sultanas is of concern, since they have been reported as producers of the highly toxic Ochratoxin A (OTA). In this study, 18 strains were analysed by HPLC for OTA production in pure culture on Yeast Extract Sucrose agar. Nine out of 12 tested strains of *A. carbonarius* and four out of six tested strains of the *A. niger* aggregate produced OTA. The ITS-5.8S rDNA RFLP patterns of the OTA-producing isolates in the *A. niger* aggregate were also determined. All four isolates were classified as type N [Accensi et al. 1999], corresponding to the RFLP group '*A. niger*'. The results indicate that OTA analyses of organically grown raisins and sultanas would be of interest to the consumers.

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910 - A monitoring system for green fluorescence protein gene-transformed *Fusarium oxysporum* in melon seedlings

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Using melon seedlings at the cotyledon stage and genetically marked fungi, a system for monitoring pathogenic and nonpathogenic *Fusarium oxysporum* was devised in the present study. Protoplasts were prepared from three formae speciales (*melonis*, *radicis-lycopersici* and *fragariae*) of *F. oxysporum* and transformed with a synthetic gene for green fluorescence protein. Transformants were primarily isolated in the presence of hygromycin B and then screened by the emission of bright green fluorescence. Roots of melon seedlings were inoculated with fluorescing microconidia of these fungi, and fungal infection behavior was traced. Using fluorescence microscopy, we directly observed not only the fungus at the root surface, but also the mycelia elongating in the trachea of roots. Both pathogenic and nonpathogenic fungi germinated and hyphae elongated superficially on the surface of root. Only pathogenic fungi caused root necrosis at the inoculation site. Hyphae grew within the stem to induce constriction or cracking of lower hypocotyls, then causing wilting of the seedlings. Infection behavior of genetically marked pathogenic and nonpathogenic *F. oxysporum* could be successfully monitored after inoculation of cotyledons of seedlings.

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911 - Biological control of bluestain in logs using an albino bluestain fungus

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Bluestain causes significant economic losses to the Canadian forest industry. We examined the feasibility of using an albino bluestain strain (Cartapip^{TM}, now renamed Sylvanex^{TM}) of *Ophiostoma pilferum* to control bluestain in lodgepole pine logs. Cartapip and four challenge bluestain fungi were sprayed alone, and in sequence, on two types of wounds artificially produced on pine billets. When inoculated alone, Cartapip colonized fresh lodgepole pine and did not cause stain. The challenge fungi alone caused significant stain. However, when the challenge fungi were applied 2 or 10 days after Cartapip, the stain was negligible in most cases and Cartapip outcompeted the fungi. Field work using commercial size logs was done in 2000 and 2001 in Alberta, Canada. Cartapip suspension was sprayed on logs and the bluestain development quantified and compared with logs sprayed only with water. Statistical analysis of the data after 12-13 weeks of storage showed that Cartapip significantly reduced the amount of bluestain. After 6 weeks of summer storage in 2001, Cartapip-treated logs remained almost spotless compared to the considerable stain found on non-treated logs. After 13 weeks of storage there was some stain development in Cartapip-treated logs but the amount was significantly less than in non-treated logs. The product, and the concept of using albino isolates to control stain, therefore have potential for industrial use.

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912 - Expression of fruit body color in *Pleurotus* spp. hybrids derived from pairings of compatible neohaplonts

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The monocaryotic components (neohaplonts) of 10 *Pleurotus* spp. strains (ECS127, ECS187, IB67, 1E202, IE200, IE201, INI8, POROS, RP y P15) producing fruit bodies of contrasting colors were obtained by chemical dedicaryotization. By pairing these neohaplonts, 5 intersterile groups were recognized. Four strains belong to the first group, IB67 (gray), IE200 (white), PORO (pink) y RP (pink) and their neohaplonts showed compatibility factors AmBm, and AnBn, respectively. In the second group, a white (IE201) and a pink (IE202) strain were found; their nehohaplonts could have either completely different compatibility factors AoBo and ApBp or the complementing factors of the first group, AmBn and AnBm. In the third group, 2 gray strains, INI8 and ECS127, were found with compatibility factors AqBq and ArBr, while a yellow strain, ECS187, was found in the fourth group with compatibility factors AsBs and AtBt. Finally, a gray strain, P15, was found in the fifth group with compatibility factors AuBu and AvBv. Thirteen hybrid dikaryons were obtained by cross mating neohaplonts from groups 1 and 2 and they were grown on a commercial *Pleurotus* sp. substrate (feremented straw). Gray fruit bodies were obtained from 11 hybrids and, unexpectedly, yellow fruit bodies were produced by two hybrids resulting from matings of neohaplonts from a white strain (IE201) and a pink one (IE202). Regulation of gene expression or extrachromosomal factors to explain such results is discussed.

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913 - New plant pathogenic fungus from the sterile white basidiomycete complex

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An isolate of Sterile White Basidiomycete (SWB) was obtained from the visually healthy roots of buffalo grass (*Pennisetum landistinum*), growing in a natural bush land in Perth, Western Australia. The greenhouse pathogenicity tests revealed that the fungus is able to infect 12 different plant species. This isolate was compared with a strain of SWB, obtained from the American Type Culture Collection (ATCC 28344), originated from Florida, United States, which has been reported as an aggressive pathogen on 16 genera of agricultural crops. Mycelial features, growth rate, as well as pathogenicity pattern and inoculum potential of these two pathogens were distinctly different. In addition, sequence analysis of the Internal Transcribed Spacer (ITS) regions, 5.8S and the first 800 bp of the 25S ribosomal RNA genes strongly supported that the Australian fungus was clearly different from the American isolate. The sequence data confirmed the previous report by another research group that the American SWB strain belonged to *Marasmius graminearum*. Further studies are carried out in order to resolve the taxonomic position of the Australian SWB.

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914 - The biodiversity of microfungi from selected organically produced fruit and vegetable commodities from Norway

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The mycofloras of four organically produced fruit and vegetable commodities; dried fruit, strawberry, corn and carrot, for sale in Norway were examined. The species of fungi obtained from each food commodity were mostly saprophytic. Xerophilic fungi such as *Eurotium* spp., *Aspergillus* spp., and *Wallemia sebi* were isolated in many dried fruit samples along with fungi capable of food spoilage such as, *Rhizopus* spp. and *Zygosaccharomyces rouxii*. Mycotoxin- producing *Aspergillus flavus* was also isolated, and aflatoxins were also detected in low levels in these samples. The fruit spoilage pathogen, *Botrytis cinerea*, was frequently isolated from all strawberry samples along with two other strawberry pathogens *Idriella lunata* and *Colletotrichum gloeosporioides*, which were less frequently isolated. Most fungi obtained from corncob kernels were aerial contamination species such as *Cladosporium* and *Epicoccum*, Zygomycetes and yeasts. The fungi obtained from carrot samples were common saprophytic species frequently reported from soil and roots, although carrot spoilage pathogens such as *Thielaviopsis basicola* and *Rhizoctonia carotae* were also isolated in low numbers.

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915 - Physiological regulation of biomass and polysaccharides production by medicinal mushrooms

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Agaricus nevoi, *Inonotus levis*, *Phellinus igniarius*, *Ph. robustus*, *Pleurotus ostreatus*, and *Tremella mesenterica* biomass and polysaccharide production in dependence on the carbon and nitrogen sources have been investigated. All strains grew well in the presence of glucose, maltose or mannitol in the medium yielding in shake flasks experiments up to 13-18.9 g l^{-1} of crude biomass-polysaccharide ethanol precipitate. Among carbon sources studied, glucose appeared to be the best for the polysaccharide production by *I. levis*, *Ph. igniarius*, *Ph. robustus*, *P. ostreatus*, whereas *T. mesenterica* produced the highest level of polysaccharide during growth in the presence of sucrose and mannitol. It has been shown that the yield of polysaccharides correlated with carbon source concentration in the medium. Among nitrogen sources studied, organic compounds and ammonium sulfate

stimulated fungi growth and polysaccharides production. The best polysaccharide producers, *I. levis* and *T. mesenterica*, were cultivated in BioFlo Fermentor 2000 (New Brunswick, USA). In this case yield of crude protein-polysaccharide products reached 23 and 27 g l^{-1}, respectively. Data on the dynamic of fungi growth in fermentor indicate that in the culture of *T. mesenterica* biosynthesis of polysaccharide parallels to fungus biomass growth, while in the case of *I. levis* polysaccharide synthesis mainly occurs in the later logarithmic phase of growth.

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916 - Mechanism of wilting in oak mortality in Japan caused by *Raffaelea quericivori*

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Mass mortality of oaks (mainly *Quercus serrata* and *Q. crispula*) has been appeared along the Japan Sea since 1980's. Blockage of xylem sap ascent induced by infection of a fungus *Raffaelea quericivori*, which is vectored by ambrosia beetle *Platypus quercivorus*, is considered to have close relationship with this mortality of oaks. Lower stems of *Q. serrata* and *Q. crispula* seedlings were inoculated with *R. quercivori*. Dye treatment revealed that blockage of sap-flow occurred only around the inoculation points. Symptoms during wilting after the *R. quercivori* inoculation and those after cutting of lower stem were similar. Further, supplying water into stem lengths cut from upper half of the seedlings just after the appearance of wilting also delayed the further development of wilting in both of them. Water conductivity in the stem was measured with a high pressure flowmeter (HPFM) after the fungus inoculation onto *Q. crispula* seedlings, which survived after the inoculation. A parameter of chlorophyll fluorescence, Fv/Fm, decreased slightly in several seedlings. Conductivity was not changed in the upper part of the seedlings, though conductivity aroud the inoculation points was conspicuously reduced in fungus-inoculated seedlings, especially ones with lowered Fv/Fm. Our results suggested that upper part of the seedlings was not directly affected by the fungal inoculation, and that wilt was induced by the blockage of sap ascent around the inoculation point.

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917 - *Penicillium* fungi from *Picea glehnii* seeds protect the seedlings from damping-off

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We have postulated that germinating seeds and seedlings of *Picea glehnii* might be protected from pathogens by seed-epiphytic fungi that produce antimicrobial compounds. We screened seed-epiphytic microorganisms that produced antibiotics against pathogenic *Pythium vexans* by the agar-on-paper method. Of the 149 isolates, 13 fungi showed high antifungal activities and they were identified as *Penicillium* fungi. Three isolates (*P. cyaneum*, *P. damascenum*, and *P. implicatum*) showed high antifungal activity and they produced patulin, citrinin, palitantin and frequentin, respectively. In the co-inoculation test of three *Penicillium* species and *P. vexans* to *P. glehnii* seedlings, the number of surviving seedlings which were inoculated with *P. vexans* 5 days after pre-inoculation with *P. damascenum* increased compared with those inoculated with *P. vexans* alone. Dense mycelial growth of *P. damascenum* was observed microscopically around the roots of the inoculated seedlings. Furthermore, the antifungal and phytotoxic compound citrinin produced by *P. damascenum* was detected as a component released around the roots of the normally growing fungus-inoculated seedlings. We proposed two possible mechanisms for the protection of *P. glehnii* seedlings by *P. damascenum* from *P. vexans*; first, chemical protection by an antibiotic agent, citrinin produced by *P. damascenum*, and second, the occupation of the space around the *P. glehnii* roots by the *P. damascenum* mycelia.

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918 - Growth inhibition for wood decaying fungi by lichen mycobionts

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About 400 strains of lichen-derived cultures induced natural thalli and spores are maintained in our laboratory and we previously indicated that they showed several biological activities. In natural conditions, lichens may oppose mushroom on the bark. Screening of growth inhibition of two wood decaying fungi, *Trametes versicolor* (L. ex Fr.) Pilat and *Fomitopsis palustris* (Berk. et Curt.) Gilbn & Ryv. in 46 strains of lichen mycobionts cultured. Two cell-aggregates of each mycobiont strain were placed oppositely on agar-plates of malt-yeast extract medium, glucose peptone medium and potato dextrose medium in a Petri dish and pre-incubated at 20C in the dark. After a month, one agar block of malt-yeast extract medium on which a wood decaying fungus grew was inoculated in the center of the agar-plate on which its mycobiont grew and maintained at 20C in the dark. After a week, inhibitory effect on growth of a fungus by lichen mycobionts was judged from the extent of its inhibition circle. Three mycobionts of *Acarospora fuscata*, *Arthonia cinnabarina* and *Ramalina exilis* inhibited the growth of both fungi. The growth of *T. versicolor* was inhibited by mycobionts of *Stereocaulon sorediiferum* and *Dibaeis* *absoluta*, on the other hand that of *F. palustris* was done by those of *Cladia aggregata*, *Haematomma puniceum* and *Xanthoria elegans*.

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919 - Wood stain and causal fungi on Canadian hardwood species

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In most countries, trees are harvested into logs and stored in a log yard for a certain time before sawing into lumber. Logs left lying on the ground during warm weather are vulnerable to attack by sapstaining fungi. Wood sapstain is caused by several groups of fungi that penetrate deeply into wood with dark pigmented hyphae. Hardwood species are used to a greater extent in value-added wood products, reducing sapstain in these species has a significant economical impact. This study investigated the speed of sapstain development in Canadian hardwood species and the major fungal species involved in this discoloration. Trees of sugar maple and yellow birch were felled in June 2001. Logs were transported to and stacked in a sawmill within two weeks after felling. The evaluation was conducted every 2 weeks. Logs were sampled into discs, stain development on these discs was examined, and isolation and identification of staining fungi were followed up. The results showed that stain was detected on logs 3 weeks after harvesting and logs were stained less than 5% of wood in 5 weeks. After 13-week storage, most sapwood of logs was stained. Yellow birch was more susceptible to fungal stain than sugar maple, and log ends were more stained than the internal sections. The frequently isolated sapstaining fungi were *Ophiostoma piceae*, *O. piliferum*, *Leptographium* sp., *Aureobasidium pullulans*, *Alternaria alternata*, and *Cladosporium cladosporioides*.

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920 - Production, formulation and application of a bioprotectant for wood protection

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Moulds, stain and decay cause serious problems on wood utilization. Though a growing volume of wood is kiln-dried, the market for green exports remains significant, and environment-friendly wood protection is required to replace traditional chemicals. As a solution to this problem, Forintek Canada Corp. developed a bioprotectant for protecting logs and green lumber from moulds, stain and decay, which was granted a US patent. The method relies on an albino fungus, *Gliocladium roseum*. One objective of the project was to assess the technical feasibility of large-scale production and utilization of this bioprotectant. Fermentation in 150L fermentors was achieved within three days. The spores and mycelia of the fermented fungus were formulated into a powder form. A shelf-life study indicated that this bioprotectant did not lose any of its vigour after one-year storage at -20 °C. Large-scale application and efficacy tests were carried out on 2400 boards in a sawmill; the lumber tested was a mix of 2"x 3" x 8' black spruce and balsam fir boards. The treatment was applied in two different manners: a) with a spray system normally used for chemical application, and b) by dipping in a tank. After three months storage at the mill, 100 percent of the treated board were found to be acceptable as compared to only 36 percent for untreated controls.

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921 - Medicinal mushrooms as inhibitors of angiogenesis

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Medicinal mushrooms have been an important source of therapeutic substances for the treatment of various human illnesses including cancer. In Japan, few polysaccharide antitumor agents have been developed such as lentinan, krestin, and schizophyllan. Angiogenesis is an essential component of the body's physiology and contributes to the pathogenesis of a variety of diseases such as psoriasis, rheumatoid arthritis, and cancer. Our objective is to select a medicinal mushroom extracts with anti-angiogenic and antitumor activities, monitoring ability of selected mushroom extract to interfere with the proliferation of endothelial cells and other tumor cell lines. Our interest is on selecting mushroom extracts that promote apoptosis of the appropriate cell lines. We developed rapid, accurate and reliable assays that allowed us to detect different events of apoptosis. More than thirty species of edible and medicinal higher basidiomycetes were evaluated. Screening data of selected medicinal mushroom extracts will be presented. Selected mushroom extracts or fractions that show promising activity in the screening assays will be further evaluated in animal models.

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922 - Identification of viruses from diseased edible mushrooms, *Pleurotus ostreatus*, *Pleurotus eryngii* and *Flammulina velutipes*

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Natural viral epidemic was observed in the commercial farms of edible mushroom, *Pleurotus ostreatus*, *Pleurotus*

eryngii, *Flammulina velutipes* in Korea. The disease always accompanied the presence of isometric virus particles. Using purification procedures involving Tris-EDTA buffer extraction, PEG-NaCl precipitation, differential centrifugation, and equilibrium centrifugation in CsCl gradient (1.585 g/cm3), we have obtained three isometric viral particles, one of 27 and two of 34 nm in diameter, with different densities from *P. ostreatus* and *P. eryngii*. The 27 nm particle, denser than 34 nm particle, encapsidated ssRNAs as its genome. There were two kinds of lighter particles with the same size of 34 nm which encapsidated dsRNA. Coomassie Brilliant Blue stained polyacrylamide gel (12%) electrophoresis showed different polypeptides of coat proteins of Mr 29, 71, and 62 kD in viral coat proteins, respectively. These results demonstrated that there are at least 3 different kinds of isometrc viruses whose genomes were ssRNA or dsRNA in *P. ostreatus* and *P. eryngii*. The *Flammulina velutipes* harbored different spherical ssRNA viruses. In an attempt to prove that the viral complex is the causative agent for the disease symptoms we cured the viruses from diseased spawn. The virus-cured spown formed normal mushroom and recovered from the epidemic. Therefore, the phenonena of mushroom malformation and reduced harvest in the mushroom farms could be of viral nature.

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923 - Comparison of the aflR gene sequences of strains in *Aspergillus* section *Flavi*

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Aflatoxins are polyketide-derived secondary metabolites produced by *Aspergillus parasiticus*, *A. flavus* and *A. nomius*. Human health and economic benefit are most affected by the toxic effects of aflatoxins. The aflR gene is a regulatory gene of aflatoxin biosynthesis and encodes a protein containing a zinc cluster DNA binding motif. Although *A. oryzae* and *A. sojae*, used in fermented food and ingredient manufacture, never have a record of producing aflatoxin, they have shown the presence of aflR gene. In this study, 34 strains belong to the *Aspergillus* section *Flavi* were examined and the aflR gene of 23 strains of these strains were successfully amplified and sequenced. No PCR products of aflR were found in five strains of *A. sojae* and six strains of *A. oryzae*, these results suggest that the aflR gene might not exist or significantly different in some strains of *A. sojae* and *A. oryzae*. The sequenced aflR genes of the 23 strains have 96% similarity, especially the zinc finger DNA-binding domain are highly conserved. The aflR gene of *A. sojae* has conspicuous character, an extra CTCATG fragment inserted and a C to T transition change cause earlier termination of the encoded AFLR protein. The differences between *A. parasiticus*/*A. sojae* and *A. flavus*/*A. oryzae* were also found in some bases of aflR gene. Although the aflR genes have no obvious difference between *A. flavus* and *A. oryzae*, some differences may exist in aflatoxin producing and no producing strains.

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924 - Sixty-one alleles of the *ALS7* open reading frame in sixty-six *Candida albicans* strains: *ALS7* is a hypermutable contingency locus

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The *Candida albicans ALS* (agglutinin-like sequences) family of genes encodes agglutinin-like proteins believed to play a role in adherence of the yeast to endothelial and epithelial cells and is discussed as a virulence factor of this important fungal pathogen of humans. Using 66 *C. albicans* isolates, representing the major phylogenetic lineages in a worldwide collection of 266 infection-causing isolates, we discovered >= sixty-one different alleles of the *ALS7* open reading frame. Differences between alleles were largely caused by polymorphisms in two nonadjacent repeats, the so-called tandem repeat domain (21 different types occurred) and the so-called VASES domain (21 different types). *C. albicans* is diploid, and combinations of *ALS7* alleles generated >=50 different genotypes. All alleles tested were expressed in human patients; parts of some open reading frames were transcribed in both directions. Isolates representing a more pathogenic general-purpose genotype cluster (Schmid et al., 1999, Microbiology 145: 2405-2414) had a higher number of tandem repeats than other strains; of the 21 types of VASES region found, two were largely exclusive to these strains, the remaining almost exclusive to non-cluster strains. Our results suggest that *ALS7* is a hypermutable contingency locus, important for the success of *C. albicans* as an opportunistic pathogen of humans.

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925 - Arid element in the lichen flora of Kugitangtau (Turkmenistan)

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Kugitangtau is the South-West ridge of the Pamir and Alai mountains and is a constituent part of the Central Asian mountain province. In the flora compositions of lichens have been revieled 240 species, 5 varieties and 12 forms. The analysis of Kugitangtau lichen flora according to geographical element have been done and 9 elements have been singled out: arid (68 species - 28,3% of total species, multiregional (36-15%), eurogolarktical (30-12,5% boreal (18-7,5%), hypoarkto-montane (12-5,2%), arkto-highland (11-4,5%), mediteranean (5-4,5%), montane (12-5%) and non-moral. Among them the species of arid element prevail which refer to 21 genera of 10 families. They include 45 species epilities, 19-epigeides, 8-epiphytes and 2 migrating, but some of them (6 species) can be observed both on the soil and rocks. Turklmenistan is situated in the arid zone, therefore it is natural that the highest percent of Kugitangtau lichen flora includes the arid species. The area spectrum of the arid element accounts for 18 names. This element is mainly represented by xeromeridional, desert and steppe species.The bulk of the arid element is formed by species with central asian (12), european and asian (9), asian(8), xero-merideonal-golarktical (7) and golarktical (6) type of area.

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926 - Population genetics of *Marasmius oreades* in a Norwegian sand dune ecosystem analyzed by DNA amplification fingerprinting

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The population genetics and spatial structure of *Marasmius oreades* was studied at the molecular level. Basidiocarps were collected from fairy rings from the sand dunes on Peninsula Lista in SW Norway. Samples were collected after mapping of fairy rings. DNA amplification fingerprinting (DAF) with arbitrary oligonucleotide primers was used to study genetic relationships between the basidiocarp samples. The fungal populations contained a high number of genotypes and each fairy ring generally represented a separate genet. Both cluster and phylogenetic analyses of the DAF products established relationships between fairy rings and showed that genetically similar basidiocarps were found close to each other. Overall results showed, however, only a weak correspondence between genotype and spatial distribution and no correspondence between genotype and composition of the surrounding vegetation.

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927 - Impact of monsooning of coffee on fungal community structure, enzyme profiles and toxins

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Monsooning of cofee beans is a semi-solid state fermentation process occurring over a 8-10 week period under high humidity conditions of South-west peninsular India. The moisture content of beans undergoing monsooning increases by up to 20%, consequently significant variation in the microbial populations were observed between the non-monsooned and monsooned beans. Total populations of bacteria and fungi varied significantly in these two treatments in both cultivar Arabica and Robusta. Dominant species included *Aspergillus niger* and *A. ochraceus*. Hydrolytic enzyme assays showed that there were significant differences between coffee types indicative of physiological activity of different fungal communities. The level of ochratoxin in naturally and controlled monsooneing was less than 5 ppb. The implications of these results for developing controlled monsooning systems are discussed.

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928 - Promoted growth of cabbage seedlings inoculated with arbuscular mycorrhizal fungus

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Arbuscular mycorrhizal (AM) colonization promotes the growth of host plants. Non-host plants (Brassicaceae, Chenopodiales and Polygonaceae etc.) are not infected and do not build symbiosis with AM fungus. However, the growth of cabbage seedlings (*Brassica oleracea* L. var. *capitata* L., cv. Earlyball) was significantly promoted by the inoculation of AM fungus (*Gigaspora margarita*), though no fungal infection of the root was recognized. The factor of the phenomenon was investigated in terms of the experiment process, the phosphorus condition of the soil and the species of AM fungus. The growth of seedlings was the same to control (no addition of inoculum) in spite of addition of autoclaved inoculum to rhizosphere. This observation shows that the extender included in inoculum did not play a role of source of the nutrients. By addition of P fertilizer to soil, the growth of seedlings at high-P-soil was more vigorous than at low-P-soil and growth promotion by inoculation of AM fungus was additively maintained. All the three AM fungus species (*Gigaspora margarita*, *Glomus fasciculatum* and *Glomus mosseae*) used in experiment promoted the growth of cabbage seedlings significantly. However, the level of the promotion varied among the three species. These results suggest that the existence of AM fungus in the rhizosphere improves the soil condition for plant growth, and it provides a new approach for the plant-AM fungus symbiotic system.

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929 - Interactive effects of nutrient applications and arbuscular mychorrhizal colonization on little bluestem grass (*Schizachyrium scoparium*)

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A manipulative field study was conducted in 1985 to evaluate the impacts of supplemental nutrients (N, P, K and Ca+Mg) on little bluestem grass (*Schizachyrium scoparium*) with a high (20.2 ±1.6%) or low (2.6 ±0.7%) level of colonization by arbuscular mycorrhizal fungi (AMF). Low-colonized plants were grown in autoclaved soil and outplanted into fumigated soil in the field, while high-colonized plants were grown in unsterilized soil and outplanted into unfumigated field soil. Originally, data were analyzed by multiple univariate analyses of variance. Previous conclusions drawn from these analyses were that bases (Ca+Mg) were the limiting nutrients in this system. A reanalysis of the data was performed by means of multivariate statistics. New interpretations suggest that P was the limiting nutrient in this system, since application of P increased growth of *S. scoparium* when AMF colonization was low. However, no effect of P application on growth was found for plants with high levels of colonization. This interaction is explained by the cost of having a fungal partner. Thus, in this sand prairie system AMF restricts growth of *S. scoparium*. In addition, application of bases enhanced AMF colonization under natural conditions.

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930 - Phytopathological characteristics and environmental impact on the different fungi of the *Gaeumannomyces*/*Phialophora* (G/P) complex

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The G/P complex contains fungi that cause take-all diseases on cereals as well as apathogenic fungi. To study the interactions of different groups, about 2000 isolates from numerous geographic locations in Germany were differentiated into species and varieties as well as on the intravarietal level using RAPDs. Based on this strain collection, the phytopathological behaviour of representative isolates was evaluated on several cereal varieties. Beside differences between species and varieties we found a high variation in aggressiveness, especially to wheat, among isolates of var. *tritici*. Whereas one RAPD group of the var. *tritici* consisted exclusively of high pathogenic isolates, the other one contained pathogenic, apathogenic and even growth promoting isolates. In order to predict the potential risk of take-all it is necessary to analyse both the composition of the G/P fungi population and the impact of different environmental factors on the abundance of respective fungal groups. In our study we monitored spatial occurrence and distribution of species, varieties and subgroups of the G/P-complex as a function of soil variables, weather conditions and crop rotation over 4 years. Data show that the various fungi analysed displayed clearly different claims concerning environmental factors. Actual weather and several soil parameters were determined as the most important discriminating influences. Results may provide a useful basis for detailed improvement of prognostic models.

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931 - The direct effect of nitrogen as a mechanism for change in ectomycorrhizal fungal communities

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The mechanisms causing shifts in ectomycorrhizal fungal (EMF) communities exposed to increased nitrogen (N) by atmospheric deposition or fertilization are not understood. The effects of N increase could be direct, or could be due to ancillary effects such as altered pH or a change in limiting nutrients. This study examines the direct effects of increased N by examining the response of EMF communities to a 16-year fertilization experiment in an oak savanna. The experiment consists of two levels of nitrogen fertilization (5 and 17 g N m^{-2} yr^{-1}) and unfertilized plots. Each fertilization treatment also receives equal background levels of P, K, Ca, Mg, and S added to offset ancillary effects of N. Fertilization has increased soil N, held pH constant and not caused limitation by other nutrients. Meanwhile, fertilization 1) decreased the abundance of aboveground EMF sporocarps for all species except *Russula amoenolens* (which increased 7 fold); 2) held belowground EMF richness constant as measured by morphotype and PCR-RFLP methods; but 3) altered the dominant fungi in the EMF communities: Unfertilized EMF communities are dominated by species with extensive external hyphae, notably *Cortinarius* species, while heavily fertilized communities are dominated by those that lack external hyphae, primarily *R. amoenolens*. These data show that the direct effect of N addition may be the driving mechanism behind the dramatic shifts in EMF communities in ecosystems that experience N deposition.

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932 - Ligninolytic enzymes and biodegradation during the interactions of white-rot fungi and soil microorganisms

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White-rot fungi are able to degrade lignin and related compounds. Under natural conditions, the process occurs in the presence of other microorganisms. Introduction of microorganisms to liquid cultures of *Trametes versicolor* led to the increase of activity of the ligninolytic enzyme laccase. High increase was achieved with soil fungi *Trichoderma harzianum* (2810% of control), *Penicillium rugulosum* (1940%), *Fusarium reticulatum* (1690%), *Cephalosporium sphaerospermum* (840%), and *Humicola grisea* (480%). The increase was lower after addition of bacteria - *Escherichia coli* (310%) and *Bacillus subtilis* (190%), or the yeast *Endomyces magnusii* (220%). After one-week cultivation with *Trichoderma harzianum*, the mycelium of *T. versicolor* was killed, which was accompanied by the decrease of Mn-peroxidase activity. Increase of laccase activity is a common response - it was found also in the white-rot fungi *Abortiporus biennis*, *Coriolopsis occidentalis*, *Pleurotus ostreatus*, *Pycnoporus cinnabarinus* and *Trametes hirsuta*. It might be involved in active defence, since some products of laccase exhibit antimicrobial activities. Increase of laccase activity correlated with the increase of decolorization of the synthetic dye Remazol brilliant blue R. It seems, that interspecific interactions can affect the biodegradative activity of white-rot fungi *in situ*. This work was supported by the Grant Agency of the Czech Academy of Sciences (B5020202).

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933 - Population structure of *Ceratocystis fimbriata* from Congo, Colombia and Uruguay, determined using microsatellite markers

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Ceratocystis fimbriata is a haploid ascomycete that causes serious diseases on a wide range of plants, world-wide. Very little is known regarding the population biology or origin of this important pathogen. The aim of this study was to use 11 polymorphic PCR-based microsatellite markers previously designed for *C. fimbriata*, to examine the population structure and genetic diversity for different populations of *C. fimbriata*. Populations consisting of 32 isolates from each of Congo, Colombia and 22 from Uruguay were studied. High genetic diversities for all populations were observed (H = 0.48-0.31; G = 24-48%). Colombia was the most diverse population consisting of 82% of the total, and 53% of the unique, alleles. Genetic differentiation between populations was great (G_{ST}= 0.39) and minimal gene flow was observed (Nm = 0.77). I_A, PTLPT, and linkage disequilibrium tests to determine mode of reproduction showed little evidence for recombination within populations. *C. fimbriata* appears to reproduce primarily without outcrossing. UPGMA dendrograms showed that the Colombian population was more distantly related to the Congo and Uruguay populations than they were to each other. Some of the groups in the Colombian and Uruguay populations were genetically similar to isolates from the Congo. Results of this study show that African isolates of *C. fimbriata* originated in Latin America. Moreover, evidence indicates that Latin America is also a likely area of origin for *C. fimbriata*.

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934 - Studies in the life cycle of *Puccinia glechomatis*

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Puccinia glechomatis DC. is a microcyclic rust fungus on *Glechoma* spp. (Lamiaceae) forming only telia and basidia (spore states III, IV). The species is a native of Eurasia but naturalized in North America where it is spreading since the early 1990s. We studied the life cycle of the species by propagating the fungus on *G. hederacea* (ground-ivy) in the greenhouse, by various inoculation and germination techniques and by using the light microscope. Nuclear conditions were documented by epifluorescence microscopy and DAPI as DNA specific fluorochrome. The complete life cycle requires c. 20 d. After karyogamy in teliospores both cells germinate with a phragmobasidium. In basidia meiosis produces four nuclei, which move in the developing basidiospores. Further mitosis in the basidiospore may result in up to four nuclei. All but one nucleus degenerates during germination of the basidiospore. The germ tube penetrates the epidermis on the upper leaf surface and develops a rich haploid mycelium especially around stomata. Fusion of haploid hyphae occurs could not be documented. Our studies indicate that the fungus is homothallic.

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935 - Preliminary study of NE Portugal's macrofungal communities

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In Portugal, as in many other countries, there are few studies concerning macrofungi and there is much to be known. The NE Portugal is no exception, and habitats like oak forests and serpentine soil sites have not yet been explored. The present study takes place in two different habitats, a *Quercus pyrenaica* stand and a *Quercus rotundifolia* in serpentine soil stand. The objective is to characterize the macrofungal communities present in both habitats and relate them with soil and vegetation variables. We expect to find different mycota and that the influence of environmental factors is different in the two habitats.

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936 - Interspecific interactions between saprotrophic basidiomycetes: Effect on gene expression and enzyme activity

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Saprotrophic basidiomycetes rarely form monospecific populations in the soil or organic substrata that they inhabit, therefore interspecific mycelial interactions continually occur. Gross outcome can either be deadlock (where neither species gains headway) or replacement (where one species wrests territory from the other). Irrespective of the outcome, dramatic morphological changes may occur in both mycelia. Morphological changes are correlated with differences in physiology and enzyme production, and may occur distant from the site of interaction. Currently, there is little or no information on spatial and temporal changes in gene expression and in enzyme production in mycelia in soil, either when growing alone or during interactions. In interactions between *Hypholoma fasciculare* and *Phlebia radiata*, we test the hypothesis that: (1) there is spatial and temporal variation even in mycelia growing alone, related to morphological differentiation and presence of substrates; (2) massive changes occur in the vicinity of interactions; (3) changes also occur elsewhere in support of the interaction front.

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937 - Genetic sieves in sexual reproduction: sexual spores of *Aspergillus nidulans* have lower mutation load than asexual spores

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Whatever the evolutionary forces are for the widespread occurrence of sexual reproduction, Mendelian transmission is interwoven with it. This system ensures maximal uncertainty for the transmission of genetic information, thereby preventing parasitic genes or sequences to gain a transmission advantage. A disadvantage of this system is that both low quality and high quality alleles have equal chances of being transmitted to the progeny. Clearly, evolution should favour processes within an individual that alleviate this disadvantage either by selection on gamete producing cells, on gametes or on support-relying zygotes. We introduce the term 'genetic sieves' for these processes. The haploid homothallic ascomycete fungus *Aspergillus nidulans* is excellently suited to study genetic sieves due to important properties, which we will explain on our poster. We predict that 'genetic sieves' in the sexual reproduction cycle prevent or slow down the accumulation of deleterious mutations. We tested this in a mutation accumulation experiment for both sexually selfing and asexually reproducing lines. In this poster we present the result of this mutation accumulation experiment that support our prediction. From the fitness data we calculated mutation rate and mean fitness effect of a mutation using the Bateman-Mukai equations. This gives us insight in the operation of this sieve.

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938 - *Armillaria* species and the legacy of forest disturbance in Ozark Highlands landscapes of the central U.S.A.

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Relationships between disturbance regimes and diverse life forms are being studied to understand forest landscapes and their responses to management. Disturbance regimes were characterized by abrupt ring-width reductions in *Pinus echinata* and fire frequency. Disturbance indices are correlated with topographic roughness and the abundance of certain bird, reptile, soft mast fruit, *Armillaria*, and tree species. In turn, *Armillaria* spp. contribute to forest decline, functioning both as long-lived disturbance factors and as catalysts for further disturbance, in a feedback manner. Three *Armillaria* spp. occur regionally with different distributions. Nearly ubiquitous, *A. mellea* often occurs alone on thermally exposed aspects; *A. tabescens* and *A. gallica* occur typically on ridges *vs.* protected aspects in more rugged landscapes, respectively. Behaviorally, *A. gallica* fruits infrequently, produces most rhizomorphs, and causes least disease. Most root disease is caused by *A. mellea*, which fruits prolifically but produces fewer rhizomorphs. Less abundant, *A. tabescens* produces the most limited rhizomorph systems, fruits well, and causes some disease. *Armillaria* spp. take advantage of successive stress events to gradually invade infected root systems. Tree mortality, mostly *Quercus coccinea*, *Q. velutina*, and *Cornus florida*, is often associated with root crown invasion by *A. mellea* or *A. tabescens*. Greater mortality on exposed aspects is associated with the exclusive presence of *A. mellea*.

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939 - Mycology within the Georgia Coastal Ecosystems Long-Term Ecological Research Project (GCE/LTER), subproject Decomposer Consortia Experiments (DCE)

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The GCE/LTER focuses on potential effects of alteration of the quantity and quality of freshwater flow from the Altamaha River into Georgia (USA) saltmarshes. Ascomycetes are major secondary producers within Georgia saltmarshes (about 550 g m^{-2} y^{-1}). One goal of the DCE subproject is seasonal measurement of ascomycetous and prokaryotic biodiversity in/on the decaying shoots of smooth cordgrass (*Spartina alterniflora*), using both direct microscopy (ascospores) and DNA technology (base sequences in rDNA/ITS, using ascomycete-specific PCR). The DCE subproject also includes examination of genes for lignocellulose-degrading enzymes of prokaryotes and ascomycetes - the ascomycetous enzyme targeted is laccase. Selected findings follow. 1) We found no rDNA of major, cryptically occurring ascomycetes - the same three species that are the principal ascomatal producers were also the major rDNA sources (*Phaeosphaeria spartinicola*, *Mycosphaerella* sp.2, *P. halima*). 2) The species with the highest rates of ascospore expulsion (*P. spartinicola*) may not be the species with the highest biomass yield - *Mycosphaerella* sp.2 exhibited the greatest rDNA-detection peaks (T-RFLP). 3) All major and minor ascomycete species examined had one or more laccase genes (15 distinct types total), and laccase genes of the three predominant ascomycetes (+ two unidentified laccase genes) were found within naturally decaying leaf blades, with the greatest % of clones belonging to laccases of *P. halima*.

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940 - The diversity of mycophagous diptera and their macrofungi hosts

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Despite their ubiquity in nature, few studies have been conducted worldwide to determine the ecological importance of mycetophagous diptera (fungi-feeding flies). For this study 134 species from 30 families of Basidiomycetous fungi and 19 species from 11 families of Ascomycetous fungi were collected from different sites in northeastern Ohio. Different sites were selected to obtain a diversity of mushroom substrate, as well as biotic and abiotic conditions, and consisted of mature forest, mixed mesophytic forest, urban forest, and urban residential. Adult flies were reared from 87 different fungal collections. Families of Diptera that seem to include mycophagous species are: Drosophilidae, Chloropidae, Phoridae, Mycetophilidae, Sciaridae, Tipulidae, Cecidomyiidae, and Platypezidae. Several other dipteran families are probably scavengers (Anthomyiidae, Sarcophagidae), occurring only infrequently in decaying mushrooms. Many mycetophagous flies are poorly known; several have larval stages that remain completely undocumented. While some fungal species seem to host a single fly species per mushroom, most do not. How mycophagous dipteran species can avoid competition is uncertain. Unless factors are in operation to prevent it (niche partitioning, predation, parasitism), mycophagous diptera may pose a challenge to the competitive exclusion principle. Preliminary evidence suggests parasitism by species of parasitic wasps and predation by ants and beetles may play a role.

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941 - r DNA ITS-sequence analysis of ericoid endophytes from Australia and the USA

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One hundred and seventeen slow-growing, sterile root endophytes were isolated from *Leucopogon parviflorus* Lind. (Epacridaceae) collected at two coastal sites in southeastern Australia in Autumn, 2001. Twelve isolates were then selected for the sequencing study along with four root endophytes isolated from *Pieris floribunda* (Ericaceae) collected in the southeast of the United States. All sixteen sequences along with sequences from the Northern Hemisphere mycorrhizal fungi *Hymenoscyphus ericae* and *Oidiodendron maius* and the closest GenBank matches were aligned using programs made available by the Australian National Genomic Service. Most Australian sequences from this study clustered with unnamed, sterile, isolates from previous studies of the Epacridaceae, not related to *H. ericae*. Three of the Australian sequences matched closely (96%) with *O. maius* and conidia produced confirmed them as *Oidiodendron* spp. Two of the fungi isolated from *P. floribunda* were 99% similar to *H. ericae* sequences in the GenBank the other two fungi clustered with Australian dark sterile isolates.

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942 - Effect of arbuscular mycorrhiza (AM) on seedlings of six endemic *Mimosa* species from semiarid south-central Mexico

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We report on the effects of arbuscular mycorrhiza (AM) on the growth of seedlings of six endemic *Mimosa* species: *M. adenantheroides*, *M. calcicola*, *M. lacerata*, *M. luisana*, *M. polyantha* and *M. texana* var. *filipes*. Seeds were collected directly from plants. In the greenhouse, germination was carried out in pots containing the soil of the localities where the species occur. Ten seedlings per treatment per species were examined. Treatments were applied once per week: control, benomyl (B), phosphorus (P) and B+P. Shoot length was measured twice per week. Plants were harvested after seven months. Both root and shoot length were measured, dry biomass quantified and shoot phosphorus content; and AM root colonization percentage. Seedlings treated with B had a significant lower growth and less biomass than the other seedlings. Phosphorus content was higher in the seedlings treated with P and P+B. The highest AM colonization percentage was registered on the seedlings treated with P and in the control. We found a higher number of nodules in the seedlings treated with P. Based on other parallel studies, after germination, which takes place during the wet season, a fast growth favored by AM symbiosis, may increase the survival probability of seedlings in a harsh environment such as semiarid ecosystem.

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943 - Molecular ecology of root endophytes in an alpine *Bistorta vivipara - Kobresia myosuroides* tundra plant community

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A total of 210 endophyte axenic cultures from root tips of 22 plant species, including the ectomycorrhizal herb species *Bistorta vivipara* and *Kobresia myosuroides* were grouped according to culture morphology and HinfI and HaeIII restricted ITS-RFLP groups. nrDNA ITS sequences (98) from representative isolates of the 23 ITS-RFLP groups were obtained. More than 90 % of the sequences had affinities to the Helotiales (Ascomycota) and were subjected to phylogenetic analyses in PAUP* with 47 sequences from GenBank. Altogether 39.5 % of the total sample of root endophytes were referable to *Leptodontidium orchidicola*. *Phialophora finlandia* was recorded in four herbaceous plant species, i.e. *Bartsia alpina*, *Carex atrata*, *K. myosuroides* and *B. vivipara*, and most often in association with the ectomycorrhizal root tips of *B. vivipara* and *K. myosuroides*. Axenic cultures of *Phialocephala fortinii* were obtained from ectomycorrhizal root tips of *B. vivipara* and *K. myosuroides* only. The *L. orchidicola* ITS genotypes (31) were unevenly distributed in four plots investigated. Hypotheses for host specificity for fungal endophytic strains and possibilities of a common fungal network are proposed.

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944 - Effect of binucleate and multi-nucleate *Rhizoctonia* spp. on seed germination and growth of *Anoectochilus formosanus* Hayata

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Anoectochilus formosanus Hayata is a medical and ornamental orchid in Taiwan. It was considered as a 'King medicine'. Asymbiotic germination of *A. formosanus* in Hyponex # 3 agar medium (H3A) was 65%. While symbiotic germinations inoculated with binucleate *Rhizoctonia* sp. (R02) or multi-nucleate *Rhizoctonia* sp. (R04) in oat meal agar (OMA) medium were 42% and 31% respectively. But the growth of symbiotic seedlings were faster than those in asymbiotic medium (H3A). Both binucleate (R02) and multi-nucleate (R04 and R05) *Rhizoctonia* spp. could enhance the growth of *A. formosanus* plantlets in vivo. The binucleate *Rhizoctonia* sp. R02 could be *Rhizoctonia callae*. By hyphal anastomosis tests, the results showed that multi-nucleate *Rhizoctonia* spp. (R04, R05, R06, R07, R09) belong to AG-6 group. From RAPD data, using UPGMA clustering, and light microscopy observation showed that that R03 and R08 were *Fusarium* spp., and the other multi-nucleate *Rhizoctonia* spp. were *Rhizoctonia solani*. It was suggested that the inoculation of binucleate or multi-nucleate *Rhizoctonia* spp. for *A. formosanus* plantlets in vivo could promote their growth in practical use.

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945 - Ecology of micro-fungi in mangrove sediments of the Ganges river estuary in India

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Tidally inundated riverine sediments in the tropics at outfall are inhabited by mangroves, the climax formation of hydro-halophytes. Population dynamics and ecology of micro-fungi in surface sediments of four floristically different, nutrient limited mangrove succession stages at the Ganges river estuary in India are reported. Population density and species diversity of micro-fungi were inversely

related to salinity and inundation levels and positively with species diversity and nitrogen content of the sediments. None of the 46 species of micro-fungi isolated was halophile, only adaptive halotolerance was observed in most of them. While population of most halotolerant species declined with increasing salinity, the same for some remained constant and for a few increased. Although ED50 salinity concentration for in vitro growth retardation of the isolates varied between 8.2 - 14.5 dSm-1, they differed in growth dynamics from their mesic counterparts between 3.0-5.5 dSm-1, suggesting a lower salinity tolerance limit for physiological function than for their ecosystem presence. Comparison of the population fraction of cellulose decomposer and insoluble phosphate solubilizer fungi, their diversity and distribution, in vitro decomposition-solubilization ability and limits of salinity tolerance with that of sediment bacteria indicated that the fungi were no less significant as a population group of carbon and phosphorus cycling microorganisms in the extreme saline environment.

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946 - Comparison of the post-fire dynamics of the ectomycorrhizal community in two *Quercus ilex* stands in northern Spain

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A comparative study of the post-fire recolonization of ectomycorrhizae in a evergreen oak stand (*Quercus ilex* L. subs. *ballota* (Desf.) Samp.) has been carried out in two *Quercus ilex* stands located in Nazar and San Cristóbal (Navarra, Spain) respectively. The research in our group has been focused during the last three years on the description and identification of ectomycorrhizae in burnt *Quercus ilex* stands, and in adjacent areas which were not affected by fire and act as control plots for the study. In 1993 a stand in Nazar burnt, but it was not until 1998 that the study started. On the contrary, the study in San Cristóbal started immediately after the stand had burnt (in the year 2000), thus enabling us to analyse the mycorrhizal communities in the first stages after the fire. Therefore we have been able to compare the regeneration in both stands and the species composition five years after the fire and immediately after the fire. The sampling of ectomycorrhizae has been done in Nazar seasonally for three years while in San Cristóbal the monitoring of the mycorrhization has been done according to the same methodology during one and a half years so far. The description of several of the most important morphotypes of ectomycorrhizae found in both stands are provided, including photographs of different macroscopic and microscopic features of the mycorrhiza.

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947 - Influence of mycorrhizal inoculum on clonal propagation in *Epacris impressa* Labill.

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Members of the Epacridaceae are traditionally difficult to propagate and are in decline in parts of Australia. Attack by soil-borne fungi has led to some species of Epacridaceae in Western Australia being listed as endangered. Seed is nearly impossible to germinate and cuttings often have a strike rate as low as 10%. Previous studies have shown that introduction of soil collected from beneath adult plants showed improved health and survival of cuttings of several epacrid species. In this study cuttings were grown in either potting mix alone or potting mix containing either mycorrhizal inoculum or soil from beneath adult plants collected in the wild. Plants were grown under glasshouse conditions for 20 weeks and monitored for health and survival before harvesting. Strike rate and mycorrhizal status was then determined. Statistical analysis of results showed no significant difference between treatments and no mycorrhizas present in the roots of any cutting in any of the treatments.

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948 - The development and use of co-dominant SNPs and SSRs in the study of gene flow in Scottish late blight populations

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Potato blight is the most serious diseases of the potato industry worldwide. Infecting both the foliage and tubers, it reduces yield and quality of ware and seed potato crops. A survey of Scottish potato blight (*Phytophthora infestans*) populations from 1995-1997 yielded 500 isolates from over 80 disease outbreaks. The isolates were characterised by mating type, fungicide sensitivity and AFLP fingerprinting. These data were examined alongside cropping details to determine the population structure in the context of existing disease management. Both mating types were found and considerable AFLP diversity observed. Three sub-populations were identified with higher ratio of the A2 mating type within one group. Evidence of occasional sexual recombination, and thus gene flow was observed.

Since the production of long-lived sexual oospores has an epidemiological and evolutionary significance that impacts disease management, it is important that we understand *P. infestans* population biology. At SCRI we have developed a range of co-dominant SSR and SNP markers for this purpose. They are being used to examine the fine-scale diversity of Scottish populations, form the basis of a 'molecular toolkit' for examining populations worldwide and have utility in tracking isolates in epidemiological studies.

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949 - ITS-sequence study of root endophytes isolated from Alpine Epacridaceae

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Three hundred and forty sterile fungi were isolated from the roots of five species of Epacridaceae (*Epacris paludosa*, *E. microphylla*, *E. petrophila*, *Leucopogon montanus* and *Richea continentis*) collected in Jan 2002 from Mt Kosciuszko National Park, NSW, Australia (-36°27', 148°15', Elevation 2228 m). Fifty isolates were selected for sequencing studies and each was grown in liquid culture, harvested and DNA extracted. The universal primers ITS1 and 4 were used to amplify the ITS 1, 5.8s and ITS2 regions of the rDNA. Sequences were edited and used to search the database for similar matches. All fifty sequences plus the closest GenBank matches were aligned using programs made available on the Australian National Genomic Information Service and the resulting alignment analysed using PAUP. The phylogenetic tree created was similar to those produced in previous studies of epacrid endophytes isolated from plants growing at sea level. None of the fifty fungi isolated had similar ITS sequences to the known ericoid endophyte *Oidiodendron maius*.

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950 - Life forms of *Rhizoplaca baranowii* (Poelt) Golubkova

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The Project presented aims in comparing the 2 morphotypes or life forms of the lichen *R. baranowii*. Studies are based on published data as well as herbarium materials and field observations in Altai Mts (Russia, Mongolia). *R. baranowii* is endemic to Altai Mts and is known from the few localities in high mountain steppe habitats. All specimens can be divided into 2 groups based on their morphology 'subplacoid' (P) and 'spheroid' (S). Most specimens in majority of sites belong to the 'S', and this is the morph that falls into original description of the species by Poelt. 'P' is characterized by the unattached plates up to 12 mm in diam with down-turned margins. The main peculiarity of 'P' is its dorsiventral structure. Further with time the open part of the black hypothallus is becoming smaller and finally disappearing. Transitional forms between 'P' and 'S' are found, but both forms can't be treated as subsequent stages in the life span as they are reproducing by fragmentation independently giving rise to the same form respectively. Reproduction by apothecia is known only for 'S'. The series of transitional forms between 'P' and 'S' illustrates in our opinion the evolutionary process of evolving of spheroid thallus from unattached plates. Ecologically both morphotypes discussed are the life forms induced by the differing environmental conditions. Population analysis and comparison between the habitats of 'S' and 'P' proves that sheep grazing largely induces the latter form.

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951 - Morpho-productive and genetic diversity of a collection of *Pleurotus eryngii* isolates

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The evaluation of phenotypic and genetic variation of *P. eryngii* var. *eryngii*, *P. eryngii* var. *ferulae* and *P. eryngii* var. *nebrodensis* germplasm is a prerequisite to correctly distinguish groups for traits of interest and to identify strains with high yield potential. In this study, a population of 142 strains, belonging to the above varieties and having different Italian geographic origins, was studied for quantitative, qualitative and molecular traits. An experimental design with three blocks and three replicates for each strain within block, was used to estimate trait parameters. High significant differences were observed among strains for number and weight of sporocarps, while no significant differentiation was observed among geographic origins and taxonomic groups. By using qualitative traits, a degree of differentiation was only observed with the Sardinian and *nebrodensis* strains respectively. Some non-commercial strains performed better than the commercial ones in terms of yield. On average, yield per strain (basidiocarp weight) was more correlated with number of fruit bodies than with their size. The most stable traits across replicates were basidiocarp 'number' and 'weight' per strain. It follows that such traits can be used for an efficient genetic selection of the best yielding strains. Both minisatellites and RAPD markers performed very well in identifying each strain and in discriminating the different taxonomic groups.

952 - Ecological specificity of *Laboulbenia* (Laboulbeniales, Ascomycetes) through transmission experiments and artificial infections

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Host specificity of *Laboulbenia slackensis*, and congeneric taxa, was studied on living hosts and under controlled conditions. From abiotic factors, soil composition and relative humidity have the strongest impact on the fungus population growth. The best growth conditions are close to natural conditions. Transmission of *L. slackensis* is affected by copulation, host population density and spore longevity. Direct transmission is promoted by host copulation and increasing host population density. It surpasses indirect infection as spores are short-lived and soilborne infections rare. Both transmission types enhance isolation and promote specialization as they reinforce intraspecific and obstruct interspecific transmission. Successful artificial infections with *L. slackensis*, *L. flagellata*, *L. giardii*, *L. vulgaris* and *L. pedicellata* were obtained on new carabid hosts, providing that optimal growth conditions are met. Hosts from non-carabid families could not be infected, suggesting that physiological barriers are present, but active on higher taxonomic levels. The specialization of *Laboulbenia* species, with a narrow natural carabid host range, is explained by (1) a physiological dependence and obligate ectoparasitism to Carabidae, (2) a certain degree of isolation due to the lack of interspecific transmission (time and space) between potentially suitable carabid hosts and 3) the adaptation of the ectoparasite to a specific environment which is rigorously chosen by the host.

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953 - Fungal biodiversity in truffle beds in Navarra (Spain): Ectomycorrhizae and hypogeous fungi

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Since 1993 we have been studying the ectomycorrhizae of truffle beds in order to know more about the evolution of mycorrhizal colonization in plantlets artificially inoculated with *Tuber melanosporum*. The richness of the ectomycorrhizal community increased considerably during the first sampling years, and we realised soon how important the process of natural colonization was. Apart from monitoring the permanence of the inoculated mycorrhizae of *Tuber melanosporum* in field conditions, we have recorded other *Tuber* mycorrhizae, as well as mycorrhizal morphotypes belonging to the genera *Genea*, *Scleroderma*, *Hebeloma*, *Pisolithus* and many *Tomentella*-like morphotypes. Although the identity of most of the mycorrhizal morphotypes is still unknown, the detailed morphological and anatomical descriptions carried out will probably enable us to identify the fungus forming the mycorrhiza in future studies. On the other hand, we have also studied the sporocarps, paying special attention to hypogeous fungi and gasteromycetes due to their special importance as ectomycorrhizal species. The ectomycorrhizal morphotypes characterized and the sporocarps collected in truffle beds will be presented, together with some comments about the possible link between them.

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954 - Above- and below-ground views of tomentelloid fungi in a *Quercus ilex* stand in northern spain

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Since autumn 1998 we have carried out seasonal samplings of ectomycorrhizae in a *Quercus ilex* stand located in Navarra (N Spain). The ectomycorrhizal morphotypes found have been described and identified when possible, yielding a total of 43 morphotypes. After three years of study, one of the most outstanding facts is the great amount of *Tomentella*-like ectomycorrhizae found, which account for ca. 30% of the total mycorrhized root tips analysed. Apparently, the situation changes considerably when we consider the above-ground community. Few studies have paid attention to resupinate fungi when collecting ectomycorrhizal carpophores, thus inducing a mismatch between the species composition above- and below-ground. Nevertheless, a closer look at the ectomycorrhizal carpophores, considering especially the resupinate basidiomata, shows there is not such a big mismatch in our study site. Tomentelloid fungi seem to be well-adapted to survive in dry environments, such as the Mediterranean region in which our study site is located, thus occurring abundantly both as ectomycorrhizae and as carpophores. A preliminary catalogue is presented, including the list of tomentelloid fungi found both in the above- and below-ground communities as well as descriptions and pictures of the ectomycorrhizal morphotypes and the basidiomata.

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955 - Biosorption of copper by *Auricularia polytricha*

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The biosorption of copper ions has been studied using lyophilised mycelium of*Auricularia polytricha* (SMR 0055 of International Bank of Edible Saprophytic Mushrooms of National Council of Research) in a batch system. This isolate was chosen for its ability of producing large amounts of biomass able to adsorbe metal ions. Experiments were performed in a pH range 2 - 6 at temperature within 15 - 35 °C, using different amounts of mycelium (100, 200, 300, 400 mg) in the presence of 1, 5, 10, 30, 60 ppm of copper. Biosorption equilibrium was established in 5 minutes at pH 3 and 25 °C. The biosorption is pH-dependent but indipendent from temperature. The results comfirm the previous ascertained ability of *A. polytricha* to chelate heavy metals. The optimal condition for copper biosorption were determined at pH 3, when 100 mg of biomass were incubated with 30 ppm of copper. Fungal biomass was also immobilised on a PVA matrix for further applications on heavy metal polluted streams.

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956 - The co-introduction of Australian ectomycorrhizal fungi with eucalypt plantations in the Mediterranean. Potential environmental risks

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A growing evidence supports the recent dispersion of Australian ectomycorrhizal fungi with plantations of eucalypts and Australasian acacias worldwide. The effects of such introduction on the natural ecosystems have been poorly studied. In the Iberian Peninsula, such co-introduction seems to facilitate the adaptation of eucalypts to the soils of plantation sites. Consequently, the replacement of eucalypts for native sclerophyllous species is difficult and causes high costs in areas where eucalypts were extensively planted, such as the Natural Park of Monfragüe in Cáceres (Spain). In eucalypt plantations close to Monfragüe, we have found fruit-bodies of ectomycorrhizal fungi specific to Australasian trees, such as *Hydnangium carneum*, *Hymenogaster albus*, *Hysterangium inflatum*, *Setchelliogaster rheophyllus* and eucalypt-specific strains of the *Pisolithus* species complex. Other exotic ectomycorrhizal fungi, unable to fruit outside its natural geographic range, can have gone undetected belowground. Whether these exotic fungi extend beyong the eucalypt plantations will depend on their compatibility with native hosts and their ability to compete with native mycosymbionts; i.e. of the Australian species *Laccaria fraterna* (=*L. lateritia*), which was found in Mediterranean shrublands under ectomycorrhizal *Cistus* species. Further studies will be necessary, if we are to predict potential risks involved with the large-scale use of eucalypts in the Mediterranean.

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957 - Development of extraradical mycelia of ectomycorrhizal fungi and interaction with saprotrophic and root pathogenic fungi in soil microcosms

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Our recent studies of mycelial systems in soil microcosms using combined image analysis, radioisotope tracers and digital autoradiography, have provided new insights into their structure and function. Nutrient foraging and transport by mycorrhizal mycelia, together with the patterns of carbon allocation from plants to mycorrhizal mycelia have been visualised and quantified using 33P and 14C tracers. Fungal morphology (mycelial fanning and cord formation) and area cover of ectomycorrhizal mycelial systems of *Paxillus involutus* and *Suillus bovinus* varied between isolates and species over 27d. Encounter with pine litter patches significantly increased mycelial cover to 230% of controls. Extraradical mycelial morphology also influenced ingress by saprotrophic fungi. Dense mycelial systems of *P. involutus* resisted invasive cords of *Phanerochaete velutina*, but led to allocation of host-derived carbon away from the interaction zone. In contrast, the more aggregated mycelia of *Suillus bovinus* had less mycelial density, vigour and allocation of host derived carbon to the entire extraradical mycelium when over grown by *P. velutina* mycelia, compared to controls. We are currently investigating the mechanisms by which extraradical mycelia may also provide protection from migratory and non-migratory root pathogenic fungi. We hypothesise that differences in extraradical mycelial morphology and function will determine degree of protection of host roots from pathogen infections.

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958 - Amplified Fragment Length Microsatellites (AFLM): A simple method to develop microsatellite markers in fastidious microorganisms applied to AM fungi

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Microsatellites are abundant, highly mutable arrays of simple DNA sequence repeats which are powerful molecular markers to study population and evolutionary biology. Developing microsatellite markers for organisms that cannot, or are not, easily manipulated under laboratory conditions can be difficult because sequence information is needed. To overcome this problem in the arbuscular mycorrhizal (AM) fungi *Glomus etunicatum* and *Gigaspora gigantea*, global amplification of the genomes of each species were performed using linker-adaptor-PCR from single spores. Amplified fragments were enriched for microsatellite motifs using biotinylated oligonucleotides and recovered by magnetic streptavidin beads. The recovered fragments were reamplified, run on denaturing polyacrylamide gels, and sixteen selected bands were excised, cloned, and sequenced. Seven microsatellite motifs were detected from six clones (efficiency rate of 43.8%). Primers were designed for all putative microsatellite loci and most were successfully amplified from three single spore preparations and from pools of 5, 10, and 20 spores after global amplification. We term this technique amplified fragment length micosatellites and propose that they can be useful markers for fastidious microorganisms, such as Glomalean fungi. However, the technique can also be used to isolate microsatellite loci in any organism.

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959 - Componets and techniques of spore sampling from determining presence to molecular biology

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Spore samplers, from a simple petri dish or glass slide to complex volumetric samplers, have been used to determine the presence, concentration, and identification of airborne fungal spores since the beginning of mycological studies. The simplest of spore traps that collect spores by gravity deposition, have been used primarily for identification of spores present in the air. Volumetric samplers provide the added advantage of measuring the concentrations of fungal spores under existing wind conditions within a given air mass. Flow meters that provide variable adjustments in the rate of air intake of the sampler may be used to obtain iso-kinetic volumetric samplers. Small personal samplers have been developed for use by individuals with specific allergies to obtain levels of allergens that individuals may encounter on a daily basis. Techniques for identification of fungal spores collected by volumetric samplers using molecular markers are being developed. This presentation identifies various types of samplers that have been used in studies of identification, production, release, local dispersal and long distance transport, survival, and deposition of mycological material important in disease epidemics. Photos or sketches of many of the samplers used in research in our laboratory are shown including photos of the initial samplers used in private aircraft or on board ships. Major advantages and disadvantages of each type sampler are discussed.

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960 - Monitoring a non-pathogenic strain of *Fusarium oxysporum* on tomato plant using Green Fluorescence Protein gene marker

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Non-pathogenic strains of *Fusarium* are known organisms to effectively reduce the incidence of wilt diseases caused by the pathogenic isolates of this genus. Competition for nutrition in soil and rhizosphere, induced systemic resistance and competition for root colonization have been suggested as modes of action involved in such antagonistic effects. Here a non-pathogenic strain of *Fusarium oxysporum*, previously demonstrated to be an effective inhibitor of the tomato wilt pathogen caused by *Fusarium oxysporum* f.sp. *lycopersici*, was genetically transformed with both hygromycin B resistance and a gene for green fluorescence protein (GFP). Stable transformants with bright green fluorescence and resistant to hygromycin B were obtained. Several transformants with similar growth rate and colony morphology to the wild-type isolate were further used for the inoculation of tomato seedlings, in order to study their behavior on the host and the pattern of colonization of the roots. The growth of fungal mycelia on the root surface, penetration into the root cortex and colonization of this tissue were observed using a fluorescence microscopy. Re-isolation of the transfromants from surface sterilized root and stem tissues of inoculated tomato plants, 4 weeks after inoculation, indicated that the fungus was able to extensively colonize its host tissues.

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961 - Dynamics of microbial communities associated with submerged *Typha angustifolia* litter: effects of litter manipulations on microbial decay processes

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In wetlands, most of the plant biomass enters the detrital pool to be decomposed by microorganisms. Prior studies of wetland plant decay have often used litter that has been prematurely harvested and/or manipulated (e.g, oven-dried) prior to submergence. We examined the effects of litter manipulations, such as oven drying and litter age (senescent vs. standing-dead), on microbial assemblages associated with decaying litter of *Typha angustifolia*. Plant litter of *T. angustifolia* was harvested after senescence and after a period of standing-dead decay. Litter of both age groups were air-dried and oven-dried (80 °C), placed into litter bags (1 mm mesh), and submerged in the wetland. Litter bags were retrieved periodically and analyzed for mass loss, microbial biomass, fungal production rates, and rates of microbial respiration. Several microbial parameters differed markedly between the litter treatments. Fungal biomass (ergosterol) associated with oven-dried litter was 62% lower than air-dried litter during the first 114 days of decay (i.e., 83 ±53 and 220 ±43 µg ergosterol g^{-1} AFDM, respectively). Rates of fungal production and microbial respiration exhibited similar trends. Initial rates of microbial respiration showed a 5-fold difference between oven-dried and air-dried treatments (ca. 10 vs. 70 µg CO2-C g^{-1} AFDM h^{-1}, respectively). These results point to contrasting patterns in microbial dynamics during emergent macrophyte decay as a result of litter manipulations.

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962 - Community structure and diversity of below ground ectomycorrhiza fungi in Danish beech (*Fagus sylvatica*) forest with different cropping history

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The aim of this work is to compare the below ground community of ectomycorrhiza fungi in Danish beech forest sites with different cropping history. Six locations in Grib Skov, Northern Zealand, Denmark were studied. A monoculture of 100 years old beech trees are growing on all six locations, and the cropping histories of these locations are well described since early 1800s. At this time 200 years old naturally regenerated beech were growing in the locations. This tells us that beech have been continuous at least for 400 years, and probably for many more hundreds years at the locations. At three of the locations the continuity of beech was broken from about 1830 to 1900 with Norway Spruce (*Picea abies*). All the six locations were replanted with beech again in 1900. The different cropping histories give us a unique chance to study, whether broken continuity after 100 years can have an impact on the ectomycorrhizal community. Previously, the under story herbal communities at these same locations have been shown to differ between the continous and non-continous plots (Flemming Rune, Danish Forest and Landscape Research Institute, unpublished data) In 2001, at late spring and early autumn soil cores were taken in the six plots. From the samples ectomycorrhiza fungi on the roots are sorted by a combination of morhotyping and ITS-RFLP and identified by sequencing. The first results on the ectomycorrhizal community structure at these sites will be presented in this poster.

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963 - Seasonal distribution of higher filamentous fungi in a subtropical estuary

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Little is known of the distribution of higher marine fungi along tropical and subtropical estuarine salinity gradients and how species composition may change seasonally. To examine these trends, five stations were set up along the New River Estuary in south Florida, USA. Salinity, dissolved O2 and pH were measured bimonthly. Substrates for collection were submerged panels of white oak (*Quercus alba*) and Douglas fir (*Pseudotsugu menziessi*). One pair per station was collected every three months, incubated and observed for fungi. Twelve species were identified (4 Ascomycetes, 8 Deuteromycetes). The Ascomycetes *Halosphaeria quadricornuta* and *Verruculina enalia* were the dominant species by frequency of occurrence. Some species showed a physiological preference for higher salinity waters. The terrestrial species *Alternaria* sp., *Aspergillus* sp. and *Penicillium* sp. were isolated from the low salinity station and *Trichocladium achrasporum* was isolated only from the high salinity location. Despite no apparent patterns of seasonal distribution, successional patterns were discernible. Maximum species diversity occurred during the first three months which is consistent with previous findings. Compared to temperate studies species diversity was low suggesting interference behavior. Many species observed at low frequencies are cosmopolitan or temperate. Our observations indicate that salinity and temperature directly influence species composition and abundance in subtropical estuaries.

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964 - The impact of global warming on the mycorrhizal fungal community of a Canadian High Arctic site

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The impact of global climate change is hypothesized to be more prevalent in arctic than temperate ecosystems. Experiments have shown that increase of temperature by the use of open-top chambers (OTCs) has impacted plant growth and phenology. However, data on the impact of global warming on the mycorrhizal fungal community are scant. The objective of this study is to examine the impact of increased air and soil temperature on the mycorrhizal fungal community. Three sites, each containing three plots, were chosen at Alexandra Fiord, Nunavut Canada. Air and soil temperatures were increased using 1-m diameter OTCs, which have been on the sites for at least five years. Plots within 2 m of the OTCs were used as ambient controls. Dominant plant species *Cassiope tetragona*, *Dryas integrifolia*, *Salix arctica*, and *Saxifraga oppositifolia* were harvested from warmed and control plots. Root tips were randomly selected for DNA extraction and morphological analysis. Mycorrhizal fungal communities were assessed with terminal restriction fragment length polymorphism (T-RFLP) analysis, a technique that has previously been applied primarily to bacterial community analysis. Representative fungal isolates were sequenced to determine phylogenetic affiliations. Comparisons between warmed and ambient plots will be presented.

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965 - Mycorrhiza of blueberry and control of *Verticillium* wilt on strawberry: Two examples of rhizosphere microbial steady states in agricultural land use

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The roots of blueberry are mycorrhizal, but on sandy agriculturally used soils, the mycorrhizal symbiosis has to be stabilized by a specific management of the mulch layer which provides organic C- and N-compounds. In long-term plot studies, a high sensitivity of this symbiosis depending on the mulch layer, particularly tree species of wood chips and time of application was found. Thus, the microbial population structure of the rhizosphere of variety 'Reka' varied considerably. In one plant rhizosphere, the microbial community was similar to the rhizosphere community of a natural peaty soil which was correlated with the highest fruit mass. This leads to the assumption that the mycorrhizal symbiosis of highbush blueberry depends on an appropriate fungus-bacteria-rhizosphere community which can be influenced by the management of the mulch layer. Strategies to achieve an appropriate microbial steady state can be used to control the *Verticillium* wilt on strawberry. The effect of different *Verticillium* strains and antagonistic bacteria is going to be analysed under controlled climatic conditions and in field trials. On different agriculturally used sites, *Verticillium* strains will be isolated to select the regionally best adapted antagonistic bacteria to control the *Verticillium* wilt.

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966 - Influences of T4-lysozyme producing potato plants on the endophytic fungi of the roots studied by a classical and a molecular approach

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T4-lysozyme expressed in potato plants seems to be a promising strategy to enhance the plants' resistance against phytopathogenic bacteria like *Erwinia carotovora*. However, lysozyme has unspecific effects on other bacteria and on fungi. The monitoring of bacterial and fungal rhizosphere communities of transgenic and non-transgenic plants with a cultivation-independent molecular fingerprinting technique (PCR-DGGE) revealed in no cases a plant line dependent influence. The lysozyme is secreted into the apoplastic space where its concentration should be higher than in the rhizosphere. Therefore, the influence on endophytes which live inside of the apoplast could be stronger than that on the microorganisms in the rhizosphere. Endophytic fungi were isolated from roots of the parental and a transgenic line after surface sterilisation. The isolates were cultivated, identified and characterised by molecular methods. Here significant differences between the parental and the transgenic line could be found: the roots of the parental line were colonised with *Verticillium dahliae* around three times higher than those of the transgenic line. Additionally the surface sterilised roots were examined by PCR-DGGE. Fingerprints of the endophytic fungi could be shown for the first time. To associate the bands to fungal isolates clones were generated and sequenced. The results of the two methods of investigation are compared and discussed.

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967 - Substrate selectivity of corticioid fungi from *Nothofagus* forests in Patagonia (Argentina)

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Corticioid fungi (Aphyllophorales, Basidiomycetes) grow on almost every type of woody substrate, but species usually show preferences on certain features of the substrate such as the decay stage and/or the part of the tree involved. These preferences determine the spatial and temporal arrangement of these organisms along the degradation process. During an intensive floristic survey of Corticiaceae growing on 3 *Nothofagus* spp. in the Patagonian Andes forests, the habitat preferences and the degree of selectivity of 42 species in relation to three substrate features (decay stage, part of the tree and diameter of the detritus) were evaluated. Decay stage was classified into six categories according to hardness and other features. Part of the tree was classified as bark, sapwood, hardwood or the combination of bark/sapwood or sapwood/hardwood. Diameter was classified as 0-5, 6-10, 11-20, 21-30 or >30 cm (from small branches to big trunks). The differential distribution (not aleatory) of the species according to the characteristics of the substrate was statistically tested (X^2). 95% of the species showed a differential distribution in relation to the decay stage, 64% in relation to the part of the tree and 54% in relation to the diameter. In order to evaluate the degree of selectivity, the range of categories where a species was recorded for each variable was analyzed. Species with a range ≤ 3 were considered highly selective for that variable.

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968 - Adaptive spatiotemporal distribution of soil microfungi in 'Evolution Canyon' II, Lower Nahal Keziv, Western Upper Galilee, Israel

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We describe and interpret spatiotemporal micromycete community structure and adaptive complexes to xeric and mesic contrasting microclimates in the soils of 'Evolution Canyon' II, western Upper Galilee, Israel. A total of 192 species belonging to Zygomycota (9 species), Ascomycota (13 species), and mitosporic fungi (170 species) were isolated. The fungal communities on the xeric south-facing slope (SFS) demonstrated significantly greater diversity (species richness, Shannon index, and evenness) than on the mesic north-facing slope (NFS). Seasonally, winter slope communities were less heterogeneous. Forest localities on the NFS and in all seasons and the shady locality on the SFS in the winter were overwhelmingly dominated by mesophilic *Penicillium* species. The sunny locality on the SFS was characterized by a dominance of melanin-containing fungi that was most pronounced in the summer and by high occurrence and abundance of thermotolerant *Aspergillus* and *Fusarium* species. Ascomycetes and zygomycetes were the minor components in all local mycobiota studied; sexual ascomycetes, being stress-selected fungi, were more abundant than 10 fold in the soil of the SFS, with the peak of abundance in the sunny summer community. The results demonstrated a microscale adaptive spatiotemporal inter- and intraslope divergence in soil mycobiota structure. Microclimatic natural selection appears to be the major factor affecting soil fungi diversity patterns and spatiotemporal dynamics.

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969 - Structural diversity of fungal populations in soil under the influence of different farming management systems

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Although it is known that fungi play a vital role for ecosystems, only few studies were published so far showing fungal diversity and dynamics in soil. Mainly in agricultural ecosystems fungi are essential for the soil quality, as many species are able to degrade complex organic compounds, supply plants with nutrients and serve as biocontrol organisms against plant pathogens. However some strains can also act as plant pathogens. Therefore we investigated the influence of precision farming (a new agricultural technique, focusing on the heterogeneity of a field site and adapting the amount of fertilizer given to the yield expected in a particular plot). For comparison samples were also taken from plots, where fertilizers were applied using conventional techniques. To access the different fungal populations culture-dependent and culture-independent methods were used. Fungal cultures were isolated on three different media and examined by means of microscopy and a genetic fingerprinting method (interLINE, PAGE). Additionally DNA was directly extracted from the soil samples and clone libraries of the 18S rDNA were established. Yet the data of the culture-dependent and the culture-independent methos show a seasonal influence as well as an influence of the high- and low-yield areas. Besides also a direct influence of the agricultural management systems on the structure of the fungal populations was detected.

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970 - Microbial companions of an introduced tree: needle endophytes of Siberian larch (*Larix sibirica*) in Finland

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Endophytes are organisms inhabiting plant tissues without causing symptoms. The distinction between pathogens and endophytes is not clear as some endophytes are pathogenic for other plants. Some pathogens also show periods of latency. In single hosts pathogenicity or endophytic behavior may be determined by only one gene. Plants are frequently introduced to novel geographic areas in the modern world. It is not known how commonly endophytes are co-introduced with their plant hosts. This information should, however, be available as nonindigeneous plants may transport fungal endophytes pathogenic to native plants. We analysed needle endophytes of Siberian larch in its natural distribution area in Russia and in old or young stands in Finland, where the tree does not occur naturally. 880 isolates were collected, and identified to species level based on colony morphology and DNA-analyses. The co-introduction frequency of endophytes turned out to be high; only one common (in Russia) endophyte was missing from one Finnish stand. In contrast, several rather common endophytes observed in Finland lacked from the natural stand in Russia. We also analysed genetic variation in one endophyte species.

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971 - Interspecific fungal interactions and recognition in decayed wood

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We compared interspecific interactions in fresh beech wood and beech wood partly decayed by the primary decay agents, *Eutypa spinosa, Fomes fomentarius, Stereum hirsutum* and *Trametes versicolor*. The fungi used in the experiments included five secondary decay agents, common on beech wood in advanced decay, as well as the aforementioned primary decayers. The experiments were carried out using sterilised, recolonised wood and lasted for seven months. Interactions involving secondary decayers were affected by wood type, with outcomes changing from complete replacement to deadlock in at least three species combinations. Most of the secondary decayers were able to replace some of the primary decayers. However, several of the secondary decayers were unable to colonise sterilised wood decayed by *Trametes versicolor* or *Stereum hirstutum*. This lead to a new series of experiments in which the effects of exudates from fresh and partly decayed wood were investigated with respect to hyphal extension rates in a larger range of secondary decay fungi. Sterilised wood pieces were placed on 0,5% malt agar, opposite to small plugs containing the test fungi, which showed very variable growth responses to the various wood types. Most species increased hyphal extension rates as response to wood decayed by *Eutypa spinosa* and *Fomes fomentarius*, while wood decayed by *Stereum hirsutum* resulted in reduced or completely lacking growth in a majority of species.

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972 - Physiological characteristics of symbiotic fungi associated with the seed germination of *Gastrodia elata*

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This study was carried out to investigate the possibility for seeds germination of *Gastrodia elata* using symbiotic fungi. Since seeds of *G. elata* are very small and lack an endosperm and other nutrients, their germination is difficult without requirement for external nutrients. Out of twenty six isolates collected from protocorms of *G. elata* and roots of native orchids inhabited in wild, two strains (H-2 and H-21) were observed to stimulate the seed germination of *G. elata*. The seed germination of *G. elata* was excellent on oak tree leaves medium. The optimal conditions for mycelial growth of symbiotic fungi were 25 °C and pH 6.0, respectively. The mycelial growth of H-2 strain was excellent on YMA medium, while h-21 was poor on PDA medium. In case of carbon sources, the mycelial growth of H-2 and H-21 was good on media supplemented with glucose and dextrin, respectively. Calcium nitrate was good for mycelial growth of H-2 strain as a nitrogen sources, whereas urea was effective to H-21 strain.

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973 - Classification of wood decay fungi isolated from durable wood species, ekki

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Ekki (Azobe, Bongossi; *Lophira alata* Banks), a native African wood species, is known as a large durable species and has been utilized without preservative treatment for outdoor architectures such as bridges in EU. In Japan, however, several bridges of ekki wood are decayed in a short time after construction. In this study, as the first step of the elucidation of decay in ekki wood, basidiomycetes isolated from damaged members of ekki bridges were classified by PCR-RFLP. Decayed wood pieces and fruit bodies from ekki bridges were incubated on potato-dextrose-agar plates containing antibiotics. Twenty-six isolates of basidiomycetes were obtained from different areas in Japan. One of the isolates was identified morphologically as *Perenniporia tephropora* (Mont.) Ryv. (*Loweporus tephroporus*). Genomic DNAs were extracted from isolates using the benzyl chloride method. The whole inter specific transcribed (ITS) regions (ITS1, ITS2, and 5.8S) were amplified by PCR. The PCR products were digested with 4 restriction enzymes to obtain RFLPs. Restricted fragments were separated by electrophoresis in 8% of polyacrylamide gel. Comparing fragment patterns, 14 isolates were identified as *P. tephropora*. To obtain further molecular information on the decay basidiomycetes, we undertake to determine sequences of 18S rDNAs.

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974 - *Aspergillus* section *Flavi* in the United States: characterization of soil populations from agricultural fields

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Species belonging to *Aspergillus* section *Flavi* often produce aflatoxins and cyclopiazonic acid, mycotoxins that contaminate preharvest peanuts, corn and cottonseed. Soil populations of *A. flavus*, *A. parasiticus*, *A. nomius*, *A. tamarii* and *A. caelatus* were examined over a large geographic area within the United States and in greater detail within a single field. A transect was established from New Mexico to Virginia, and species from section *Flavi* showed distinct patterns in their geographic distribution. *A. flavus* (large sclerotial morphotype or L strain) was the most widely distributed species, but *A. flavus* var.

parvisclerotigenus (small sclerotial morphotype or S strain) was primarily confined to eastern Texas and Louisiana where cotton is intensively grown. *A. parasiticus* was most common along the southern Atlantic coast, particularly in areas where peanuts are cultivated. *A. tamarii* and *A. caelatus* occurred at low incidences along most of the transect, and *A. nomius* was detected only in the Mississippi River Delta region (Louisiana and Mississippi). Individual vegetative compatibility groups of *A. flavus* and *A. parasiticus* were widely distributed along the transect. In a single peanut field from southwestern Georgia included in the transect, soil populations of *A. flavus* and *A. parasiticus* were genetically diverse, as indicated by the large number of vegetative compatibility groups.

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975 - Isolated *Pinus contorta* seedlings in coastal dunes are supported primarily by fungi with high host specificity whose spores are carried in deer feces

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Pinus contorta seedlings are establishing in sand dunes on the coast of Oregon, U.S.A. in a primary succession pattern. The seedlings establish at least 100 m away from the continuous forest and thus establish without the benefit of an existing ectomycorrhizal network. Over 120 species of fungi in 37 genera have been recorded fruiting along the edges of the continuous forests and spores from these fungi may disperse to the isolated seedlings via wind, water or mammals. We identified EM fungi associated with the roots of the isolated seedlings and found that they are colonized almost exclusively by `suilloid' fungi: *Suillus brevipes*, *S. umbonatus*, *S. tomentosus*, *Rhizopogon fuscorubens*, *R. occidentalis*, and *R. vulgaris*. Deer commonly traverse the dune system from the surrounding forest past the isolated seedling areas and their fecal pellets contain large amounts of spores. Spore slurries made by bringing up individual fecal piles with variable pellet numbers in 100 ml of water and were found to contain between 1×10^6 and 1×10^8 spores/ml. In a bioassay, seedlings inoculated with 20 ml of slurries made from fresh fecal piles became colonized by the same fungi observed on the field-collected seedlings. These results suggest that 1) deer can vector suilloid spores to areas where mycorrhizal networks are non-existent, 2) suilloid spores may lie dormant in soils until pine seedlings germinate, and 3) in contrast to other early succession work, pine seedlings establish and are supported by fungi with high host specificity.

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976 - Effect of root endophytes on the germination of seed of the Australian terrestrial orchid *Caladenia formosa* G. W. Carr

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This study investigated two seed germination methods to determine which would maximise the production of plants of the endangered Australian orchid *Caladenia formosa* for use in re-establishment in situ. Root endophytes were isolated from adult plants in the wild at various stages in the orchid life cycle (budding, leafing, flowering, capsule production and senescence). Seed was germinated on minimal (Oatmeal Agar) and complex (PA5 containing coconut water) media with and without root endophytes under axenic conditions. Germination and subsequent growth was recorded at monthly intervals for a period of twelve months. Mycorrhizal status of the seedlings was determined by SEM. Seed grown on minimal media inoculated with mycorrhizal fungi from the leafing, budding and flowering stages gave fastest (within one month) and maximum (>50%) germination rates. Seed grown on the complex media did not germinate in the first month. Symbiotic seedlings grown on the minimal medium were defasked and survived to produce tubers under glasshouse conditions.

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977 - Effects of *in vivo* sub-culturing on changes in morphology and virulence of the entomogenous fungus *Metarhizium anisopliae*

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One of the major drawbacks with fungal biocontrol agents is that they can lose virulence if maintained on artificial culture media. This attenuation of virulence has been reported by several workers and is a major concern in the development of insect-pathogenic fungi for pest control. Understanding the factors responsible for this phenomenon will contribute important information for development of media to stabilise or increase virulence. The effects on pathogenicity of *Metarhizium anisopliae* (Metsch.) Sor. isolate V208 following one passage through the heterologous insect-host *Myzus persicae* and 11 *in vitro* successive subcultures were investigated. Attenuation of virulence as measured by LT_{50} (median lethal time) was manifested in rapid decline with recovery and slight decline after recovery. Similarly, sporulation of attenuated

subcultures was poor with some subcultures ultimately becoming sterile. Significant decline in germination was observed in subculture 3 with slow recovery by subculture 11. Most studies to date report a gradual or rapid decline in virulence with recovery only being achieved by passaging through an insect. Here recovery was shown in the absence of passaging. Bioassays alone do not reveal the full variation in expression of attenuation. In an attempt to identify more overt attributes of attenuation, particular attention was focused on the physiological and biochemical factors, which may account for the attenuation observed.

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978 - Detection of double-stranded RNA from *Helicobasidium mompa* and *Rosellinia necatrix*

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Helicobasidium mompa and *R. necatrix* cause root diseases of many woody plants including fruit trees. Diseased plants show stunting of shoots and yellowing of leaves but are seldom killed unless intensive cultural practices are used. As the first step to exploit double-stranded (ds) RNA for biological control of the diseases, fungal strains were examined for the presence of dsRNA. In *H. mompa*, 72.1% (158/219) of strains had dsRNA whereas 24.4% (66/270) in *R. necatrix*. Electrophoresis revealed the presence of diverse dsRNAs in both fungi with 1 to 12 bands, ranging from ca. 2 to 15 kbp. Hyphal tip isolation was mostly unsuccessful to remove dsRNAs except that found in *R. necatrix* with 12 dsRNA segments, which was considered to be a member of the Reovididae. Single spore isolates of *H. mompa* from basidiospores were all dsRNA-free. Single basidiospore isolates were not pathogenic. Single ascospore isolates of *R. necatrix* did not contain dsRNA and were as virulent as those from vegetative hyphae. Sexual reproduction functions to remove dsRNA in both fungi. In *R. necatrix*, ascospore offsprings are considered capable of survival in nature since they are pathogenic. This is, however, not the case with *H. mompa*; single basidiospore isolates may not survive in nature. The difference in the detection frequency of dsRNA between *H. mompa* and *R. necatrix* is, at least partly, ascribed to the difference in the efficacy of sexual reproduction to establish dsRNA-free offsprings in the field.

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979 - Ecological characteristics and contemporized dynamics of ectomycorrhizal ammonia fungi in warm temperate forests in Japan

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We studied successional speed and dry weight of fruitbodies to clarify the correspondence between the successional phases of ammonia fungi: the early phase (EP) and the late phase (LP) and nutritional types: saprophytic and ectomycorrhizal (ECM). Urea treatments were conducted to induce fruiting of them in two ectomycorrhizal forests in warm temperate Japan. Ten EP and six LP species developed their fruitbodies. LP species fruited more in biomass than EP, and each fruitbody of LP species was larger than that of EP. ANOVA proved that fruiting of EP species was affected by treatment season or season-vegetation, that of LP by season-vegetation or vegetation. EP had short and one fruiting phase, while LP had long and multiple phases. Those ecological characteristics of EP and LP are corresponding to saprophytic and ECM species, respectively. Consequently, we measured the contemporized dynamics between ECM formation and fruitbody development. We found 1) the damaging effect of ammonia released from urea on ECM; 2) the ECM of two ammonia fungi, *Alnicola lactariolens* and *Hebeloma vinosophyllum*, by PCR-RFLP analysis; 3) the increase and subsequent decrease of the ECM of the two fungi preceded the processes made by their fruitbodies; and 4) the existence of ECM fungi which do not develop fruitbodies in urea-treated soil, which resulted in the extension of the definition of ammonia fungi from referring solely to fruiting fungi to including proliferating and non-fruiting ones.

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980 - Evaluation of the accessibility of ribosomal RNA regions to fluorescently labelled probes for *in situ* yeast identification

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Fluorescence *in situ* hybridisation (FISH) with probes targeted at the ribosomal RNA has been successfully applied to the rapid and specific identification of microorganisms in their natural habitats. However, the target site may not be easily reachable by the probe due to structural constraints, leading to weak fluorescence intensities even when using pure cultures under appropriate assay conditions. To circumvent this problem and optimise probe design, it is advisable to determine the accessibility of the different rRNA regions to FISH. In this work we studied the accessibility of the D1/D2 domains of the 26S rRNA of *Saccharomyces cerevisiae*, with a set of ca. 30 Cy3-labelled oligonucleotide probes covering the full length of this region, using flow cytometry. To evaluate whether the information obtained for *S. cerevisiae* could be extrapolated to other yeast species, a subset of probes belonging to different fluorescence intensity classes were hybridised to selected yeasts that had a full complementary target site for those probes. The results indicated that the *S. cerevisiae* rRNA accessibility model provides a useful guidance tool for the design of species-specific yeast probes. To test this hypothesis and illustrate target site selection, we designed a DNA probe that specifically binds to the rRNA of basidiomycetous fungi, including yeasts, but not to ascomycetes.

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981 - Habitat diversity in the Aphyllophorales of tropical forests, Albertine Rift, western Uganda

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The fungiflora of East Africa is extremely rich in a range of genera and the number of species though large areas remain totally unexplored (Pegler, 1977). Results on habitat diversity of the Aphyllophorales from an ongoing Ph. D. research will be presented. The taxonomy follows Alexopoulos & Mims (1996), and, Ryvarden & Johansen (1980). The study area, the Albertine Rift, with regard to non-fungal research,high habitat/species diversity with many endemic taxa. Its northern section lies within the postulated movement of forest species eastwards from a major refugium in eastern Democratic Republic of Congo, and, Cameroon/Gabon in western Africa during the pleistocene period. The fungi are systematically collected from different forest types taking seasonality, substrate/host and altitude into account.

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982 - Influence of vegetation types on the distribution of tropical forest poroid fungi, Uganda. (Preliminary results)

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Kibale forest covers an area of 560 km^2 with an altitudinal range 1,110 - 1590 m. Its annual rainfall is 1,100 - 1600 mm, a tropical climate with rainfall peaks in March - May and September - November. The vegetation can be classified as Medium Altitude Evergreen forest, Medium Altitude Semi-deciduous forest, Mixed Forest Communities, Grassland and Swamp. Though there are 85 species of polypores known from Uganda, systematic collecting has not been done and most of the country remains unexplored. In Kibale forest, three forest sites with distinct vegetation types, are at different altitudes and have different annual rainfall totals, are systematically sampled in the last month of each rainy season. Ecological studies conducted in temperate forests have indicated no differences in fungi species distribution within a forest. However due to high diversity of both habitats and species in tropical forests, the results could be different. Data collection and determination of collected material are going on.

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983 - Intraspecific diversity of *Thysanophora penicillioides* and genetic structure in the species population

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We investigated the intraspecific variation of *Thysanophora penicillioides*, a widely distributed saprophytic hyphomycete analyzing the sequence data of rDNA ITS regions to address a possibility of geographic isolation between regional or local populations. We collected 325 isolates from decaying *Abies* needles in 20 local populations in a wide range of Japan. From regional populations in Europe and North America, 30 and 5 strains isolated were also collected, respectively. As a result of analysis of the sequence for all isolates, base changes or indels were found in 30 sites in ITS regions, and 18 haplotypes distributed in seven lineages were detected. In Japan, five lineages were detected and major lineages were found widely in local populations. One major lineage which was not detected from Europe and North America dominated in most Japanese local populations and accounted for about 65% of all Japanese isolates. From European population two lineages were detected and also two lineage from North American population. Only one lineage was common between Japan and Europe and no lineage between Japan and North America, while one lineage was common between Europe and North America. These results suggest that the genetic structure of Japanese population is different from European and North American populations. Probably, the distribution of individual lineage found in this study is limited to a relatively area, though the fungus produces small, dry, wind-dispersed conidia.

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984 - Belowground ectomycorrhizal community structure along a local nutrient gradient in a boreal forest in Northern Sweden

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The belowground ectomycorrhizal (ECM) community structure was investigated along a local nutrient gradient in a boreal forest located in Betsele in Northern Sweden. The ECM taxa colonising root tips in the O horizon were identified using morphological characterisation and PCR-

RFLP of the ITS region of the fungal rDNA. The ECM community changed along the investigated transect. A *Russula* sp., *Tylospora* spp., a *Tomentella* sp., and *Cenococcum geophilum* were the most commonly found ECM taxa along the transect. Species not producing conspicuous sporocarps constituted a high proportion of the belowground ECM community. *Tylospora asterophora*, *Amphinema byssoides* and all taxa identified as *Cortinarius* spp. were associated with the nutrient-rich end of the transect, whereas the *Russula* sp. and *Piloderma* spp. were associated with the nutrient-poor section of the transect. Nitrogen availability, pH and supply of base cations appeared to be the most important soil factors controlling the ECM community structure in the O horizon of the Betsele site.

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985 - The impact of ash fertilization on the biomass of roots and ectomycorrhiza of coniferous trees

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Wood ash from the forest industry constitutes a considerable waste problem, and recycling it back to the forest is of great interest. Ash could be seen as a valuable resource since it contains most of the elements needed for plant nutrition and growth. The environmental issue concerning ash is that it stimulates nitrogen mineralization, increases soil pH and also contains heavy metals. Several aspects concerning ash fertilization have been studied for these reasons, especially the processes in forest soil, like microbial activities, nutrient mineralization and responses of various soil organisms. We studied the biomasses of the roots and ectomycorrhiza in a dry, nutrient poor Scots pine (*Pinus sylvestris*) forest in Muhos, northern Finland before and after fertilization with wood ash. The results after the first season were unexpected, as both these biomasses slightly increased due to strong ash application, 9000 kg ha^{-1}. For getting more thorough picture and long term view, the sampling repeated three years later. Another field experiment started in Evo, in Southern Finland in a more nutrient rich Norway spruce (*Picea abies*) forest. We also established a microcosm experiment for estimating the capacity of mycorrhizal root tips and extramatrical mycelium in storing N and thus alleviating the high spatial and temporal variation in nitrogen mineralization in forest soil after ash fertilization.

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986 - Effect of grassland plant species on soil fungal communities: a microcosm study

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Grasslands are the main vegetational class in Ireland and are of major significance in agriculture. Although intensification has been central to increased productivity in Ireland, there is concern globally and within the EU about the sustainability of such practices and a need to understand at a basic level how grasslands function. It is known that fungi play a major role in grassland ecosystem processes such as carbon, nitrogen and other nutrient cycles. In this study, microcosms were planted with seven grassland species representing the most commonly found plants in both unimproved, species-rich grasslands, and in improved, fertilised grasslands, which are typically dominated by only a few plant species. After harvest, shifts in the fungal community structure of the rhizosphere soil of each plant species were assessed. The ergosterol content of each soil sample was measured in order to get an indication of fungal biomass. In addition, samples were analysed using TRFLP (terminal restriction fragment length polymorphism). The resulting profiles, which indicated the number of fungal species per sample, were compared between plant species. In general, fungal communities were not significantly altered by plant species. The similarity of fungal profiles was even more striking when compared to bacterial profiles for the same soils, which differed significantly according to plant species type.

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987 - Dynamics of VA mycorrhizal fungi in burned Japanese red pine forests in the Setouchi Destrict in Western Japan

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Many large forest fires have occurred in the Japanese red pine forests of the Setouchi District in Western Japan. We investigated the seasonal changes of infection rate, numbers of spores and sporangiocarps, and composition of spore types of VA mycorrhizal fungi at the burned and the vegetation naturally recovered sites (BNR sites,7 and 22 yrs after the fires) and the burned and planted site (BP site, 7 yrs after). 1. Infection rate is about 90% irrespective of the sampling months at the BNR-22 yrs site. Infection rates at the BNR-7 and BP-7 yrs sites showed the similar trends, 70-90% in July-September, and then gradually decreased to 50-60% in January. 2. Number of spores at the BNR-22 yrs site is always higher (400-900/10 g dry soil) than those at

the BNR-7 and BP-7 yrs sites, and rapidly increased from November to January. At the BNR-7 yrs site the number gradually decreased from 400 in July to 200 in January. At the BP-7 yrs site, on the other hand, the number gradually increased from below 100 in July to 200/10 g dry soil in January. 3. Number of sporangiocarps at the BNR-22 yrs site was always higher than those at the BNR-7 and BP-7 yrs sites, in the range of 70-110/10 g dry soil, and showed the higher level in January. At the BNR-7 and BP-7 yrs sites the number is always very low (below 10/10g dry soil). 4. At the BNR-7 and BP-7 yrs sites *Gigaspora* and *Scutellospora* types, at the BNR-22 yrs site *Glomus* and *Sclerocystis* types were typically observed.

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988 - Seasonal changes in an ectomycorrhizal community associated with beech (*Fagus sylvatica*)

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The seasonal fluctuations of species abundances in an ectomycorrhizal (EM) community associated with beech (*Fagus sylvatica*) are being studied. Throughout 2001 intensive sampling was performed in a 15 x 15 m plot in Lille Bøgeskov, located in mid Zealand. Soil cores were taken at eight harvest times covering the growing season from March to November. The sampling in this plot will be continued in 2002-2003. Fungal species forming EM on the beech roots are sorted by morphotyping and ITS-RFLP and then identified by sequencing. In the fall 2001, basidiocarps of EM species were sampled in the plot (collected and identified by Morten Christensen, KVL, Denmark). The basidiocarps are being sequenced in order to create a reference sequence database to aid in the identification of the root tips. As other EM communities the beech community turns out to be species rich. So far, the two harvests from March and May 2001, which has been most thoroughly worked through, has yielded approx. 40 species. Some of the more abundant species found are *Cortinarius* species, Russulaceae spp., chanterelloid spp. including *Clavulina cristata*, *Cenococcum geophilum*, thelophoroid spp. and *Piloderma* spp. While some species e.g. *Lactarius subdulcis* are present throughout the growing season others e.g. a yet unidentified chanterelloid sp. are absent in the spring harvests but are then becoming dominant in the fall. Possible explanations for the observed seasonal trends will be discussed.

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989 - *Rhizopogon* spore bank communities: Species distribution within and among Californian pine forests

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In this study we examine the distribution of *Rhizopogon* species in spore banks from five Californian pine forests. Four of the forest sites were discontinuous populations of *Pinus muricata* while the last was a *Pinus ponderosa* stand in the Sierra National Forest. *Rhizopogon* species were isolated from a soil-bioassay using *Pinus muricata* as host followed by isolation from individual root tips with typical *Rhizopogon* ectomycorrhizal morphology. The cultures were screened by ITS-RFLP and from each site unique patterns were sequenced. These sequences were then compared with sequences derived from identified sporocarp material. Bioassaying proved to be an efficient way to bring *Rhizopogon* species into culture as 50% of the pots contained ectomycorrhizal tips with *Rhizopogon* like morphology and half of these pots then resulted in axenic *Rhizopogon* cultures. Members of all the major pine-associated sections were represented within the isolates. Our results showed that *Rhizopogon* spores are homogeneously distributed within local forest areas while there is significant structuring at the regional scale. High spore longevity and homogenization by soil and water movement may explain their distribution within local forest areas while the latter observation fits with an expectation of limited dispersal because of the hypogeous fruiting of *Rhizopogon* species.

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990 - Monitoring of white-rot fungus during bioremediation of dioxin-contaminated soils

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Bioremediation is a low-cost treatment alternative for the cleanup of dioxin-contaminated soils and -fly ashes, when pollution spread broad-ranging. An interesting fungus was isolated from white rotten wood of a broadleaf tree from Kyushu Island in Japan, named strain MZ-340. This isolate has high ability to degrade dioxin. We have tried to use the strain MZ-340 for bioremediation of dioxin-contaminated soil on site. On the other hand, we have to consider that this trial has potential problems to bring biohazard on natural ecosystem, when this organism has been naturalized. Therefore, we have to develop a monitoring system for the introduced fungus with the examination of bioremediation. The bioremediation of the dioxin-contaminated soils by the fungus MZ-340 has been examined. We have developed a PCR-based assay to detect strain MZ-340 reliably on bioremediation site. DNA, isolated by series of CTAB and organic solvent extractions followed by silica column purification from the site, was amplified by the PCR using specific primer derived from ITS sequences of the strain MZ-340. We successfully monitored the transition of the population of the strain MZ-340 on the bioremediation site. The relationship between the population of the fungus and the effect of bioremediation by this fungus will be discussed.

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991 - Vegetative compatibility among *Botrytis cinerea* strains in Israel

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A total of 21 *B. cinerea* strains originating from five host plants at 10 sites throughout Israel, were tested for mycelial interaction. Complementation between auxotrophic selenate-resistant mutants (*sul*) mutants, and antagonism (barrage) between wild type strains served as criteria for compatibility: Complementation indicates that the parent strains are compatible, whereas the barrage indicates that the strains are incompatible. *sul* mutants resistant or sensitive to chromate were paired on MM. Wild type growth of complementary heterokaryons was always observed between different mutant phenotypes derived from the same parent, as well as in some inter-strain pairings. Most isolates were placed into the same group based either on positive complementation reaction between their mutants, or, more often, on positive reaction with mutants of bridging strains, compatible with two or more isolates that were not compatible directly. Barrage between wild type isolates, in the form of a zone of sparse mycelium with or without dark pigmentation of the agar along the line of confrontation, was observed in about 35% of pairings. Although isolates were often incompatible with each other, all of them were eventually placed into one group based on their compatibility with a common third strain (bridging), confirming the data of heterokaryon compatibility by *sul* mutants. *sul* mutants were pathogenic to bean and strawberry and could be used as marked strains in epidemiological studies.

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992 - Effect of the *Pinus strobus* invasion on the mycoflora of litter needles

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Mycoflora of pine litter and trash (needles, twigs, cones) was studied in the National Park Czech Switzerland (Czech Republic). A comparison of a native pine species (*Pinus sylvestris*) and an invasive pine species (*Pinus strobus*) was done at two localities in a well preserved *P. sylvestris* forest and in a growth of *P. strobus*. An experiment using litter bags with needles was arranged for a succession study. Between years 1999 and 2002, the litter bags were exposed in litter of both the pine species. Needles from the litter bags were cultivated in damp chambers. Frequencies of occurrence of recorded fungi were analysed using multivariate analysis and analysis of variance. Sixty-three species of ascomycetes in both teleomorph and anamorph stage were recorded. Two species: *Tympanis neopythia* Ouell. & Piroz. and *Pseudocercospora deightonii* Minter were found for the first time in the Czech Republic. Most of the recorded fungi colonised litter of both the pine species but reached different frequencies. Results of the analysis proved significant differences of the frequencies between the pine species, seasons (spring and autumn) and the localities. The following species were recorded in high frequencies: *Selenosporella curvispora* G.Arnaud, *Chloridium* state of *Chaetosphaeria preusii* W.Gams & Hol.-Jech. and *Anavirga laxa* B.Sutton. However, according to the literature they are uncommon at pine litter.

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993 - Spatial distribution of fruitbodies of macrofungi in pine forests with *Vaccinium myrtillus* in northern Latvia

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Investigation on spatial distribution of fruitbodies of agaricoid and boletoid fungi was carried out during 1993-1997 in three *Pinus sylvestris* stands in the northern Latvia. *Vaccinium myrtillus* and mosses dominated the ground cover of forests. 25 permanent subplots, each of 2m x 20m, were established at each forest site. Each subplot was divided into 40 lesser microplots with size of 2m x 0,5m. Counting of fruitbodies was performed 5-6 times per year during August-October. A total of 92 species were found in the plots during the 1993-1997. The annual number of species and their productivity considerably varied from year to year. The fruitbodies of some species appeared in the same or in the adjoining microplots year after year. The distribution of fungal species was patchy. Neither more than 3-4 (usually 0-2) species were found at the same microplot simultaneously, though a total number of recorded species per microplot varied from 0 to 9. A negative correlation among species was found. Fruitbodies of some species, such as *Cortinarius croceus* and *C. semisanguineus*, were spread rather evenly through all subplots. Fruitbodies of some other species were localized in a few microplots only, and their appearance often were related to the discriminative features of these microplots-soil disturbance, presence of decaying wood or other substrates, as well as the type of vegetation. However, no spatial patterns of fruitbodies distribution were found for the major part of species.

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994 - Leaf decomposition by aquatic fungi in heavy metal polluted habitats

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The long history of copper mining and smelting in the district of Mansfeld (Central Germany) resulted in high loads of heavy metals in streams, lakes and even groundwater [1]. We followed decomposition of *Alnus glutinosa* leaves in two streams, one with a high (H4) and one with a moderate (H9) load of these metals. In H9, mass loss closely followed an exponential decay curve; in H4, leaf mass remained constant after a very rapid initial decay during the first 4 weeks. Fungal biomass, estimated by ergosterol measurements, rose more quickly and to higher levels on H9 than on H4 leaves. Conidium production by aquatic hyphomycetes was reduced in site H4. Despite the huge differences in conidium production between the 2 streams, fungal species numbers during decomposition were similar. After 4 weeks of stream exposure, leaves showed greatly increased levels of heavy metals [2]. Leaves exposed in site H4 layered with an inconsistent assemblage of biofilm material, fungal hyphae, extraneous particles of sediment and metallic precipitate. The plant material was remarkably well preserved. A relatively high leaf decay rate may indicate a fungal community that has successfully adapted to the ambient heavy metal levels. [1]KRAUSS, G. et al. (2001) Aquatic hyphomycetes occur in hyperpolluted waters in Central Germany. Nova Hedwigia 72: 419-428 [2]SRIDHAR, K.R. et al. (2001) Decomposition of alder leaves in two heavy metal polluted streams in Central Germany. Aquat. Microb. Ecol., 26: 73-80.

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995 - Fungi and insects - an ecologically important relationship in a mountaineous rain forest in South-Ecuador

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During two research periods at the Estacion Scientifica San Francisco near Loja in South-Ecuador, we discovered that specific ecological relationships between fungi and insects are an important factor influencing coevolution between the two groups. Especially beetles from the Staphylinidae have been observed on Agarics. Beetles from the Ptiliidae have been discovered to be very well adapted to the hymenial morphology of Polyporaceae s.l.

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996 - Microscopic fungi associated with oak bark beetle (*Scolytus intricatus*) in the Czech Republic

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During 1997-99, surface mycoflora of *Scolytus intricatus* was studied with respect to its role in transmission of microscopic fungi. Samples were collected from five localities in central Bohemia. Investigation was focused on all different stages of life cycle of the beetle: eggs, larvae, adults before emergence, adults in generation and maturation feeding (altogether 500 samples) and also on galleries (400 samples). Insect samples were washed by sterile water in ultrasonic cleaner. Suspension and insect were separately inoculated onto Petri dishes with malt-extract agar. The most frequent fungi associated with *S. intricatus* appeared to be *Candida rhagii*, *Geosmithia* spp. and *Penicillium* spp. A great attention was paid to occurrence of ophiostomatoid fungi. Two species were determined: *Ophiostoma piceae* s.l. and *O.* cf. *prolifera*, both considered to be saprotrophs or weak pathogens. Frequency of all these fungi varied depending on the locality and the stage of life cycle of the beetle. During this study other interesting micromycetes were isolated, belonging to phytopathogenic fungi (e.g. *Diplodia mutila*), wood colonizing fungi (e.g. *Acrodontium crateriforme*, *Cytospora* sp., *Libertella* sp., *Phomopsis* sp., *Ramichloridium anceps*, *Phaeoacremonium* spp.), nematophagous fungi (*Esteya vermicola*), entomopathogenic fungi (e.g. *Paecilomyces farinosus*) and many litter and soil fungi (e.g. *Acremonium* spp.). The study was supported by the Grant Agency of the Czech Republic (No. 203/97/0037).

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997 - Study on fungal communities in mediterranean pine woods: First results

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Fungi can be regarded as useful bioindicators of forest ectotrophic stability; mycological studies can be useful for predicting the fate of forests subject to different types of stresses. Integrated researches on fungal communities and their environment provide useful informations about the ecology and distribution of fungi in space and time; such informations are preliminary for studies on the conservation of fungal flora and for the statement of global change effects. This type of informations are available for many taxa in central and northern Europe, but is largely lacking for those in the Mediterranean area. The European Confederation of Mediterranean Mycology (CEMM.ae) recognized the need to fill these gaps and in 1999 research was begun on fungal communities of *Pinus* sp. pl. woods by mycologists of the three member countries (France, Italy and Spain). Here, the first results of this work are reported. Various species linked to pines or to the Mediterranean environment were found. Some taxa present in the Red Lists of central and northern European countries were also present in the studied areas. The percentage of mycorrhizal species was calculated for each permanent plot. This percentage was found to reflect forest health in northern and central Europe. Low values of this index for some of the studied pine woods suggests that they are in a state of decline. Classification and ordering of the relevés showed affinities between the fungal communities.

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998 - Interaction of arbuscular mycorrhizal fungi with plant pathogens on *Coffea arabica* L.

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Interactions between AM fungi and a pathogen of coffee leaves were investigated. First, we evaluated three coffee cultivars for differences in the amount of AM extraradical hyphae. Extraradical hyphae were significantly less in Caturra than in Pacas and Borbón cultivars in all locations. In another study, we compared the percentage of mycorrhizal root length among paired coffee seedlings with and without *Cercospora coffeicola* lesions. We found that seedlings with pathogen lesions on the leaves had significantly greater mycorrhizal colonization than seedlings without lesions. In a third study, we evaluated effects of mycorrhizae and soil nutrient levels on plant growth and development of leaf lesions caused by *C. coffeicola*. This experiment used the cultivars Caturra and Borbón with four nutrient treatments: a control, 25 ppm added N or P, and 25 ppm each of added N and P. Growth in Caturra only responded to fertilization while growth in Borbon only responded to mycorrhizae. The results also suggested that there was a higher cost of mycorrhizal association in Borbón than in Caturra. There were no significant differences in lesion development among treatments in two replicate experiments.

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999 - Community structure of ectomycorrhizal fungi beneath fruitbodies of *Xerocomus subtomentosus*, *Suillus luteus*, *Lactarius rufus* and *Scleroderma citrinum*

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The goal of our studies was to determine the correspondence between above- and below-ground views of species composition of ectomycorrhizal fungi in Scots pine (*Pinus sylvestris*) stand in Poland. We sampled mycorrhizas from soil cores taken directly below fruitbodies of some ectomycorrhizal fungi, which appeared with differentiated abundance on tested plots. *Suillus luteus*, *Lactarius rufus* and *Scleroderma citrinum* were among the most abundant fruitbodies and *Xerocomus subtomentosus* belonged to rather rare specimen. All viable ectomycorrhizas removed from cores taken directly below fruitbodies were classified into morphotypes based on color, size and type of ramification. On average 5-6 different ectomycorrhizal morphotypes were selected under each species and mostly one or two morphotypes dominated. RFLP analysis of the ITS region to identify species in ectomycorrhizal communities confirmed morphological typing. A few ectomycorrhizal tips belonging to *X. subtomentosus* and *S. citrinum* were detected in cores taken under these fruitbodies. In samples taken beneath *S. luteus* and *L. rufus* root tips bearing mycorrhizas of these species were lacking. The results suggest that root tips of several ECM species coexist within fruitbody of each particular species.

1000 - Switching partners: ecto- and arbuscular mycorrhizal fungi on seedlings of three oak species

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Interestingly, an increasing number of researchers have shown that under field conditions, some *Quercus* species may host both ecto- and arbuscular mycorrhizal fungi. This ability to host arbuscular mycorrhizal (AM) fungi may allow oaks to successfully establish beyond the ectomycorrhizal (EM) root zone of mature oaks. In this study, we considered how established EM and AM communities might affect colonizing oak seedlings. Three oak species (*Quercus agrifolia*, *Q. garryana*, and *Q. douglasii*) were transplanted into a Southern Californian

oak (*Q. agrifolia*) ecosystem. After a year in the field, the seedlings, accompanied by soil cores from their immediate environments, were harvested and analyzed. We found that the position of planting strongly determined the EM or AM status of the seedling; seedlings transplanted under a mature oak canopy become colonized with ectomycorrhizal (EM) fungi, while those planted into surrounding grassland areas typically showed arbuscular mycorrhizal (AM) colonization. Morphotyping and RFLP data showed that the presence and position of EM species on mature oaks strongly predicted EM diversity on seedlings. Several species of EM fungi dominated the site and were present on both seedlings and mature trees, although many rare types were also found. The diversity and identity of mycorrhizae associated with transplanted oak seedlings appear to be largely regulated by position of planting, and not by any mycorrhizal preferences of the three oaks species.

1001 - Population structure of *Heterobasidion annosum* in birch plantations, replanted on infested sites

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The persistence of *Heterobasidion annosum* on infested areas and its transfer to a new forest generation was studied in three forest sites with similar histories. The sites represented *H. annosum* disease centres (each 0.2 ha in size) in 50-60 year-old *Pinus sylvestris* stands, which were clear-felled and replanted with *Betula pendula* 25 years previously. Fungal isolation from trees and stumps on each site encompassed both replanted *B. pendula* and surrounding *P. sylvestris* from the previous generation. A total of 121 isolates of *H. annosum* was obtained from spatially separated resource units. All of them belonged to the P intersterility group. Each isolation locality was put on a local map. Vegetative compatibility tests with the isolates revealed extensive territorial clonality: from the first site, 23 isolates clustered into 5 clones (4.6 isolate per clone), from the second - 63 into 6 (10.5), and from the third - 35 into 5 (7.0). Three largest clones consisted of 30, 18 and 10 isolates and covered the respective areas up to 25, 32 and 48 m across. It was not uncommon for the territorial clone to cover areas that encompassed both *P. sylvestris* and *B. pendula* forest generations. The results of this study show that *H. annosum* is able to persist on infested sites for decades and readily attack replanted trees of the new forest generation. This is the first extensive study dealing with the population structure of *H. annosum* P group in birch stands.

1002 - *Chytridium lagenula* Scheffel is a parasite of *Tribonema gayanum* Pash. (Xanthophyceae)

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Development of *Chytridium lagenula* was observed in the cultures inoculated by water samples. This parasite develops only on the alive cells of alga. We observed development of *C. lagenula* on *Tribonema gayanum* and *T. vulgare* Pasch. We did not observed development of this parasite on dead cells and there was not growth on the pine's pollen and on the free medium (without alga), that is why we think, that *C. lagenula* is obligate parasite of algae. *C. lagenula* has a sessile upright or slightly titled sporangium with a thin smooth wall. Growing sporangium is globular, ripening pear-shaped, 8-13 µm length, 4-6 µm breadth. Zoospores of the parasite are globular with one big oil droplet. The diameter of zoospore is 3-4 µm. *C. lagenula* is able to form globular resting spores, 6 µm in diameter. Endobiotic system of *C. lagenula* consist of a rhizoid. Also we have a facts about quantity circulation of *C. lagenula* in nature. The number of infectious units of *C. lagenula* was evaluated in Ladoga lake and adjacent water bodies. It was 10 stations examinated. *C. lagenula* was detected in 4 stations examined. Quantity of infective units (sporangium, zoospores) was varied from 0.1 to 0.36. (It is need to take into account, that concentrations were low, because water was filtered). Reservoir it is possible to speak, that *C. lagenula* is widespread parasite of alga in nature, which mainly inoculate genus *Tribonema*.

1003 - Single sequence repeats markers reflect population diversity and geographic barriers of *Ceratocystis polonica* in Eurasia

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The blue stain fungus *Ceratocystis polonica* and its associated bark beetle vectors, in particular *Ips typographus*, cause huge losses to spruce (*Picea abies*) in Eurasia. The aim of this study was to develop Single sequence repeats markers (SSRs) in order to determine the population structure and genetic diversity of *C. polonica* in Europe and Japan. RAMS primers were used to target

SSRs regions. The amplified PCR products were cloned, sequenced and analyzed for SSRs. Primers were designed, flanking the SSRs found in these amplicons. Seventy-five isolates from four populations were tested with ten SSR markers. A high gene diversity was found (h=0,56). Analysis of the G statistics showed a low degree of population structure in Central Europe and a high level of gene flow between populations (Gst=0,02, Nm=17,17). In contrast, a high population subdivision was found between the Japanese and the European populations, indicating strong geographic isolation. These data based on *C. polonica* closely resemble those recently produced for its insect vector, *I. typographus* (Stauffer et al., Mol. Ecol. 8, 763-773). The SSR primer pairs could also amplify PCR products from six other *Ceratocystis* spp. from conifers in the *C. coerulescens* complex. Results from this study suggest that quarantine strategies in Europe should consider the entire range of genetic diversity on the continent and that movement of *C. polonica* and its vectors between Europe and Japan should be avoided.

1004 - Differentiation of the macrofungal communities in high diversity forest ecosystems of central Balkan peninsula

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To study macrofungal communities in forest ecosystems we chose a canyon in Western Serbia where the vegetation showed very high biodiversity and differentiation due to the climatic conditions. Three ecological groups of macrofungi (wood and litter decayers and mycorrhizal) were detected by observing sporocarps during 6 years. Plant and fungal community differentiation were examined comparing three techniques of multivariate analyses. To determine the influence of abiotic factors on differentiation of plant and macrofungal communities we used a calibration method for humidity, temperature, soil acidity, available nitrogen and light intensity. We used canonical correspondence analysis for analysing influence of abiotic parameters, as well as for analysing influence of dominant phanerophytae species on differentiation of macrofungal communities. Four vegetation complexes were described: xerophyllous forests of black hornbeam, forests of xerophyllous oaks, mixed mesophyllous forests of black hornbeam and mesophyllous beech forests. The highest macrofungal diversity, as well as the best community structure and differentiation were detected in oak and beech forests. The main factors that influenced differentiation of plant communities were temperature, available nitrogen and light intensity. Abiotic factors showed much lower influence on differentiation of macrofungal communities. The main factor for this process was presence of specific phanerophytae species.

1005 - Fungal associations of mycorrhizal roots of conifer seedlings in Lithuanian forest nurseries

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Morphological and molecular identification of fungi associated with mycorrhizal roots was conducted on 60 pine and spruce seedlings in 5 forest nurseries in Lithuania. The plants were either grown in nursery beds as bare root seedlings, in plastic trays or in wrapped polyethylene (WP) containers. Bare root greenhouse seedlings were also examined. In total, 5364 & 12441 root tips were investigated for pine and spruce, respectively. Of the pine roots, 2131 (39%) were mycorrhizal, and 6527 (52%) of the spruce roots. The cultivation system had a profound impact on mycorrhizal colonization for both tree species. In pine, it was most abundant in the nursery bed bare root system (49%), while in spruce - in the WP containers 72% of the roots were mycorrhizal. In total 8658 mycorrhizal roots were morphotyped, and 7 species were found on pine and 8 on spruce roots. On pine an unidentified ascomycete was most common (58% of mycorrhizal tips), while on spruce a basidiomycete *Amphinema byssoides* colonized 54% of the mycorrhizal tips. Isolation of the fungi into pure culture was attempted from 8163 mycorrhizal root tips, yielding 625 isolates, which were divided into 144 groups according to morphological characteristics of the mycelium. One representative of each mycelial group was subjected to sequencing of the ITS region of the rDNA and 43 fungal species have been determined using the NCBI BLAST. The most common species were *Phialocephala fortinii* (29%) and *Phialophora finlandia* (37%).

1006 - Rhizomorph Foraging Patterns of *Armillaria gallica* and *A. mellea* from the Ozark Mountains of the central U.S.A.

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Our research is driven by the need to understand inter- and intra-specific interactions of co-occurring *Armillaria* genets with respect to oak deline. Foraging patterns of *A. gallica* and *A. mellea* rhizomorphs were measured in three experimental series: 1) temporal pattern changes measured in a uniform environment (agar); 2) pattern responses to woody baits measured in sand; and 3) pattern responses to the presence of neighboring genets of the same or different species, measured in sand. Four response metrics were used: fractal dimension, number of foraging tips, total rhizomorph length, and total rhizomorph surface area. Preliminary analysis indicates that rhizomorph foraging by

A. gallica was stochastic where target woody food bases were placed laterally with respect to the colonized source; however rhizomorph foraging was significantly directed when the target woody food base was placed above or below the source. Presence of con-specific neighbor genets altered the allocation of rhizomorph length and the number of foraging tips without affecting fractal dimension for both *A. gallica* and *A. mellea*. Similarly, neighboring genets of different species did not influence the fractal dimension of *A. gallica* or *A. mellea* rhizomorph systems. Thus, *Armillaria* rhizomorph system pattern is influenced in abundance and physical location in the landscape by adjacent genets of the same or different species, while the inherent foraging pattern remains unresponsive to such influences.

1007 - Micro-evolution in the ectomycorrhizal fungus *Suillus luteus*

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Previous research has shown that populations of the ectomycorrhizal fungus *Suillus luteus* found in areas polluted with high levels of heavy metals (Zn, Cd) have acquired adaptive tolerance to elevated levels of these metals. Furthermore, these populations showed a reduced genetic variability, as measured by ISSR markers, compared to control populations from unpolluted sites. In this study we aim to analyse the microevolution of populations of *Suillus luteus* in areas polluted with heavy metals. Therefore, the genetic structure of these populations, as well as the structure of populations from unpolluted areas, the amount of gene flow between these populations and the occurrence of bottlenecks will be described using microsatellite and AFLP markers. In a later stage, mtDNA markers will also be used, after having resolved the inheritance mode of the mitochondria in *S. luteus*. After identification of genes related with the heavy metal tolerance, natural selection will be investigated on a molecular level using these genes and the selectively neutral molecular markers.

1008 - Genetic diversity within French populations of *Tuber melanosporum* Vittad.

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Truffles are ectomycorrhizal ascomycetes and edible fungi of economic value. They have been the focus of numerous studies of genetic diversity. Certain species show a high genetic diversity (e.g. *Tuber uncinatum*), whereas others have low polymorphism (e.g. *T. melanosporum*). *T. melanosporum* was regarded as a clonal species without any geographic genetic structure. We have analyzed the genetic diversity of *T. melanosporum* populations from different geographic regions in France by looking for polymorphic loci. We have used RAPD (Random Amplified Polymorphism DNA) profiles, genomic sequences previously identified as SCAR (Sequence Characterized Amplified Random) and sequences from the internal transcribed spacers (ITS) of nrDNA. The RAPD phenotypic analysis and the sequence analysis of genomic tags have confirmed the genetic homogeneity of this species. RAPD phenotypes can not be correlated to their origin region. However, we have found some polymorphic populations and, according to the Shannon's index, isolates from 'Bourgogne' were the most variable. Sequencing of the ITS allowed us to identify four haplotypes (I, II, III and IV) in 47 ascocarps. Haplotypes I and II were the most frequent and they were found in every populations except for haplotype I in 'Lorraine'. On the other hand, haplotypes III and IV were more scarce and restricted to particular French regions (e.g. Haplotype IV was found only in 'Lorraine') suggesting a genetic structure in *T. melanosporum*.

1009 - *Ceratocystis* wilt of *Acacia mearnsii* in Uganda

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Ceratocystis wilt caused by *C. albofundus* is a severe disease of *Acacia mearnsii*, in Africa. In a recent survey, this disease was found on wounded *A. mearnsii* in South Western Uganda. Diseased trees showed extensive discoloration of the wood, dieback, gummosis, wilting and death. Previous studies of the gene diversity of a South African population using CAT_5 marker and 8 microsatellite markers revealed a high gene diversity. The high gene diversity and presence of *C. albofundus* on native *Protea* species has led to a hypothesis that it is native to South Africa. The aim of this study was to determine the gene diversity of a Ugandan population and compare it to that of the South African population. 36 isolates, were obtained from *A. mearnsii* trees in South Western Uganda. Eight microsatellite primers pairs previously designed for *C. fimbriata* were used to amplify the microsatellite rich regions of the genome. Analysis of the microsatellite data revealed 7 loci to be polymorphic. Nei's gene diversity (H) showed that the South African population is slightly more diverse than the Ugandan population with a value of 0.4320 compared to 0.4082. Results further revealed that very few alleles are shared, with little gene flow between the two populations. The high gene diversity in both populations strongly suggests that *C. albofundus* is native to the African continent and not necessarily only to South Africa, as previously suggested.

1010 - Roles of ascospores in the white root rot fungus, *Rosellinia necatrix*

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Rosellinia necatrix Prilleux, the ascomycetous white root rot pathogen, causes destructive damage to numerous woody and herbaceous plants, especially to fruit trees, throughout the world. The fungus produces teleomorph rarely on diseased plants in nature, and the roles of ascospores in the life cycle remains unclear due presumably to the scarcity of teleomorph production. In this study, stromata produced on root fragments were studied to elucidate the roles of ascospores. Stromata were produced on 23 out of 47 diseased root samples from Japanese pear, grapevine and *Chloranthus glaber*, which had been placed in a hollow on the ground surface in the shade of trees and covered with rice straw. Also, stromata were obtained on mulberry twigs inoculated with two out of six isolates. Readiness of stroma production was not related to host plant species, dates of treatment, or the presence of double-stranded (ds) RNA. In 18 single ascospore isolates from six stroma samples, mycelial interactions between isolates from the same samples were incompatible, and the isolates were pathogenic to *Lupinus luteus* with a few exceptions. dsRNAs in vegetative hyphae of 10 stroma samples were not transferred to single ascospore isolates. Thus, ascospores in *R. necatrix* are effective in extending genetic variation, producing pathogenic offspring and eliminating infectious factors such as dsRNA.

1011 - Population dynamic of *Pyrenochaeta terrestris* on the basis of cropping patherns and infection percentage in onion fields

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Studies on population dynamic of the fungus on the basis of infection, indicated that the cropping patherns having a significant effects in infection of the fungus. 441 onion fields were taken into considerations during 1997-98, where there was five manin cropping patherns including alfa-alfa (*Medicago sativa* L. usually for 4-5 years), cereals (wheat, barley, rice and seldom millet), vegetables (tomato, bringal, mask-melon, cauliflower, cabbage, etc), orchard (where the fruit trees were up rooted and became under cultivation) and onion frequently for the last four years before to onion. A hundred onion plants were up rooted near maturity stage and their roots were examined for the presence of the infection based on infection percentage of the roots in three scoring scales (>10%, >25% and more than 25% infection). The higher infection was observed in frequent onion growing fields, fallowed by alfa-alfa with 36.16 and 27 percent infection respectively, where the orchard soil had the least infection with 2.83 percent infection on onion roots. Out of which 12.58, 9.58 and 0.50 percent were in the ranges of more than 25 percent respectively with a significant effects, where cereals and vegetables were in a separate group. These results revealed that the build up of population inoculum is an improtant factor in natural enivronments, but the more inportant one is the population potentiality, which can measured infectionally and/or differentially with a distinct scoring scales.

1012 - Coprinaceae in wood and straw degradation: implications for ecology and biotechnological use

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Coprinaceae have been defined as a family of about 800 species, following morphological and physiological characteristics such as lamella and spore colour and their abilities to self-digest upon fruit body maturation. Recent molecular analysis suggests Coprinaceae are not monophyletic but comprise four different genera (1). Coprini are usually saprotrophic on soil or dung of herbivores. Species growing on decaying wood or plant debris in soil and a few plant pathogens have also been described. Here we present an overview on species relating to wood and straw degradation. Enzymes implicated in wood and straw degradation (phenol oxidases including laccases; peroxidases; xylanases) have been described in a few species. Biotechnological use have been made of *C. cinereus* peroxidase (2) and one laccase (3) - genes for at least six different laccases are known (3,4). It is thought that at least some of these enzymes link to developmental processes, for example fruiting body development (*C. congregatus*; 5) and spore formation (*C. cinereus*; 6). Our experimental work aims at elucidating wood degrading abilities of various Coprinaceae, at making use of enzymes in biotechnology and at determining functions of enzymes in fungal development. (1) Redhead et al. 2001 Taxon 50:203; (2) Kauffmann et al. 1999 J Biotechnol 73:71; (3) Yaver et al. 1999 AEM 65:4943; (4) Bottoli et al. 1999 J Microbiol Meth 35:129; (5) Choi et al. 1987 Mycologia 79:166; (6) Vnenchak, Schwalb 1989 Mycol Res 93:546.

1013 - Physiologic races of stem rust of wheat in Egypt during 1994 - 1996 seasons

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The problem of stem rust incidence has been solved since the release of resistant wheat varieties in Egypt. Also, the early sowing of wheat varieties made the problem less serious. However, the appearance of new races is expected. Therefore, continuous identification of physiological races of the causal organism is required. In the course of this study, identification of stem rust physiologic races was carried out during two successive seasons (1994-1995 & 1995-1996) using the North American wheat stem rust monogenic differentials. The results obtained revealed the presence of different physiologic races in Egypt during both seasons of the study. Races RT-, RR-, TR-, TT-comprised 92.02% of the total isolates. While, races RT-, RR-, TR- and TT-comprised 7.98% of the isolated races in 1994/1995 season. On the other hand, in the second season (1995-1996) races TT-, RT-, RR-, MT- and QF- comprised 47.04% of the total isolates. While, the rest were 29 races comprised 52.96% of the isolated races. The great variability of the physiologic races showed the dynamic state of the pathogen. Also, showed that wheat plants are liable to be attacked by different races from different sources.

1014 - Frequency of virulence of *Puccinia triticina* on wheat in Egypt during 1998-2001

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Leaf rust of wheat is common under the Egyptian conditions due to the favorable environmental climate for disease incidence and development. Many wheat varieties were discarded because of their susceptibilty to the disease due to the appearance of new virulences and the dynamic state of the causal organism. The frequency of virulence was studied against 32 local genotypes and 45 monogenic lines for leaf rust resistance in addition to the highly susceptible cv. Thatcher. The results revealed that more than 75% of the obtained races were virulent to six genotypes i.e cvs. Giza 139, Giza 155, Giza 157, Giza 160 and Giza 164. Whereas 50-75% of the total races were virulent to 15 genotypes i.e. cvs. Giza 162, Giza 163, Giza 165, Giza 167, Sakha 8, Sakha 92, Sids I, Sids 2, Sids 3, Sids 4, Sids 5, Sids 9, Gemmieza 1, Gemmieza 3, and Sakha line 202. Less than 50% of the total races were virulent to 11 genotypes i.e. cvs. Giza 168, Sakha 61, Sakha 69, Sakha 93, Sids 6, Sids 7, Sids 8, Gemmieza 5, Gemmieza 7, Gemmieza 9 and Sakha line 206. On the other hand, no virulence was detected against Lr 19. Low frequecies were found to Lrs; 15, 21, 24, 28, 29, 36, 38, 39, 40, 41, 42, and 43. Therefore, these genes should be considered in breeding programme for leaf rust resistance. High frequencies (more than 80%) were found against Lrs; 1, 2c, 3, 12, 13, 14 a, 22b, 23, 33 and Lr B whereas, frequencies from 51 - 80% were found on the rest of genes.

1015 - Contribution to the knowledge of the endophytic mycoflora of roots of sessile oak (*Quercus petraea*)

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The composition of endophytic mycoflora of roots of sessile oak (*Quercus petraea*) was studied. This research was conducted in six oak stands in Krivoklátsko region in Central Bohemia (Czech Republic). The fungi were isolated from fine roots (0.1-0.3 cm diam) and from peridemal bark, subperidermal bark and wood of medium roots (2-5 cm diam) and coarse roots (0.5-1 cm diam). After surface sterilisation the roots were cut into segments and placed onto malt extract agar. More than 60 species of microscopic fungi were identified from the samples. Fungi occurred most frequently in fine roots and in peridermal bark of medium and coarse roots. Wood of coarse roots was colonised at least. The most frequent fungi were *Phialophora* cf. *fastigiata*, *Cryptosporiopsis radicicola*, *Cystodendron*-like hyaline anamorph, dark septate endophyte, *Umbelopsis nana*, *Trichoderma viride*, *Oidiodendron griseum*, *Chloridium preusii* and *Sporidesmium* cf. *anglica*. Species of genera *Sporidesmium*, *Chloridium*, *Chaetosphaeria* and *Menispora* were mainly isolated from the fine roots. Rhizomorphs of *Armillaria* and different mycelia of Basidiomycetes were recorded in some roots. Bacteria were common in roots. The study was supported by the Grant Agency of the Charles University (No. 243/1997/B BIO/PøF).

1016 - Biodegradation of drying oil claddings by micromycetes

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In the drying-related industry the oil components are of great utility for covering wood, metal and other materials and constructions. And there is the problem of its complete

degradation after some period of service. It is known that natural and synthetic polymeric materials undergo a process of decomposition after a period of service and the main biological agents of it are filamentous microscopic fungi-micromycetes (MM). The goal of the present study was to find out the community of MM for a maximal biodegradational activity against the drying oil cladding on the base of the natural component (linseed oil). Active strains of MM which were isolated from different polymeric materials were used to make the inoculation communities: I - including *Aspergillus niger*, *A. versicolor*, *Penicillium aurantiogriseum*, *P. funiculosum*,*Trichoderma koningii*; II - *Aspergillus fumigatus*, *A. flavus*, *Cladosporium cladosporioides*, *Penicillium frequentans*, *P. notatum*; III - *Aspergillus glaucus*, *A. terreus*, *A. ustus*, *Alternaria alternata*, *Penicillium brevicompactum*. It was found that the different communities of MM colonized oil cladding to different extent. The areas of colonization varied from 60% to 100% for II and III communities and from 40% to 50% for the I community. The inoculation by the I community resulted in maximal loss of oil cladding (up to 13.3% of the starting weight). So, the first community of MM has the maximal biodegradational activity against the drying oil cladding.

1017 - Formation of communities of micromycetes-colonizers of municipal waste during the composting

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Micromycetes (MM) are the best known decomposers of different polymeric products, which comprise the main part of municipal waste (MW). The research has been focused on mycological monitoring of MW composting and was first carried out in Russia. The results obtained demonstrated that the spontaneous communities of micromycetes-colonizers of MW were undergoing seral succession and mechanisms of species replacement in fungal communities were different. It was established that species and biochemical diversity of MM increased to the the end of the composting process. Successions of MM tended to formation of a stable heterogenic and polyfunctional community. This heterogenic climax community can withstand the environmental changes and can provide the production of a high-quality compost. Biologically high-quality compost should serve not only as a source of organic matter at soil applications but also as a source of soil suppressivness inhancement and biological control. Compost should be free from phytotoxic and pathogenic species of MM, the microbe community of the final product should be stable and include beneficial for plants species. However, it was found that final compost communities contained species with negative effect on plant and human populations. Certain complexes of MM possesing positive properties for composting and enviroment were suggested for the introduction at the definite stages of succession during composting.

1018 - Habitat differences of coprophilous organisms on moose dung

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Dung from cervids supports a species-rich community, but few studies have been done on forest-living species. In order to test whether differences in species composition may be explained by habitat-specific differences, we studied coprophilous fungi developing on moose dung of homogenous origin, composition and age in an area in northeastern Sweden. Of the 26 species of fungi found, 12 species were new to the region, 17 had never been observed on moose dung and 2 species were not previously described. We found a significant difference in species composition between the three habitats studied, with low number of species in the spruce forest and about a three fold increase in the pine forest and the open mire. Species diversity was negatively associated with degree of insect attack. This suggests that insects feeding either on the dung or the fungi (spores, mycelium) may be an important factor explaining the observed pattern. In order to test this hypothesis we need experiments including insects exclusion.

1019 - Comparative enumeration of anaerobic fungi from different herbivores

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Determination and comparison of anaerobic fungal populations from the faeces of cattle, sheep and horses were carried out. Similarities and differences, in terms of population density between the faeces and rumen digesta of same individual were also assessed. The effects of using different substrates in the most probable number (MPN) dilution series on the population density were also studied. The MPN technique was used to enumerate fungal populations and counts were expressed as thallus forming units (Log_{10} tfu g DM^{-1}). Mean value of MPN counts obtained from fresh faeces were as follow; cattle = 5.61; sheep = 4.41 and horse = 4.76. Fungal counts obtained from horse and sheep were similar ($P > 0.05$), whilst cattle contained significantly more anaerobic fungal tfu in their faeces than either sheep or horse ($P < 0.05$). The mean MPN values of faeces and ruminal digesta of 3 cattle were as follows; fresh faeces = 5.22; frozen faeces = 4.92 and fresh ruminal digesta = 5.31. MPN values obtained from fresh faeces, frozen faeces and fresh ruminal digesta were similar (P > 0.05). The mean values from faecal and ruminal samples of 3 cattle for different substrates were as follow; glucose = 5.11; cellobiose = 5.23; xylan = 4.75; wheat straw = 5.53 and cellulose = 5.11. Wheat straw gave significantly (P < 0.05) higher viable counts than glucose, xylan and cellulose. Population densities, obtained when glucose, cellulose and xylan used, were similar (P> 0.05).

1020 - Ectomycorrhizal fungal dynamics of one- and two-year-old Norway spruce seedlings after planting to mounded forest clear-cut

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Container Norway spruce seedlings were planted on two clearcut and mounded *Myrtillus* type sites in Central Finland. Seedlings were planted on mounds and on untreated spots between mounds. Seedlings were sampled before and one and two growing seasons after planting for detailed measurement of root and shoot attributes and for morphological and molecular characterization of ectomycorrhiza (ECM) in roots grown out into the soil. Different morphological groups were subsampled for PCR-DGGE analysis of 18S rRNA gene region in order to separate ECM in detail. During the first growing season, one-year old seedlings grew better in shoot height than the two-year-old. However, root egress from containers into the surrounding soil was greater in the two-year-old seedlings during both first and second summer after planting. The outgrown root length and the number of root tips per length were greater on mounds than on untreated spots. Totally 17 different morphotypes were observed and 6 of them were present in all treatments. Neither age of the seedling nor duration in the field did affect the richness of ECM morphotypes but there was a clear temporal shift in the proportions of 3 major morphotypes. The smooth white type was the most dominant morphotype before outplanting but almost disappeared in two years in the field. In contrast, the proportion of the smooth brown type increased up to 58-78% after two years. The smooth dark brown type increased especially on mounds.

1021 - *Pleurotus* spp. characterization by microsatellite-based DNA fingerprinting

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Characterizing fungal species and varieties through microsatellite is a powerful approach to evaluate biodiversity and genetic relationships among populations. Four ISSR (inter simple sequence repeats) primers have been utilized to characterize fungal species and varieties among *Pleurotus* genus. Five strains of each species/varieties (*P. ostreatus*, *P. eryngii*, *P. eryngii* var. *ferulae*, *P. eryngii* var. *nebrodensis*) have been analyzed and ISSR data were subject to cluster analysis performed with UPGMA method using Dice and Jaccard coefficients. This analysis clearly showed two distinctive cluster, one related to *P. ostreatus* and one to *P. eryngii* var. *ferulae*, whereas less clear relationships appeared between *P. eryngii* and *P. eryngii* var. *nebrodensis*. Further investigation are in progress to analyze additional *Pleurotus* strains and to test different ISSR primers as well as to identify microsatellite-containing amplicons.

1022 - The polymorphic nature of the rRNA LSU gene of arbuscular mycorrhizal fungi (AMF) spores

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Sequencing of ribosomal RNA genes has been extensively exploited in attempts to identify and determine phylogenetic relationships and genetic heterogeneity of AMF. The heterogeneity of rRNA genes within single spores and isolates of AMF has been recognised for several years, however the magnitude of this variation has been ignored in most taxonomic and phylogenetic analyses. Sequence diversity has crucial implications for the interpretation of AMF biodiversity and the species concept in these fungi. We report here the first investigation of genetic diversity in AMF where variation has been assessed using sample sizes of several hundred sequences. The study concentrated on a well characterised species of AMF, *Glomus coronatum*, but also included three other *Glomus* species with similar spore morphologies. In total 435 clones containing the LSU D2 regions from 7 isolates of *G. coronatum* (80% of isolates currently available) as well as isolates of *Glomus mosseae*, *Glomus geosporum* and *Glomus constrictum*, were screened for sequence variation using a PCR-single strand conformational polymorphism (PCR-SSCP) technique. The variation encountered was higher than expected, 138 representative sequences were obtained and analysis indicated that most were unique. This variation could not be attributed to DNA polymerase or cloning artefacts. Implications for the species concept in AMF, the use of rRNA sequences to estimate biodiversity and in situ detection in field ecology are discussed.

1023 - Temporal distribution of ectomycorhizae in *Quercus rubra* L. plantations of the Basque Country and evaluation of their infective potential

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Due to its fast growth and the commercial value of the wood, *Q. rubra* has been used in many Basque Country plantations which have been located in *Q. robur* L. habitats. The evaluation of infection ability and density of the mycorhizal propagules is one of the main factors to take into account in reforestation and restoration programmes (Kendrick 1988). The principal aim of this study is to explore the temporal variation throughout a year (2000-01) of the species richness and diversity of ectomycorhizae in six localities. Likewise, greenhouse's experiments were carried out in order to determine the infective potential of the forest soils. Maximum values of diversity were recorded during autumn and winter; whereas the minimum was in summer. The ubiquitous *Cenococcum geophilum* is one of the few morphotypes that appears throughout the year. According to the mycorhization rate biometric and physiological variables of the seedlings grown in soil of different localities of *Q. rubra* forests were determined. A positive correlation has been found between mycorhization rate and measured variables.

1024 - Growth promoting effects of endophytic colonisation of the roots of larch seedlings (*Larix decidua*) with *Cryptosporiopsis* sp. and *Phialophora* sp.

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Symbiotic associations of endophytic fungi with their hosts vary from mutualistic to latently pathogenic. In order to discern the nature of endophytic infections of the roots, those of axenically cultured larch seedlings were inoculated either with one of two endophytes, *Cryptosporiopsis* sp. or *Phialophora* sp., previously isolated from roots of larch, or with the pathogen *Heterobasidion annosum*. All three isolates colonised the roots systemically and both inter- and intracellularly. Whereas colonisation with each of the fungi significantly improved growth of the seedlings in comparison to the non-inoculated control, the greatest improvement resulted from endophytic colonisation. However, in contrast to colonisation with the endophytes, infection with *H. annosum* also led to development of disease symptoms. Application of a mycelium extract of *Phialophora* sp. to roots of the host also improved growth of the roots and seedlings. The capability of all three fungi to produce IAA may explain the growth enhancing effects of the mycelium extract and of colonisation. Additional virulence factors may be responsible for pathogenicity of *H. annosum*. In conclusion, endophytic colonisations of the roots of larch with *Cryptosporiopsis* sp. or *Phialophora* sp. are mutualistic symbioses and we hypothesise that endophytic colonisation of the roots, in contrast to that of the shoots, is generally systemic and mutualistic1. 1Boyle, Götz, Dammann-Tugend & Schulz (2001). *Symbiosis* 31: 259-281.

1025 - Spatial and temporal genetic variation among arbuscular mycorrhizal fungi from undisturbed vegetation

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Mycorrhizal roots from *Hieracium pilosella* in a Danish coastal grassland were sampled in May, August, October and November along a 50 m transect. Arbuscular mycorrhizal fungi in the roots were characterised from partial LSU rDNA sequences obtained by nested PCR. In the specific PCR, the primers were designed to amplify a clade within *Glomus* including species as *G. mosseae, G. caledonium*, and *G. intraradices*. Three sequence types were found to be common. One type showed high similarity with *Glomus intraradices*, whereas the other could not be grouped with known AM species. The sequences showed some variation, but the same sequence could be sampled from several plants along the transect. Most of the variation in sequence types could be attributed to the sampling time. The results showed that the high degree of sequence variation reported from spores, could not be found in roots from undisturbed vegetation systems.

1026 - A novel cotton swab sampling method for fungal spores

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A sampling method for continual monitoring of fungal spores, from various surfaces, such as bins in silos, harvesters and other farm implements has been developed with the use of standard cotton swabs (Johnson & Johnson Cotton balls ™). The swabs are used to lift all spores from the surfaces sampled. The swabs are then washed with a surfactant and, if necessary, washed through a series of sieves to eliminate particles of irrelevant sizes. The residue, in the form of a pellet, is obtained by centrifugation. The top surface of the pellet is mounted on microscope slides (usually 2) to which a drop or two of lactophenol has ben added. The slides are sealed with nail varnish and studied. With this method one can monitor the presence of particular spores and diseases in bulk grain can consequently be monitored successfully. Spores from diseases that are dificult to detect in the field, as well as other fungi present in the grain and particularily smuts and rusts, can be confirmed using this technique. This method could be extremely cost effective as the samples can be taken by a lay parson, sent by post and analysed. The overhead expenses are kept to a minimum. The only drawback is that a surface can only be sampled once, giving a retrieval rate of about 90%, which is ideal for monitoring compared with other grain washing methods.

1027 - *Ceratocystis albofundus*, an African fungus causing a wilt disease on exotic *Acacia mearnsii*

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Ceratocystis albofundus is a relatively newly discovered fungus that causes a rapid wilt disease of economically important *Acacia mearnsii* trees. Until recently it was known only from South Africa, on exotic Australian *Acacia* spp. and on native *Protea* spp. In 1999, the fungus was discovered in Uganda and a year later, it was also found in Kenya. In both cases it was associated with vascular wilt of *A. mearnsii* trees. Although based on only three records, *C. albofundus* has been found on native *Protea* spp. in South Africa, leading to the hypothesis that it is native to this country. Recent studies on the population diversity of South African isolates using a range of microsatellite markers, have shown high levels of genetic diversity in *C. albofundus*, similar to that found in other native *Ceratocystis* spp. The genetic diversity of a Ugandan population has also been studied, showing that the Ugandan population has a similar diversity to that in South Africa. These studies lead us to believe that *C. albofundus* is native to the African continent. It appears to have acquired the ability to infect exotic *Acacia* spp., causing serious losses to the forestry industry. Surveys of native *A. mearnsii* in Australia have failed to yield *C. albofundus* and we do not believe that it is present in that country. Our studies suggest that the fungus poses a serious threat to *A. mearnsii* in its native range and that quarantine measures should be imposed to prevent its spread to Australia.

1028 - Endophytic fungi from *Cassia fistula* L.

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Cassia fistula, known as Khoon (Thai) or Golden Shower, is a deciduous tree which is found all over Thailand. It is often planted as an ornamental, but is also an important medicinal plant. Historically all parts of the plant have been used in native remedies. The leaves of the plant are used for curing boils, coughs and skin diseases such as ringworm while the roots can be used to treat snake bites and dysentery. During the current study it has been found to contain a wide range of fungal endophytes. Isolates from three separate geographical locations in Thailand are compared and the data discussed in relation to specific habitat. A significant number of isolates have been recognised as members of the Xylariaceae and identification of a number of these has been confirmed following teliomorph induction. Testing of the biological activity of a selection of the isolates indicates antibacterial ability and, in a few cases, antifungal. Herbicidal and insecticidal activity has also been demonstrated in a number of cases. We have recently identified fluoroglucinol as a major metabolite from one of the isolates and this has been shown to exhibit antifungal activity.

1029 - Ectendomycorrhizal communities of *Pinus sylvestris* seedlings from forest nurseries in Poland

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Scots pine is the most common tree produced in bare-root nurseries in Poland. Mycorrhizas present on 1-0 and 2-0 *Pinus sylvestris* seedlings produced in bare-root nurseries situated in north-west part of Poland were estimated. Most seedlings were spontaneously colonized by naturally present ecto- and ectendomycorrhizal symbionts. However ectendomycorrhizal colonization very often exceeded the occurrence of ectomycorrhizas. Predominance of ectendomycorrhizas was significantly correlated with nitrogen fertilization. Fungal communities associated with ectendomycorrhizas of tested seedlings were assessed by a combination of morphological and molecular techniques. Morphological typing of mycorrhizas based on the structure and colors of their external hyphae, fungal mantle and anatomical analysis distinguished several ectendomycorrhizal morphotypes. To identify these mycorrhizas, fungal rDNA was amplified using polymerase chain reaction and digested with endonucleases (*Hinf*I,

*Mbo*I, *Taq*I). Morphologically variable complex of ectendomycorrhizal morphotypes consisted from at lest three symbionts giving different RFLP pattern. The fungal taxa involved as dominating ectendomycorrhizal symbiont were ascomycetes *Wilcoxina* sp. Investigation is under way to clarify the systematical position of all ectendomycorrhizas found.

1030 - Diversity and function of ectomycorrhiza during transformation of forests

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During the transformation of forests biological niches are created that are thought to result in enhanced biological variety. In the present study diversity and function of mycorrhizas were investigated in a pure stand of Scots pine (*Pinus sylvestris* L.), in a chronosequence of Scots pine stands that were underplanted with beech (*Fagus sylvatica* L.) and in a pure beech forest. Mycorrhizal diversity was higher in the pure stand of beech compared to the pure stand of Scots pine. Beech trees in the mixed forests had dominating morphotypes that were very similar to the ones dominating in Scots pine. Therefore, it seems plausible that mycorrhiza coenosis of Scots pine is influencing the coenosis of beech in Scots pine stands being transformed into mixed stands of Scots pine and beech. However, trees in the pure beech stand were mycorrhized with other types. These types were distributed in even dominances. Along the chronosequence, frequency per soil volume and nutrient amount of beech mycorrhizas increased. Nutrient uptake was higher for mycorrhizas of older beech than for mycorrhizas of older pine. This indicates that older beech trees are able to accumulate specific nutrients in the upper mineral soil and the forest floor layer. This observation supports the hypothesis of the so-called 'base-pump effect' of the older beech trees.

1031 - PCR analysis reveals diverse fungal community in smut sori of *Anthracoidea* spp. on host plants, *Carex* spp.

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Twelve *Anthracoidea* populations representing six species were collected from natural populations of *Carex* spp. in Finland. The aim of the study was to use RFLP markers from the rDNA ITS region to complement a previous study on isozyme variation of these species. Twelve smut sori from different inflorescences in each population were examined under a light microscope before DNA extraction from teliospores and PCR amplification. Six PCR samples per population were analysed using three restriction enzymes. The results revealed considerable variation between sori in size and number of PCR products and RFLP patterns. Also the size of RFLP bands often exceeded the size of the original PCR product. All samples were analysed in high resolution gels which showed that many single bands were actually two bands almost of similar size. A set of samples producing one and multiple bands was chosen for further analysis. Single bands were isolated, cloned and sequenced. ITS sequences were compared to sequences in the GenBank database. Most bands pointed to totally different organisms than smuts, viz. *Cladosporium* spp. together with some other fungi. None of these fungi has previously been found in association with smuts. True *Anthracoidea* sequences turned out to be the longest ones detected and are among the longest fungal ITS sequences reported. The nature of the previously unknown fungal community in *Anthracoidea* sori, and possible relation to the smut fungus, will be discussed.

1032 - Homothallism in *Cordyceps militaris*

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Strains from mono-ascospore, mono-part-spore and mono-conidium of *Cordyceps militaris* produced stromata from a species of lepidopteran pupae. Hyphal bodies (yeast-like vegetative stage) of *Cordyceps militaris* were injected into the pupal haemocoel of *Spodoptera litura* and stromata formation was observed. *C. militaris* were isolated in July 1995, the season of the stromata production of this fungus, from the air by placing agar plates in a beech forest. Originally, four isolates were used for experiments. Hyphal bodies of the four isolates were injected to the host pupae independently, and four mono-ascospore-strains were established from their stromata. Three out of the four mono-ascospore strains produced stromata. By re-isolation from a stroma-produced-strain in the experiment above, five mono-ascospore-strains and three mono-part-spore-strains were established. The two out of the five (mono-ascospore) and all the three strains (mono-part-spore) produced stromata. Seven mono-conidium strains were established from the two among the original four isolates. Four out of the seven strains produced stromata. Both a part spore and a conidium contained a nucleus by ultrastructural observation. From these findings, we

considered that *C. militaris* has homothallism. For this entomopathogenic fungus, homothallism is thought to be effective character for producing stromata against rare chance to encounter host insects.

1033 - Some saprobic fungi from *Typha* and their competitive interactions on leaves

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Typha latifolia is a major detritus producer in many wetlands. A diverse group of fungi decompose this matter, but how they work to partition the substrate spatially and chemically is unknown. *Psathyrella typhae*, *Talaromyces ucrainicus*, *Hymenoscyphus repandus*, *H. scutula*, *Hymenopsis typhae*, *Phoma* sp., *Cladosporium herbarum* and *Sclerotium* cf. *hydrophilum* were isolated from *Typha* leaves at various stages of decomposition, and their enzymatic abilities tested using a suite of in vitro tests. Corresponding mass loss determinations using natural substrate showed little correlation for many species, indicating in vitro tests may not accurately represent decompositional abilities in vivo. We also looked at competitive interactions between two common saprobes from *T. latifolia*, *P. typhae* and *Sclerotium* cf. *hydrophylum*, by using differential morphology (presence/absence of clamps, hyphal diameter) and enzymatic abilities (pH tolerance and ability to acidify casamino acid medium) to disinguish their relative development in co-inoculated *Typha* leaf fragments. Even though *Sclerotium* grew faster, *P. typhae* was the superior competitor, able to inhibit and invade substrate already colonized by *Sclerotium*.

1034 - From orchids (*Neottia nidus-avis*) to forest tree roots: the mycorrhizal web of the Sebacinaceae (heterobasidiomycetous '*Rhizoctonia*')

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Mycoheterotrophic (MH) plants are achlorophyllous organisms fed by fungi. Up to now, only homobasidiomycetous ectomycorrhizal fungi have been found in MH plants, while several MH orchids are probably associated to *Rhizoctonias*, heterobasidiomycetous fungi that are often parasitic or saprobic. We investigated the MH *Neottia nidus-avis*, a European orchid supposedly associated to a fungus of the *Rhizoctonia* complex. 61 *Neottia* root systems were analysed by amplification and sequencing of the fungal rDNA, showing a specific association to a subclade of Sebacinaceae. Ectomycorrhizae (ECM) growing near *Neottia* roots were shown, through rDNA sequencing, to be also colonized by the sebacinoid associates of the orchid (83% of the typed ECM). Additional data suggests that the same genets colonize the *Neottia* and neighbouring tree roots. *Neottia* therefore confirms the emerging features of MH plants, specifically associated with tree-colonizing fungi. Up to now, available data did not exclude that sebacinoids are saprobic or mycoparasites on ECM. Our TEM analysis shows that they form typical ECM, with dolipore ultrastructure supporting the molecular identification of the fungus. Sequencing of the rDNA ITS of the host plant showed that sebacinoids are not tree-specific, colonizing *Carpinus*, *Fagus*, *Quercus*, *Tilia*, etc. This, together with some GenBank data, suggests that Sebacinaceae is an overlooked mycorrhizal family, where many species await morphological description.

1035 - Rare mechanism of genetic recombination in *Pleurotus ostreatus* natural populations

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The higher basidiomycete fungi form panmictic populations resulting from haploid progeny hybridization. However, a dikaryon is also capable of contributing fertilizing nuclei to a haploid monokaryon resulting in a new dikaryon. Little is known about occurring diploid-haploid mating in nature due to rather difficulties in observing such phenomenon. Having analysed *Pleurotus ostreatus* population structure, we have revealed rather rare mechanism of genetic recombination via di-mon-mating in a local population. We have analysed a sample of heterokaryotic isolates collected within a log. All the heterokaryotic isolates within an individual substrate were somatically compatible and identical at 12 polymorphic isozyme loci. To examine distribution of mating incompatibility factors among basidiocarps within a single fruit body cluster on a log, matings between their monospore offspring were carried out. Some of the dikaryons were identically constituted with respect to A and B factors resulted in 25% of compatible pairings, while the others were distinguished at one of the two mating loci (75% of compatible pairings). This can be explained by occurring diploid-haploid hybridization. Dikaryotic individual occupying the substrate fuses with alien monospore germling that leads to random combination of three types of nuclei in cells of different basidiocarps within a fruit body cluster. Data on population structure,reproductive strategy will be also presented. RFBR grant 01-04-49447.

1036 - Evaluating colonization of ECM fungi at *Quercus petraea* seeds, seminal roots and at the roots of greenhouse seedlings

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In order to recultivate postmining landscapes, to diversify existing coniferous forests and to re-afforest uneconomic agricultural areas millions of young oak tree plants (*Quercus* spec.) are needed. But we are faced with many problems when cultivating the young oak plants. This is, above all, true as regards the germinating process of the acorns, as their seminal roots are often infected by pathogenic fungi. The plant raising enterprises are, therefore, facing huge costs. The project is accomplished with the Tinplant company that is specialized in the raising of containerized greenhouse seedlings of *Q. petraea* and aims at evolving and testing simple and economical methods of mycorrhization of oaks. To utilize the positive effects of ECM fungi at a very early stage of plant development, the hyphae should be established at the stored or germinating seeds already. Plant raising enterprises could look forward to better results during the seed storage and germinating stages, as compared to existing procedures. Recultivating companies could expect a lower plant mortality rate after oak tree afforestation, by making use of ectomycorrhizal fungi, suitable to the respective location.

1037 - Population structure and pathogenicity of *Fusarium oxysporum* isolated from soil and root necrosis of pea

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Forty-nine strains of *Fusarium oxysporum* were isolated from five different sample locations within two neighbouring pea fields. Of these, thirty-nine strains were isolated from soil and ten from pea plants showing symptoms of root rot. Twenty-eight of the isolates were tested for pathogenicity towards pea. Based on percentage discoloration of the roots and the stem base the isolates were divided into three groups. Seven strains were pathogenic, fourteen strains were weakly pathogenic, and seven strains were non-pathogenic towards pea. To assess the genetic relatedness of all forty-nine strains, gene genealogies were constructed from aligned DNA sequences from part of translation elongation factor, nitrate reductase, beta tubulin, and mitochondrial small subunit rDNA. Maximum parsimony analysis of the combined dataset yielded a single most-parsimonious tree containing three strongly supported clades which may represent cryptic species. No correlation was observed between the multigene phylogeny and pathogenicity toward pea, strain geographic origin and substrate (soil or plant) from which the strains were isolated. Strains of *F. oxysporum* that were either non-pathogenic, weakly pathogenic or pathogenic sometimes shared the same multilocus genotype. These results suggest that strains pathgenic and putatively non-pathogenic to pea are very closely related genetically.

1038 - Increased below-ground diversity of ectomycorrhizal fungi after removal of litter and humus determined from fungal hyphae in bulk soil

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Ectomycorrhizal fungi have declined in coniferous stands in The Netherlands over the last decades due to nitrogen deposition originating from air pollution. The effects of restoration practices on the ectomycorrhizal communities in nitrogen-enriched *Pinus sylvestris* (Scots pine) stands in The Netherlands were studied by Baar (1995). Litter and humus were removed to reduce nitrogen availability in the soils. This resulted in increased diversity of ectomycorrhizal fungi within three years. However, all data were obtained by counting sporocaps. The effects of litter and humus removal on the below-ground ectomycorrhizal communities remained unknown. In the present study the composition of the below-ground ectomycorrhizal communities in a *P. sylvestris* stand in The Netherlands was determined five years after litter and humus removal. Fungal DNA was extracted directly from soil samples and PCR was applied with basidiomycete-specific ITS primers. Samples were analyzed by DGGE to visualize diversity. To assess species composition, ITS regions of the amplified fragments were cloned and sequenced. Sequences were compared with known sequences and were analyzed phylogenetically. The analyses showed that the diversity of the ectomycorrhizal fungi below ground in the treated plots was higher than in the untreated plots. *Rhizopogon luteolus* was the most abundant species after litter and humus removal, while *Lactarius* and *Russula* species were the most common species in the untreated plots.

1039 - Molecular ecology of *Bistorta vivipara* ectomycorrhiza in alpine tundra communities

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We have studied the diversity and phylogenetic affinities of mycobionts of *Bistorta vivipara* ectomycorrhizas in four alpine tundra plant communities in Norway, using ITS1-5.8 sequences as a diversity marker. ITS1-5.8 sequences obtained by direct sequencing of 114 ectomycorrhizal root tips compared with sequences from known fungal taxa showed a high diversity of fungal mycobionts, including mycobionts from a number of distantly related taxonomic groups. The composition and taxonomic specificity of the ectomycorrhizal fungi varied considerably among the study sites. This suggests that *B. vivipara* is capable to interact with a number of ectomycorrhizal fungal partners in its many divergent habitats of the lowlands as well as in the alpine zone. Frequent members of the *B. vivipara* ectomycorrhizal symbiosis include *Tomentella*, *Russula*, *Inocybe*, *Cortinarius*, *Laccaria*, *Hebeloma*, *Cenococcum* and some unidentified members of Sebacinaceae. The observed high abundance and diversity of *Tomentella* mycobionts add to the earlier observations of importance of this genus as ectomycorrhizal former also in boreal forest ecosystems. The sebacinaceous ectomycorrhizal fungal partners showed affinity (95% similarity) to orchid mycorrhizal isolates from root tips of *Neottia nidus-avis* retrieved from GenBank.

1040 - Analysing the genetic structure of *Botrytis* spp. in lily and tulip fields using molecular markers

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Botrytis spp. that are pathogenic on flower bulb crops are generally assumed to be host-specific. On lily, *B. elliptica* produces primary necrotic lesions which potentially expand into healthy tissue. On the non-host tulip, however, *B. elliptica* can only cause primary necrotic lesions. The reciprocal holds true for *B. tulipae*, which uses tulip as its host. The mechanistic and genetic basis of host specificity of the two *Botrytis* species mentioned is unclear. Distinction between the species thus far relies on morphological characteristics. At this moment it is unknown to what extent sexual reproduction contributes to genetic variation in local populations of *Botrytis* spp. in lily and tulip fields. A sexual stage has so far been detected in *B. elliptica* but not in *B. tulipae*. The purpose of this study is to develop DNA markers to characterise the level of inter- and intraspecific heterogeneity and the amount of genetic recombination in *B. elliptica* and *B. tulipae* field isolates. To reach these objectives we will use a combination of sequence analysis and novel DNA fingerprinting methods. - This research is funded by the Dutch Technology Foundation STW.

1041 - Molecular markers associated with virulent and avirulent phenotypes in populations of *Uromyces appendiculatus* from bean fields in the Central USA

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Uromyces appendiculatus, cause of bean rust, is characterized by highly diverse virulence phenotypes. The use of race-specific rust resistance genes has been complicated by the 'boom and bust' cycle. Pinto Olathe was released as a rust resistant bean cultivar containing the *Ur-6* gene in 1979. The resistance only lasted 5 years. Resistance gene pyramiding is a strategy to strengthen race-specific resistance. Phenotyping the rust population is time-consuming and costly. Pathogen variation can also be monitored by genotyping. Previous work on molecular variation in rust pathotypes have examined geographic significance of rust races and the association between resistance and virulence patterns. In the present study we monitored the changing pattern of virulence on pinto 'Olathe' over the past 20 years using both molecular and phenotypic markers. A population of over 130 isolates from the Central USA representing both avirulent and virulent pathotypes on pinto Olathe were analyzed using molecular markers such as rep-PCR and RAPD-PCR. These markers were found to discriminate between rust pathogen genotypes with virulent or avirulent interactions with the *Ur-6* gene. In addition, cluster analysis of the molecular data using UPGMA methods showed agreement between statistical analysis and cluster associations of these pathotypes with selected markers.

1042 - Annual production of leaf-decomposing fungi in streams

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We have determined the annual production of fungi associated with decomposing leaves in seven streams in the southeastern United States using rates of acetate incorporation into ergosterol to calculate in situ growth rates and ergosterol concentrations to estimate fungal biomass. Annual fungal production ranged from 8-551 g/m^2 in these streams. Fungal production was significantly

correlated with the mean amount of leaf litter present in the streams throughout the year ($r=0.72$, $p<0.05$). Two streams that were much more retentive of leaf litter (mean standing crop of leaf litter, 330-440 g/m^2) than the other streams (20-71 g/m^2) exhibited much higher annual fungal production (185-551 vs 8-34 g/m^2). Consequently, the amount of leaf litter retained in a stream appears to be a major factor in determining the magnitude of fungal production. Mean annual fungal biomass expressed on an areal basis also exhibited a wide range in these streams (0.7-18.6 g/m^2), but was less variable when calculated as a percentage of the leaf detritus (3.4-7.6%). Fungal growth rates based on annual production/biomass ratios ranged from 8.2 to 18.6 yr^{-1}. The nutrient (N & P) concentrations in the water appeared to be a major factor affecting growth rate. Overall, these results suggest that, in streams, a significant amount of decomposing leaf litter is converted into fungal production.

1043 - Characterization of *Fusarium oxysporum* isolates from *Phoenix canariensis* (Canary Island Date Palm)

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Fusarium wilt of *Phoenix canariensis* (Canary Island Date Palm) is caused by *Fusarium oxysporum* f. sp. *canariensis*. The disease occurs all round the world including Australia where hundreds of palms have been killed. Isolates of *F. oxysporum* f. sp. *canariensis* were collected from fronds of diseased palms at sites around Sydney and different parts (non-frond) of individual palms within a site. Three techniques were used to assess diversity of these isolates; vegetative compatibility groupings, pathogenicity testing using a PCR-based technique and PCR fingerprinting using ERIC primers. The combined methods divided the isolates into one major and four minor groups, with two of the minor groups showing some bridging characters. A minority of isolates did not fall into any group and are presumed saprophytes. Some discrepancies in the expected correlation between the techniques occurred for the non-frond isolates; this has implications for appropriate tissue selection for disease diagnosis. The number of groups and the differences between them indicate a degree of diversity comparatively higher than that shown in other studies for this forma specialis and may have important consequences for disease management.

1044 - Effect of soil management strategies on the population structure and function of arbuscular mycorrhizal fungi

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Soil management systems involving soil disturbance affect the population dynamics and function of arbuscular mycorrhizal fungi in agricultural soils. Because conservation tillage reduces the soil disturbance and favours organic matter accumulation in the upper soil it should influence the diversity of the AM fungi and the soil AM hyphal network development. In this study the effect of conservation and conventional tillage on the arbuscular mycorrhizal fungal community in sandy loamy soils was investigated. Long term wheat cultivation systems with silage maize or potatoes as preceding crops and tillage with cultivator or with plough were compared concerning the population structure and function of arbuscular mycorrhizal fungi. The diversity of AM fungi within wheat roots and the diversity of the respective spore population was studied using specific primers. A nested PCR approach was used to specifically amplify sequences of the glomelean 18S subunit and the Internal Transcribed spacers. Roots and spores sampled from the four different cultivation variants during the vegetation period from April to August were directly used for the PCR analysis and for the estimation of root infection levels. Depending on the different management strategies the mycorrhizal colonization of the roots, the spore density and the population diversity varied. The interaction of the AM population with filamentous fungi esp. of the widely distributed genera *Fusarium* and *Alternaria* is discussed.

1045 - Spatial variation and fine scale structure of roots and ectomycorrhizal community in a mixed forest

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Fine scale distribution of roots and ectomycorrhiza were measured in a spruce-dominated mixed forest. Series of adjacent samples were taken from different soil layers and from decomposed logs. Within a sample all roottips of *Picea abies*, *Tilia cordata* and/or *Populus tremula* were identified and counted. Ectomycorrhizal morphotypes were separated in respect to host species and identified by sequencing the ITS regions of nuclear rDNA. The number of roottips, %(ecto)mycorrhization and ectomycorrhizal diversity was very variable in 5 cm scale, especially in logs. The ectomycorrhizal fungal community was dominated by *Cenococcum geophilum, Piloderma fallax*, *Tomentella* spp. and *Russula* spp. Fungal species tended to spread in horizontal rather than vertical direction. The relative abundance of *Cenococcum geophilum* did not differ across soil layers, but it was almost three times more abundant (% of total ectomycorrhizae) on roots of deciduous trees than on spruce. Many ectomycorrhizal morphotypes were found on one host only despite the roots of various trees intermingled. *Piloderma fallax* and *Tomentella* spp. showed a strong preference for dead wood. Based on our results, morphotyping combined with sequencing is a powerful tool for ectomycorrhizal community studies, esp. when more reference data accumulate.

1046 - Structure of mycobiota and organic matter decomposition in undisturbed biogeocenoses of the southern taiga

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The structural and functional peculiarities of soil microscopic fungi in typical biogeocenoses of the southern taiga have been characterised. Mycological analysis has been carried out in the Central Forest State Biosphere Reserve (CFSBR, Tver oblast, Russia). The information on taxonomic diversity of mycobiota has been obtained using the plating method and description of microscopic fungi species from different plant material, layers of litter and soil profile horizons in sphagnum spruce forest, bilberry spruce forest, wood sorrel-nemoral spruce forest, nemoral spruce forest, black alder grassy-swampy spruce forest. The soils of biogeocenoses differ significantly in total reserves of the organic carbon, rate of organic matter decomposition. The data obtained show high fungal diversity (Shannon index) in all biogeocenoses, but seasonal changes in this index are much greater than those related to the substrate type. The increasing of the rate of organic matter mineralization and fungal diversity have been observed only under optimal temperature and water conditions. We have tried to estimate the role of organic matter composition, and interspecies relations on the destructive activity of fungi in model laboratory experiments. Using cross introduction it has been shown quite high adaptation of fungi community to the 'native' decomposed substrate. The research has been supported by Russian Foundation for Basic Research grant 02-04-48870.

1047 - Nematode-trapping fungi in Victoria Land (Continental Antarctica)

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Nematode-trapping fungi have a world wide distribution. They were also found in Antarctica. In the present work nematode-trapping fungi isolated in Victoria Land (Continental Antarctica) are reported. Fortythree samples of soil, mosses and ornithogenic materials were collected during 1987-1999. The investigated area ranges from 74°S to 76°S and 162°E to 165°E. Nematode-trapping fungi were isolated by means of sprinkled-plate technique. A suspension of the nematode *Caenorhabditis elegans* was added as a bait. Predaceous hyphomycetes were isolated from twelve samples. Thirty strains of *Arthrobotrys* spp. and one strain of *Nematoctonus* were isolated and identified. All *Arthrobotrys* strains were morphologically similar; differences were noted in the general features of the colony (colours, zonations). They are able to capture namatodes by means of adhesive nets. The strain of *Nematoctonus* is the first record for Maritime and Continental Antarctica. It is characterized by the presence of clamp connections on its hyphae, typical of many heterocaryotic Basidiomycota. It produces adhesive trapping devices formed by a secretory cell with a characteristic hour-glass shape. The results here reported, show that nemade-trapping fungi are abundant in Continental Antarctica, above all in the moss where there are nematodes and other small organisms. On the base of their massive presence and high grade of specialization, they can be considered indigenous fungi of the Antarctica.

1048 - *In vitro* mycorrhization of *Cistus incanus* seedlings by *Tuber melanosporum* mycelium

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Establishment of mycorrhizal associations *in vitro* between *Cistus incanus* and *Tuber melanosporum* strain 1015 was studied. *Cistus incanus*, a Mediterranean flowering plant (Cistaceae), is a known symbiont of the black truffle. It can easily be germinated and rooted *in vitro*. Three growth media were evaluated for plant development, fungal growth, and mycorrhization: (i) N5, an MS medium containing one fifth the nitrogen content of the standard version, (ii) M, a minimal medium developed for *in vitro* mycorrhization of VAM, and (iii) half-strength Hoagland solution. All media were solidified with 0.8% agar. No significant differences were found between the media in respect to plant development (height, number of nodes and dry weight). The tuber mycelium grew vigorously on medium M, while on half-strength Hoagland and N5 its growth was very significantly reduced (by 62.5% and 84.6%, respectively). The incidence of Hartig net establishment was close to 100% on medium M, versus 10% and zero on half-strength Hoagland and medium N5, respectively. Direct irradiation with light as low as 25 µmol m^{-2} sec^{-1} strongly inhibited *T. melanosporum* mycelium growth and mycorrhization failed to occur, whereas diffuse light showed no effect on fungal development and enabled mycorrhiza formation. It appears that *in vitro* growth of Tuber mycelium is affected by the nature of the medium and by light intensity and correlates with mycorrhization degree.

1049 - Survival of insect target and biocontrol fungus in a greenery plantation

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The insect family Curculionidae contains some of the economically most important pest insect species in greenery production for Christmas decorations in Denmark. In particular, the two species *Strophosoma melanogrammum* and *S. capitatum* are important pest species. At present no chemical treatment of these weevils is allowed in Denmark. Biological control including the use of insect pathogenic fungi from Hyphomycetes may thus provide a potential to minimise the damage caused by these weevils. Isolates of the insect pathogenic fungi *Metarhizium anisopliae Beauveria bassiana* and *Paecilomyces* spp. were studied in the laboratory and field. In the laboratory, all tested isolates were able to infect and cause mycosis in both adults and larvae of *S. melanogrammum* and *S. capitatum*. Based on the results from the laboratory bioassays, isolates were chosen for release under field conditions in an *Abies procera* plantation, where different application strategies were used. Spatial-temporal distribution of the released fungus in the soil was monitored by cfu per g soil. Weekly sampling from emergence traps monitored survival of adult weevils. Adults were collected, counted and determined to species level and incubated individually in plastic cups to estimate the prevalence of the applied fungus as well as the prevalence of natural infections. Preliminary results proved that populations of weevils in the treated plots were reduced significantly.

1050 - Do *Piceirhiza bicolorata* mycobionts form both ecto- and ericoid mycorrhizas?

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Ectomycorrhizas of the *Piceirhiza bicolorata* morphotype were found commonly associated with *Pinus sylvestris* seedlings and mature trees in Glen Tanar Native Pinewood, Northeast Scotland. Fungal isolates from *P. bicolorata* mycorrhizas are thought to be part of the *Hymenoscyphus ericae* aggregate, and this raises the possibility that they form mycorrhizas with both the pine trees and the understorey ericoid vegetation. In this study, strains from *P. bicolorata* were isolated from soil cores collected in a 160 year-old natural stand. The mycobiont was cultured and identified by extracting genomic DNA and sequencing the PCR- amplified ITS regions. Phylogenetic analysis showed that our isolate grouped in clade 4 of Vrålstad et al. (*New Phytol.*, 2002) along with many other isolates from *P. bicolorata*-type ectomycorrhizas, including *Phialophora finlandia*. Mycorrhizal synthesis were performed to test the ability of our isolate to form ECM and ERM on *P. sylvestris* seedlings and ericoid plants respectively, and to cross infect between *P. sylvestris* + *P. bicolorata* (previously synthesized) and *Vaccinium myrtillus*. The synthesis with Scots Pine seedlings produced black morphotypes with or without hyaline tips and with emanating hyphae. Pure culture synthesis with *V. myrtillus* seedlings produced typical ERM: these were also produced in the cross infection experiments. In both cases the growth of *Vaccinium* plants forming ERM was stimulated relative to the growth of uninfected plants.

1051 - Strategies for sustainable land management: quarry recovered through truffle-culture

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Building materials come from quarries. The opening of a quarry is a geological damage and it causes the complete destruction of vegetation. Italian laws provide for each open quarry project an environmental recovery and steadying plan through soil-modelling and vegetation-planting in order to repair the damage and avoid subsequent trouble. A concrete case of quarry recovery through truffle-cultivation is analysed.

1052 - The identification and frequency of occurrence of higher filamentous marine fungi on mangroves in a south Florida mangle

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Little is known regarding the occurrence and distribution of the higher filamentous fungi on mangroves in South Florida, USA. Previous studies have demonstrated that marine fungi are an important degradative component and assume an important role in nutrient recycling systems in estuarine and near-shore ecosystems. The purpose of this study was to collect, identify and prepare a key to the higher filamentous fungi occurring in the mangle of J. U. Lloyd State Park on the southeast coast of Florida, USA. Over a period of ten months 33 species were identified including 1 Basidiomycete, 8 Deuteromycetes, and 24

Ascomycetes. The driftwood collected was mainly comprised of pieces of *Rhizophora mangle* and *Conocarpus erectus*. The predominant species, by frequency of occurrence, include the Ascomycetes *Hypoxylon oceanicum* (8.3%), *Leptosphaeria australiensis* (15.2%), *Lulworthia grandispora* (6.8%), and *Nais glitra* (8.3%) as well as the Deuteromycete *Humicola alopallonella* (7.6%). In addition, two new species of Ascomycetes were collected and will be presented. New records for Florida include the Ascomycetes *Lineolata rhizophorae* and *Massarina velatospora*. A new host record for *Phaeosphaeria gessneri* occurring on *R. mangle* is reported. Overall, the marine mycota of South Florida appears to be very similar to that reported for other tropical and subtropical regions.

1053 - An ecological study of woody and leafy endophytes in the tropical mangrove tree, *Kandelia candel*

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Kandelia candel is the only tree member of the Rhizophoraceae which occurs in the subtropical Asian Pacific mangroves. We have undertaken a preliminary survey of endophytes in woody and leafy tissues of *K. candel* in Hong Kong. Samplings were carried out in two distinct climatic period so as to evaluate possible effects on their occurrence. Four fully grown trees were selected. Wood sections were cut from branches of different diameters and separated into bark and xylem tissue segments. Leafy tissue segments were also removed from mature leaves of branches sampled. All segments were incubated in malt extract agar after surface sterilisation. A total of 987 strains of fungi was isolated from 1280 bark tissues, 1280 xylem tissues and 320 leafy tissues segments, with infection rates of 60, 12 and 25% respectively. This is represented by 22 identified genera and 28 morpho-types. The dominant species in bark included *Pestalotiopsis* spp., *Tryblidiopycnis* sp., *Xylaria* sp., morpho-types 3 and 5. Frequent xylem endophytes were *Phoma* sp., some unidentified pycnidial coelomycetes, *Geniculosporium* sp., and morpho-type 13. *Guignardia* sp. and morpho-type 26 were abundant on leaves. These initial results confirm endophytic infection in trees is not vertical and each tissue type is colonised by a distinct group of endophytes.

1054 - Lichen genotyping with fungus specific microsatellites

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Little is known about the genetic variation of lichen-forming fungi. Yet, corresponding knowledge would be required for the accurate delimitation of individuals, populations, or species and for studies of reproductive systems and dispersal strategies. This will also help to develop and evaluate measures for conserving lichen biodiversity. Lacking genetic variation and, hence, low adaptability to environmental changes such as habitat fragmentation may explain why many epiphytic lichens are declining over much of Europe. We suppose that population genetic studies on lichen-forming fungi are mainly limited by the lack of suitable molecular markers. An ideal genetic marker type for population studies of lichen-forming fungi would combine the detection of high levels of genetic variation with the fungal specific amplification of DNA. Both requirements would be fulfilled by microsatellite markers. Microsatellites consist of short tandemly repeated sequences and proved to be highly informative markers in evolutionary, population genetic and conservation biological studies. We show that highly polymorphic, fungus-specific microsatellite loci also exist in the epiphytic lichen species *Lobaria pulmonaria*. Their fungus specific nature, their potential to identify multiple alleles and their transferability make these STMS markers a powerful and reliable new tool for genetic mapping, large and small scale genetic diversity analyses of *L. pulmonaria* and related species.

1055 - Nutrients are released during mycelial interactions in soil

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Cord-forming saprotrophic basidiomycetes represent a major nutrient resevoir within forest ecosystems and appear to be highly conservative of acquired nutrients. At some stage they must release nutrients to soil, but information on this is lacking. We have investigated nutrient release in the wood decay fungi *Hypholoma fasciculare* and *Phanerochaete velutina*. These were grown in soil microcosms in the laboratory in unpaired, self-paired and competing combinations. Inocula were prelabelled 32P orthophosphate, and losses from the soil systems were quantified by analysing water which had percolated through different areas of the systems following simulated rainfall events. 32P was released in unpaired systems and, though amounting to to less than 1% of total recovery, is direct demonstration of nutrient release to soil. Interaction with self resulted in significant mobilization of accumulated P, and significant increases in losses to soil. Interaction with non-self mycelia usually resulted in greater P mobilization than in interactions with self, even in the case of interaction between different isolates of the same species. *P. velutina* and one isolate of *H. fasciculare* preferentially translocated mobilized P to the interaction zone, but a another isolate of the latter preferentially translocated P away from the interaction zone.

1056 - Does human impact influence the species composition of epiphytic macrolichen communities?

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Epiphytic macrolichen communities were sampled on lower stems (≤ 2 m) of deciduous trees in plots of size 400 m^2 along a regional macroclimatic gradient in Troms county, northern Norway. Canonical Correspondence Analysis (CCA) with variation partitioning revealed the following key factors for macrolichen communities: 1. Macroclimate was the primary factor controlling epiphytic macrolichen communities, with strictly macroclimatic variation accounting for 35% of total variation explained (TVE). 2. Two other key factors were spatial variation and tree substrate, amounting to 25.5% and 17% of TVE, respectively. 3. Human impact explained little (5.2%), forest fragmentation and natural patchiness none of the variation in species composition. The largest amount of shared variation was pooled between the sets of spatial and climatic variables, while the set of human impact variables shared no variation with other sets of explanatory variables. The reliability of species groupings was confirmed by DCA (Detrended Correspondence Analysis), showing an ecologically sound aggregation of the species optima. DCA revealed two main groups of species, characterised by differing photobionts. Key factors controlling epiphytic macrolichen communities are discussed in relation to lichen ecology, physiology and distribution patterns.

1057 - Modelling colonies of fungi - a generic process based approach

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A fundamental challenge in ecology is to derive a mechanistic understanding of biodiversity of natural communites. This challenge is particularly difficult in fungal communities since these systems are indeterminate and demonstrate a high degree of plasticity in response to both biotic and abiotic stimuli. The approach described considers fungal communities in terms of constituent colonies, and begins by identifying a generic, process based description of individual colonies. An individual-based model of fungal colony development is presented. The model considers the spatio-temporal growth of mycelia over heterogeneous substrates. The model is spatially explicit where an individual colony is represented as a number of interconnected cells. Communication among cells is facilitated via fundamental ecological processes. The processes of resource uptake, diffusion of internal nutrients within the colony and conversion of resource taken up into biomass are represented. In addition, the directional translocation of nutrients over large distances is supported. Colonies are characterised in terms of physiological traits: different colony forms have different traits; traits parameterise the model processes. Importantly, processes within the model are amenable to quantitative parameterisation from experiment. This model of development of individual colonies serves as a platform for investigations into fungal community dynamics through the inclusion of interspecific interaction terms.

1058 - The widespread occurrence of dark septate endophyte fungi in grassland communities

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This study describes unexpectedly high levels of isolation of dark septate endophytes (also known as mycelium radicis atrovirens [MRA]) from surface-sterilised plant roots from both undisturbed and agricultural mesotrophic grasslands in Wales. We have also examined spatial and temporal variables in their distribution. Most reports of these fungi have previously related to subalpine and boreal habitats. Their failure to sporulate in culture has hindered further studies. Analysis of sequence and restriction fragment polymorphism has been used to assess diversity of these fungi. Preliminary data suggesting a potential ecological role in these habitats is presented, based on microcosm studies of their interactions with other fungi isolated from the same site.

1059 - Cyanobiont specifity of lichens on Livingston Island, maritime Antarctica

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Nitrogen is a major factor of the lichen biodiversity in maritime Antarctica. At coastal habitats, there is a luxuriant lichen flora because of nutrition by the sea and animals, while the lichen flora in inland habitats is rather poor. The percentage of taxa containing nitrogen-fixing cyanobacteria either as primary or secondary photobiont is increased in inland communities. We have investigated the cyanobionts of lichens occurring at two ice-free locations on Livingston Island: inland habitats of South Bay and Byers Peninsula. The cyanobionts of two lichens with primary cyanobiont (*Leptogium puberulum*, *Massalongia carnosa*) and three species containing *Nostoc* in cephalodia (*Placopsis contortuplicata*, *P. parellina*, *Psoroma cinnamomeum*) were examined using molecular techniques and compared with free-living *Nostoc* colonies found in the area. Nucleotide sequences of the cyanobacterial tRNALeu intron were employed as genetic markers. Preliminary results suggest that different species at the same location share a common cyanobiont.

1060 - Small scale distribution pattern of ectomycorrhizal types in a red oak stand

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Forest soils, especially the organic layers are very heterogenious substrates. Parameters like water content, pH value or the organic content could vary in the range of millimetres. Fungal mycelia or ectomycorrhizae are dispersed dependent of the heterogeneity of micro-habitat conditions. Beyond, the spatial distribution of ectomycorrhizae is influenced by interspecific interactions. As a result of all these influences, the ectomycorrhizal coenosis form a mosaic structure in the soil. At present, very few data on micro-scaled distribution of ectomycorrhizal fungi are available. First investigations started in recent years. To find characteristic structures of ectomycorrhizal coenosis in different scales, is one of our goals. Therefor we map the ectomycorrhizae in a coherent soil volume. This project is established in red oak stands of former mining sites and compare these afforestations with stands growing on undisturbed soil. Soil units of 6 cm width, 100 cm length and 3 cm depth taken from the top organic soil layer are divided in subunits of two to two cm and one cm depth. These subunits are solved in water and the ectomycorrhizae are qualitatively recorded. Maps will visualize the distribution of the ectomycorrhizae. We know from investigations in Scots pine stands, that the assemblage of ectomycorrhizal types can vary between sides and that nearly the half of all combination of ectomycorrhizal types never grow together in the same micro-habitat.

1061 - The longevity of *Cordyceps humberti* on *Icaria* wasps (Hymenoptera: Vespidae)

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Cordyceps stromas persist for some time but little work has been done on longevity of individual stromas. Identification of a *Cordyceps humberti* epizootic in Nam Nao National Park, Thailand provided an opportunity to examine longevity of individual stromas. *Cordyceps humberti* and *Hirsutella saussurei* infected *Icaria* sp. The nest (ca. 80 cm long, 50 cm across) was in a tree at ca. 10 m. Infected wasps were found up to 30 m radius from the nest. Wasps were attached by their mouth-parts to the leaf margin. Bamboo was a preferred substrate for settling but dicotyledonous plants were also used. The first wasps were seen in August (1999). Thirty wasps were tagged and observations made every 3-4 dy. Five of 27 specimens were *Cordyceps* while the rest were the *Hirsutella* form. However, after 1 month, of 23 specimens remaining 13 were the *Cordyceps* form. Of 27 specimens recorded, 17 were lost within 100 dy while 4 survived 100-200 dy and 5 specimens were documented from 200-300 dy. One specimen was still present at the end of the work (513 dy). Significantly, of persistent specimens, 2 produced 2 crops of perithecia. One specimen remained in the *Hirsutella* form for 169 dy before synnemata were lost and it entered a dormant state. After ca. 15 days of dormancy new synnemata developed and ca. 30-40 dy later the *Cordyceps* developed. This work demonstrates that in *Cordyceps humberti* perithecia can be produced over an extended period with hosts providing material over more than one season.

1062 - The structure of symbiotic communities: population-level patterns of association between lichen fungi and their algal photobionts

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Morphological and molecular studies have largely rejected broad coevolutionary patterns between lichen fungi and their photobionts, but theory predicts that populations of intimate symbionts become locally coadapted to each other and to their environment. In the place of broad-scale phylogenetic congruence, we test this alternative hypothesis of fungal-algal association on a population-level geographic scale. Over 150 samples representing eight *Cladonia* species with their green algae *Trebouxia* were collected from five populations and sequenced for ITS. Three distinct well-supported clades of algae were identified, and each clade was found in each sampled population. Of the eight sampled fungi, six are restricted to a single clade of algae, and the other two fungi can be found with either of two clades of algae, even in the same population. Patterns of association between fungi and their algae differ among lichens, but in all cases, fungi appear selective, consistently associating with only a subset of all algal clades available in the local environment, regardless of where the lichens are collected. Furthermore, at the genotype level, some evidence is found for even stronger patterns of association, with four cases of single algal genotypes restricted to one fungal species in a given population. These patterns of association of fungi and algae vary but are consistent with strong fungal preference and in some cases support the model of local geographic patterns of coevolution.

1063 - *Ophiostoma* species associated with bark beetles infesting three *Abies* species in Okunikko, Japan

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Ophiostoma species were isolated from bark beetles and *Abies mariesii*, *A. veitchii* and *A. homolepis* infested with the beetles in Okunikko, Tochigi, Central Honshu, Japan. One to two *Ophiostoma* species were frequently isolated from each species of bark beetle. *Ophiostoma subalpinum* was the most common associate of *Cryphalus montanus*. This fungus is known to be the most dominant associate of *C. montatus* and *C. piceae* in wave regeneration forests of *Abies* species in central Japan. *Ophiostoma* sp. B as well as *O. subalpinum* were common fungi associated with *Polygraphus proximus*. *Ophiostoma europhioides* was isolated from *Dryocoetes hectographus* and *D. autographus* as one of the common associates. *Ophiostoma* sp. J and sp. S were frequently isolated from *D. autographus* and *O. striatus*, respectively. These fungi seem to have specific relationships with particular bark beetles. *Ophiostoma* sp. B, sp. J and sp. S have unique morphological characteristics and appear to be new species.

1064 - Investigation of phylloplane yeasts in Taiwan

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Seventy nine yeast strains were isolated from phylloplane of eight plants species in the campus of Tunghai University in Taiwan. Eleven species were identified and five of them presented on one plant only: *Kodamaea kakaduensis* and *Sporidiobolus johnsonii* on *Ipomoea cairica*; *Cryptococcus magnus*, *Rhodotorula glutinis* and *Rhodotorula* sp. on *Hibiscus rosa-sinensis*. Six yeast species distributed on different plants. *Aureobasidium pullulans* presented on *Euphorbia tirucalli* L., *Oxalis violacea* Linn. and *Hibiscus rosa-sinensis*; *Discosphaerina fagi* and *Sporidiobolus pararoseus* presented on *Calocedrus formosana* and *Oxalis violacea* Linn.; *Pseudozyma aphidis* presented on *Oxalis violacea* Linn., *Mussaenda parviflora* and *Ageratum houstonianum*; *Cryptococcus laurentii* presented on *Calocedrus formosana*, *Oxalis violacea* Linn., *Hibiscus rosa-sinensis*, and *Ageratum houstonianum*; *Cryptococcus* sp. presented on *Acer serrulatum* Hay. and *Ageratum houstonianum*. Black yeast found from all plants we investigated except *Ipomoea cairica*. Distributions of some phylloplane yeasts seem to associate with the species of plants, and some yeasts were widespread on different plants.

1065 - Identification of fungi associated with algae using PCR-amplified sequences of the 18S and 28S rDNA fragments and DGGE

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Since the introduction of molecular techniques, such as PCR and DGGE, in environmental microbiology, great steps forward in the comprehension of microbial diversity have been made. This method is now routinely used to analyse bacterial community diversity and dynamics. The application of these techniques to estimate fungal diversity and follow community structure, however, is poorly developed. Consequently, fungi are often omitted from ecological community analyses. In this study, PCR primers and DGGE conditions were optimised for use in environmental studies of marine fungi associated with macroalgae. Current fungal community analysis usually involves only PCR-amplified sequences of the 18S rRNA gene. PCR primers used for this gene show little fungal specificity, which is required to detect fungal sequences that might be rare in the environment. Five different PCR primer pairs were tested to amplify rRNA genes from 20 fungi isolated from *Fucus serratus*. Two primer pairs

(AZ24-AZ709; AZ107-AZ709) were designed for the PCR amplification of fungal 28S rDNA (covering D1, D2 and D3 regions) from marine fungi and environmental algal samples. Two other pairs for 18S (NS1-FR1; FF390-FR1) were chosen from previously published work for their fungal specificity and their ability to achieve a separation for most of the amplification products obtained from a reference collection. The remaining primer pair (FF390-NS24) was fungal specific for the 3-prime end of the 18S rRNA gene.

1066 - Exploring the transcriptome of the nematode-trapping fungus *Monacrosporium haptotylum*

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Monacrosporium haptotylum infects nematodes using adhesive knobs, which are developed on branches of vegetative mycelium. To identify genes and metabolic pathways that are expressed during development of traps and infection of nematodes, we have analyzed 4767 expressed sequence tags from three different cDNA libraries, vegetative mycelium, knobs, and knobs infecting the nematode *Caenorhabditis elegans*. The ESTs were clustered into 2006 contigs representing 2006 putatively unique transcripts. Only 7.6% of the contigs contained ESTs from more than one cDNA library, thus the patterns of genes expressed in the three libraries were significantly different. Approximately 26-37% of all ESTs displayed significant similarities to sequences found in the GenBank database. Most markedly, the frequency of transcripts related to protein synthesis was up-regulated in the knobs. On the other hand the knob library contained in comparison with the mycelium cells and the fungus infected the nematode lower frequencies of ESTs involved in cell growth, cell division and DNA synthesis, transcription, protein destination and cellular communication/ signal transduction. Among the most prevalent transcripts expressed during the infection of nematodes were ESTs displaying sequence similarities to extracellular proteases, transportes and toxins.

1067 - Identification of an HMG-domain protein implicated in nuclear migration and mating in *Coprinus cinereus*

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Mating between compatible homokaryons in *Coprinus cinereus* leads to the formation of a mycelium known as a dikaryon. In the dikaryon nuclei of both mates remain paired in each cell and are partitioned each time the cell divides via a structure known as the clamp connection. The formation and maintenance of the dikaryon is under the control of two sets of genes, the *A* mating type genes that encode a transcription factor that activates clamp cell formation and the *B* mating type genes that encode peptide pheromones and their cognate receptors, and which activate nuclear migration following compatible cell fusion and clamp cell fusion once the dikaryon is established. We are trying to understand how pheromone signalling regulates these quite distinct stages in dikaryon development. As a first step towards our goal we cloned a gene, *hmg1*, which encodes a High Mobility Group (HMG) protein. Homologous replacement of *hmg1* implicates HMG1 in nuclear migration, but not in clamp cell fusion. Δ*hmg1* strains are capable of donating nuclei to a wild type partner, but unable to receive nuclei. Homozygous Δ*hmg1* dikaryons display unusually vigorous growth, but nuclear distribution is irregular and sporulation is defective. Current studies explore the role of Hmg1 in pheromone signalling and the role it may have in regulating nuclear migration at other stages in sexual development.

1068 - Heterologous diploid nuclei in the violet root rot fungus, *Helicobasidium mompa*

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Allelic genes *hga*1-1 and *hga*1-2, which encode G protein alpha subunit from violet root rot fungus, *Helicobasidium mompa*, were sequenced and characterized. Restriction fragment polymorphism (RFLP) analysis determined that the gene is present as a single locus in the single basidiospore isolates, while strain V169 possessed both alleles of this gene. Therefore, although basidiospore isolates are dikaryon, they are homokaryon. Field-isolated strain V169, the putative parent strain, is dikaryotic heterokaryon. *hga*1-1 and *hga*1-2 segregated to almost 1:3 among single basidiospore isolates from the same fruiting body. These results suggest that one of the nuclei in the V169 parent strain is homologous diploid and the other heterologous diploid. This parent strain produced four homokaryotic and dikaryotic basidiospores on each basidium. The basidiospore germinated to homokaryotic and dikaryotic mycelium, and the homokaryon strains generated secondary mycelium by mating between compatible homokaryon.

1069 - Water relations, photosynthesis and growth of mycorrhizal bean plants under water stress

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Arbuscular mycorrhizae symbiosis often altered water relations with consequent effects on gas exchange rate and photosynthesis. The effect of arbuscular mycorrhizal (AM) colonisation on photosynthesis, stomatal conductance, water relations, leaf area and dry weight of droughted and well-watered *Phaseolus vulgaris* L. was assessed after 3

months growth in sandy loam pot culture in greenhouse condition inoculated at the seedling stage with the fungi *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe or *Glomus interaradices* Schenck & Smith, or uninoculated. During the drought period and after rewatering, leaf water potential, CO2 assimilation rate, stomatal conductance and transpiration of plants were measured. Leaf water potential, CO2 assimilation rate and stomatal conductance in mycorrhizal plants during the unwatered period were, in most cases, significantly greater than in the non-mycorrhizal plants, particularly in those infected by *G. interaradices*. There was no significant mycorrhizal effect on transpiration rate. Mycorrhizal plants were able to postpone the onset of wilting. During recovery from drought, there were significant differences in speed of recovery of leaf water potential, transpiration rate and stomatal conductance in mycorrhizal plants, but not in assimilation rate. Leaf areas and dry weight of mycorrhizal plants were generally greater than non-mycorrhizal plants after drought and recovery from drought.

1070 - Lignocellulose degradation by *Pleurotus ostreatus* in the presence of heavy metals

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The ability of white-rot fungi to decompose lignin, cellulose, hemicelluloses and a range of structurally related compound including several xenobiotics has found several technological applications. The contents of heavy metals is among the most important factors that affect the lignocellulose-decomposing system of these fungi. In *Pleurotus ostreatus*, Cu and Cd increase laccase activity, whereas other metals (Hg, Pb, Ag, Zn) have negative effect on enzyme production. Mn-peroxidase is negatively affected by the presence of Cd. Heavy metals also affect the carbon and energy-supplying system of polysaccharides-decomposing enzymes. Addition of Cd to *P. ostreatus* cultures increased the activities of endocellulase, exocellulase, and β-1,4-glucosidase, whereas β-1,4-xylosidase and β-1,4-mannosidase activity was negatively affected. During cultivation of wheat straw, the presence of Cd also decreased the substrate utilisation (weight loss). Furthermore, some heavy metals, e.g. Cd and Hg negatively affect fungal colonisation of both lignocellulose substrate and nonsterile soil at low concentrations, that do not affect the activity of ligninolytic enzymes. This has to be taken into account when applications of white-rot fungi for degradation of xenobiotic contaminants in soil are considered, since these processes are dependent upon external substrate input and effective spread of mycelia in the substrate. This work was supported by the Grant Agency of the Czech Republic (204/02/P100).

1071 - High rates of extracellular superoxide production by lichens in the suborder Peltigerineae

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Rates of extracellular superoxide radical formation were measured in 34 species of lichens from different taxonomic groupings and contrasting habitats before and after desiccation stress. All 20 species tested from the suborder Peltigerineae produce superoxide extracellularly at high rates, even when they are not stressed. In addition, some species show a burst of superoxide production during rehydration following desiccation. In general, production of high levels of superoxide and the existence of an inducible oxidative burst were best developed in species growing in wet microhabitats. Extracellular production of superoxide was almost absent from the 14 species from other lichen groups. Preliminary studies on the identity of the superoxide producing enzymes suggested that they do not possess the classical characteristics of those suggested to produce reactive oxygen species in higher plants. Patterns of superoxide production are discussed in terms of their possible role as a defence against pathogenic fungi and bacteria.

1072 - Cross talk between cAMP and calcium signalling in the filamentous fungus *Aspergillus niger*

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Very little is known about cross-talk between cAMP and calcium signalling in filamentous fungi. The aim of this study was to analyse the influence of cAMP and protein kinase A (PKA)-dependent phosphorylation on calcium signalling in *Aspergillus niger*. For this purpose, cytosolic free calcium was measured in living hyphae expressing codon-optimised aequorin whilst applying pharmacological agents which increased cAMP levels or inhibited phosphorylation by cAMP-dependent PKA. Calcium was also measured in mutant strains in which phosphorylation by PKA was increased or lacking. Our results indicate that cAMP-dependent phosphorylation regulates calcium homeostasis in *A. niger*, probably by activating calcium channels. Further evidence for cross-talk between cAMP and calcium signalling came from the analysis of a mutant in which the catalytic subunit of PKA was under the control of an inducible promoter. The consequence of PKA induction was a transient increase in cytosolic free calcium which correlated with a polar?apolar transition in hyphal morphology. A transient increase in calcium was not observed in this mutant when the morphological shift was in the opposite direction. These results indicate that PKA-dependent phosphorylation may be important for increasing cytosolic free calcium to induce a polar to apolar shift in hyphal morphology.

1073 - Biological decolorization of textile dyes by *Funalia trogii*

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Synthetic azo and disperse dyes are extensively used in textile industries. A significant proportion of these dyes is eluted in wastewaters. Biodegradation is an alternative to physical and chemical methods used for waste dye removal. In this study, the decolorization capability of a white-rot fungus *Funalia trogii* for eleven synthetic azo or disperse textile dyes were tested. Preliminary results showed that the fungus was capable of removing four dyes, Remazol blue RR, Dianix blau BRL, Dianix blau ACE and Telon schwarz by 38.6%, 50.9%, 32.8% and 54.5% respectively during a period of 5 days. For various dyestuffs, mechanism of decolorization appeared to be different. The effect of inoculum age and agitation on dye removal was investigated for obtaining maximum decolorization. Degree and type of dye removal was found to be dependent on the time of dye addition to the fungal cultures. Consequently, morphological differences in mycelial growth were also observed depending on addition time of dyestuffs.

1074 - Effect of oxygen in the production of manganese peroxidase and laccase by a newly isolated white-rot fungus *Trichophyton rubrum* LSK-27

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Fungal mycelium morphology is a key concept in the production of enzymes. Oxygen transfer is one of the most important parameters that affect the morphology, consequently the enzyme production. Our studies on the morphology and ligninolytic enzyme production of a newly isolated white rot-fungus *Trichophyton rubrum* LSK-27 constitute a very good example to this fact. Three ligninolytic enzymes, manganese peroxidase (MnP), lignin peroxidase (LiP) and laccase productions exhibited variations accordingly. In shake flasks, 3 different working volumes (wv: 10%, 25% and 50%) were studied, in which the organism exhibited significantly different pellet size and shape formation. Oxygen limited conditions (50% wv) led to the formation of large puffy balls (radii ^20 mm), however in 10% and 25% wv, cultures appeared similar in terms of pellet size and shape with a slightly flattened spheres (radii ^1 mm). Moreover, 10% cultures, exposed to least oxygen limitation, contained some hyphal growth in addition to the pellets. Our results clearly demonstrated that this microorganism preferred oxygen rich conditions for enzyme production. MnP and laccase productions were the highest at 10% and 25% wv respectively No MnP and laccase produced at 50% wv. LiP was not detected at all cases.

1075 - Elemental analysis of edible mushroom brown birch scaber stalk *Leccinum scabrum* (Bull. ex Fr.) S. F. Gray growing in various sites in Poland

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The concentrations of 13 elements such as Al, Ba, Ca, Cu, Cd, Fe, K, Mn, Mg, Na, Sr, Zn, and Hg were determined in fruiting bodies of Brown Birch Scaber Stalk *Leccinum scabrum* (Bull. ex Fr.) S. F. Gray collected in summer and autumn 2000 from different sites in Poland. Fresh mushrooms after cleanup from plants and soil were dried for several days in room temperature and further dried in electric oven at 40 degree C for 24 hours. The samples (0.2-0.5 g) of dried and powdered pooled samples of mushrooms (separately caps and stalks) were wet digested with 6 ml of concentrated nitric acid under pressure in closed TFM vessels in a microwave oven (CEM, Mars 5). The PerkinElmer Optima 2000 DV ICP-OES instrument was used for the analysis of mushrooms samples. In the case of mercury CV-AAS technique was used. The method was validated by analysing certified reference materials: CTA-OTL-1 (Oriental Tobacco Leaves, Institute of Nuclear Chemistry and Technology) and IAEA-359 (Cabbage Leaves, International Atomic Energy Agency). There were some variations between trace elements concentration and collecting places. Magnesium, zinc and manganese were determined in quite similar concentrations regardless of sampling site while in the case of other elements some variations were noted. The essential elements such sodium, potassium, magnesium and calcium were found in higher concentrations than toxic mercury and cadmium. Potassium is the most abundant amongst of the elements quantified.

1076 - Weathering in a saxicolous lichen community: A geobiological research project

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The weathering in a saxicolous lichen community growing on sandstone has been studied. Lichen and rock samples have been analysed by high performance thin-layer chromatography/thin-layer chromatography, scanning electron microscopy, and X-ray diffraction analysis, and rock samples also by X-ray fluorescence spectroscopy analysis. The study clearly indicates that lichen mediated weathering leads to fragmentation and chemical dissolution of the rock and its component minerals. Despite the heterogeneity of the rock, and the fact that the studied weathering rinds are the result of complex interactions of physical, chemical, and biological weathering processes during the postglacial period, the results show that some crustose lichen taxa are clearly more aggressive in aiding weathering than other taxa in a lichen community. There is a positive correlation between the degree of weathering and species with high amounts of hyphae within the rock. Differences in content of lichen compounds seem to be a more likely explanation for the variations in chemical weathering, than differences in oxalic acid production. The results further imply that there is an indirect relationship between weathering effect and ecology in saxicolous lichens, but whether this is a specific strategy for nutrient requirements remains unclear. It is also suggested that lichens generally increase the weathering processes, except at locations with extremely high abrasion, where they may protect the surface.

1077 - Biochemical pathway studies on the loline alkaloids of the grass endophyte, *Neotyphodium uncinatum*

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The insecticidal loline alkaloids (1-aminopyrrolizidines) are produced in mutualistic associations of *Festuca* and *Lolium* species with their respective fungal endophytes. Reliable production of *N* -formylloline (NFL) and *N* -acetylnorloline (up to a total of 1000 µg/ml) in cultures of the meadow fescue endophyte, *Neotyphodium uncinatum*, has facilitated studies on the loline alkaloid biosynthetic pathway. Based on loline alkaloid structures, it was previously suggested that lolines are polyamine products. However, our precursor feeding experiments, employing GC-MS and NMR analyses, indicated that lolines share common precursors with, but are not derived from, polyamines. Labels from 5-[^{13}C]-ornithine and 1,2-[^{13}C]-ornithine were incorporated into specific positions of the B-ring in the pyrrolizidine-ring structure of NFL, but the pattern of incorporation differed from what would be expected if a polyamine intermediate was involved. Feeding studies with 4-[^{13}C] and [^{15}N]-aspartic acid gave enrichment in the expected position of the pyrrolizidine A-ring (at C-3) and the 1-amine, respectively. Universally [^{13}C]-labeled methionine did not incorporate in the pyrrolizidine ring system, but labeled the 1-amino methyl and 1-amino formyl groups. These studies demonstrated the origins of the carbon atoms in NFL: the B ring is derived from ornithine, the A ring is from aspartic acid, and the *N*-methyl and *N*-formyl carbons are from the *S*-methyl carbon of methionine.

1078 - Cell state dynamics and pattern formation in polymorphic fungi

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One of the most topical problems of cell and complex systems biophysics is elucidation of driving forces, mechanisms and dynamics of pattern formation and growth. Modern molecular genetics do not allow to understand fully fundamental principles of morphological plasticity. The main idea of our work is to reveal and describe the generic principles of regulation of cell states dynamics in polymorphic fungi, of morphogenesis and differentiation from the point of view of self-organization theory. Experimental study of cell state dynamics in some strains of polymorphic fungi (black yeasts) has shown cell state transitions (spontaneous or induced by various culture conditions). The main morphogenetic factors (governing parameters) were revealed on the basis of experimental analysis of the data obtained for several black yeast strains. In preliminary surveys we have found that regulation of cell state switch exhibits some dynamic properties. Such properties include: 1) all-or-none transitions between discrete states (with a stochastic component); 2) reversibility vs. irreversibility of such state transitions (asymmetry of transition probabilities); 3) hysteresis (in response to transition-triggering agents); 4) autocatalysis or autoinhibition of cell state transitions; 5) symmetry breaking events. Cell state transitions (in particular, mycelial/yeast) result in changes of colony form, and consequently, in diverse development strategies and substrate colonisation patterns of fungi.

1079 - Modelling fungal growth and function in heterogeneous environments

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Modelling mycelial growth in nutritionally or spatially heterogeneous environments is particularly challenging since such growth is a consequence of small-scale processes (e.g. hyphal tip extension) that are manifest at considerably larger spatial scales (i.e. the mycelium) and each of which has important consequences for the spatio-temporal development of the fungus and its environment. We describe a mathematical model that accommodates these factors and explicitly simulates mycelial development in heterogeneous environments at both scales. The model is derived through the discretization of a previously formulated partial differential equation model. The modelled mycelial network is defined on a lattice allowing branching and anastomosis to be explicitly observed. Specifically, the model involves the number, location and orientation of hyphal tips; active and inactive hyphae (i.e. involved/not involved in translocation); internal and external substrate concentration; and spatial organization of substrate and habitable space. On calibrating the model using known properties for the species *Rhizoctonia solani*, information is provided on growth behaviour that is shown to be in very good qualitative and quantitative agreement with experimental data. Development of such an accurate model enables important predictions to be made on the functional consequences of fungal growth in heterogeneous environments and the key role of translocation within mycelia in enabling such responses.

1080 - Elements in ectomycorrhizal fungus *Paxillus involutus* (Batsch ex Fr.) Fr. from various sites in Poland

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Data on metallic elements accumulation of wild growing ectomycorrhizal fungus Poison Pax *Paxillus involutus* (Batsch ex Fr.) Fr. collected from different (contaminated and uncontaminated) ecosystems in Poland are presented. Concentrations of essential metals (e.g. K, Na, Mg, Ca, Mn, Fe, Cu, Zn, Ni) and other which have no apparent essential function (e.g. Hg, Pb, Cd, Al, Ag) were determined in caps, stalks or whole fruiting bodies. The mushrooms were collected between 1999 and 2001 from sites located in northern, central and southern Poland. Dried samples were wet digested with concentrated nitric acid in TFM vessels XP-1500 under pressure in microwave oven (Mars 5, CEM, USA). The elements were detected and quantified using atomic emission spectrometer with induced coupled plasma (Optima 2000 DV, Perkin-Elmer, USA) for some elements and with an inductively coupled plasma-mass spectrometer (HP-4500, Hewlett-Packard, USA). Accuracy of the method was assessed with standard reference materials: CTA-OTL-1, IAEA-359. Poison Pax is very common fungus and is well known to grow in symbiosis with various trees (e.g. *Pinus sylvestris*) this kind of complex research could be very useful in understanding interactions in fungus-metal-environment-tree system. On the other hand it may be one step further in investigation this specimen and its metal accumulation abilities. All these elements could be absorbed and accumulated by different physico-chemical mechanisms and transport systems.

1081 - Exchange processes across the ectomycorrhizal interface: interactions between phosphate and carbohydrate flow

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Basis of the compatible interaction between plant and fungi in an ectomycorrhizal symbiosis is a bidirectional transfer of nutrients across the ectomycorrhizal interface. However, the normal flows of P and carbohydrates through the plasma membranes into the interfacial apoplast are calculated to be insufficient to maintain the symbiosis. Therefore, conditions in the interface, which cause an enhanced efflux or a decrease in the level of competing uptake systems has been proposed by several authors (e.g. Smith et al. 1994). Presently, we have only rare information about a regulation of these transfer processes occuring across this specialized interface and the mechanisms involved in polarizing the transfer. Here, new results are presented, which show, that the carbohydrate supply by the photosynthetic host plant have an influence on the P absorption by the mycobiont and the P transfer across the ectomycorrhizal interface. By the carbohydrate flow across the interface the P allocation between different P pools in the mycobiont and the P efflux across the fungal plasma membrane into the interfacial apoplast is affected. A model system is presented, which show, in which way the carbohydrate and P transfer might be linked and how the exchange processes between both symbiotic partners might be regulated. Smith SE, Gianinazzi-Pearson V, Koide R, Cairney JWG (1994): Plant Soil 159:103-113.

1082 - The localization of nutrient transfer in the arbuscular mycorrhizal symbiosis

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The absorption of ^{33}P-inorganic phosphate and the element distribution in mycorrhizal roots of cucumber inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae* was analysed by microautoradiography and energy dispersive X-ray spectroscopy (EDXS). Arbuscules with their extensive contact area to the plasma membrane of inner cortical cells are the main site for P transfer from an arbuscular mycorrhizal fungus to its host plant. The X-ray microanalytical results indicate, that across this mycorrhizal interface possibly Na and Mg can also be transferred. However, there are no indications, that the arbuscules are the only site for P transfer. Both methods showed that P can also be released from intercellular hyphae into the interfacial apoplast. The results are discussed with respect to the effect of an arbuscular mycorrhizal infection on the nutrient supply of its host plant and the interface regions which might be involved in nutrient transfer.

1083 - Rock and mould: transformation of carbonate minerals by fungi

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In terrestrial environments, fungi serve as important geochemical agents in rock and soil. Fungi promote rock weathering and contribute to the dissolution of mineral aggregates in soil through the excretion of H^+, organic acids and other ligands, or through redox attack of mineral constituents such as Mn and Fe. Fungi can also play an active or passive role in mineral formation through the precipitation of secondary minerals, e.g. oxalates, and through the nucleation of crystalline material onto cell walls, resulting in the formation of biogenic micro-fabrics in mineral substrates. Such interactions between fungi and minerals are of fundamental importance to biogeochemical cycles including those of C, N, S and P which are important nutrients required for plant, fungal and other microbial growth. Our research has demonstrated that fungi may play an important role in the transformation of micro-fabrics in limestone and dolomite. Scanning electron microscopy and energy-dispersive X-ray micro-analysis of rehydrated limestone samples has provided direct evidence of mineralized fungal filaments. The crystal coatings on fungal hyphae exhibited different crystalline lattices and elemental composition from the initial limestone rock. Powder X-ray diffraction analysis indicated that these were probably secondary (re-distribution) carbonates, providing a novel demonstration of biogenic mineral formation mediated by fungal activity.

1084 - Toxic metals in edible mushrooms: Is hyperaccumulation caused by synthesis of metal-binding proteins?

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A common feature of many of the most popular edible mushrooms is the capability of concentrating certain potentially toxic metals to high levels in the fruiting body, thus introducing the metals to terrestric food chains, including humans. Throughout the years, several reports have revealed high concentrations of toxic metals such as Cd and Hg in wild-growing, edible mushroom species. Firstly, these reports may suggest negative effects on persons consuming large quantities of mushrooms, but they also call for a better understanding of the mechanisms involved in metal uptake, transfer and storage. Metal-binding proteins of several classes have been isolated from macrofungi and characterized previously. Some of these proteins share several characteristics with metallothioneins (MT). We here present data on a new Cd-binding protein from the popular edible mushroom *Boletus edulis*, which is a hyperaccumulator of both Cd and Hg. Radiotracer studies clearly suggest this new protein as an important binding site for Cd in this species. The presence of aromatic amino acids and the virtual absence of cystein shows that this protein does not belong to the MT family. When the Cd radiotracer was added to cytosol of *B. edulis* sampled from soils rich in Cd, more than 90% of the radiotracer was bound by the new protein, suggesting a role for the protein in Cd transport and storage. These findings are of importance in view of toxicology and food chemistry, but also environmental protection.

1085 - Metal effect on fungal toxin, genetic expression, and growth regulation: Use of RT-PCR, microarray and electrospray ionization

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The effects of the metal ions (Zn^{+2}, Cu^{+2} and Fe^{+2}), as single or in mixed treatments were observed through changes in *A. flavus* RNA, RT-PCR levels, and by differential genetic expression using microarray analysis, and also assessed by fungal dry weight accumulation, aflatoxin and OMST production. The interaction between the metal ions and the aflatoxin and/or its precursor OMST was determined by electrospray ionization mass spectrometer. The direct effects of metal ions on fungal growth, aflatoxin, and OMST synthesis, varied according the type of metal ion and expressed to either single ion or amendment of ion mixture. All ion treatments induced changes of fungal total RNA and mRNA levels and associate fungal growth, biosynthesis of aflatoxin and its precursor OMST. All mixed metal ions treatments boosted total RNA synthesis and enhanced expression of fungal RNA and the RT-PCR, as well as fungal biomass and synthesis of aflatoxin and OMST. These treatments thus induce changes in pattern of gene expression of *A. flavus* related to fungal growth and synthesis of aflatoxin and its precursor OMST. The differential genetic effects of these treatments were also clearly expressed by the microarray results. The electrospray ionization mass spectrometer analysis showed distinct differential binding of the metal ions to OMST.

1086 - Carbon and nitrogen allocation patterns in the two green algal lichens *Hypogymnia physodes* and *Platismatia glauca* in relation to nutrient supply

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We investigated resource investments in lichens, by quantifying major carbon and nitrogen pools in two green algal lichens in nutrient enriched and poor habitats. Investments between the symbionts were determined by quantifying ribitol and chlorophyll a for the photobiont, and mannitol, ergosterol and chitin for the mycobiont. Two soluble carbohydrates, arabitol and glucose, as well as amino acids and proteins were also quantified. The soluble carbohydrate concentrations were twice as high, c. 4% of thallus dry weight, and thallus N concentrations were 2-4 times higher in thalli from the nutrient enriched compared to the nutrient poor habitat. The proportion of thallus N invested in proteins was similar in the two habitats, while the amino acid pool was doubled in the fertilized thalli. This increase could be attributed to the N- rich amino acid arginine. Both lichens displayed an increased proportion of photobiont cells in relation to mycobiont hyphae in the nutrient enriched habitat. Resulting in a significantly higher ribitol to mannitol ratio, and a higher Chl a to ergosterol ratio, in thalli from the nutrient enriched habitat. Suggesting that the photobiont had benefited more than the mycobiont from the fertilization, and the increased arginine concentration suggests that the mycobiont may suffer from a relative carbon shortage. Both lichens were still healthy and alive, so the altered balance between the bionts might be an adaptation to handle the fertilization stress.

1087 - Proteinases involved in skin degradation by the oomycete pathogen *Pythium insidiosum*

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The oomycete *Pythium insidiosum* is the only stramenopile pathogen of humans and other mammals. In essence, the mechanism of tissue invasion utilized by this microorganism is identical to the process employed by every other filamentous fungus that colonizes animal or plant tissues: invasive growth depends upon the exertion of force by the hyphal apex, and the degree to which the substrate is degraded by secreted enzymes. Biomechanical experiments prove that *Pythium insidiosum* achieves a 100-fold reduction in the strength of cutaneous and sub-cutaneous tissue through proteinase secretion. In an attempt to identify the enzymes that play a primary role in tissue degradation, we have isolated and characterized a number of unique serine- and metallo-proteinases. By analyzing the effectiveness of each protein at facilitating hyphal penetration of collagen-rich membranes it is possible to distinguish between their nutritional and 'barrier-dissolving' roles.

1088 - Lichens as a model-system for symbiotic organisms under most extreme conditions

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As a consequence of the symbiotic state of lichens both the bionts are able to colonize habitats where the separate bionts would not be able to survive. The symbiosis of lichens reflects a high degree of complexity and plasticity. The combination of both enables these organisms to colonize most extreme habitats. Besides the already well investigated microorganisms lichens are good model-systems to examine adaptation strategies to most extreme environments. Because of the symbiotic nature of the lichens a 3-component-system can be used for investigations: the mycobiont, the photobiont and the lichen itself. Our investigations are based on such a system. The influence of different doses of UV A, B, C on the vitality of fungal (mycobiont) spores and the germination process has been investigated. The spores are cultivated on a variety of different subtrates to testing the influence of the UV radiation. The influence of vacuum conditions has been investigated. The aim of this research is to test the reaction of a symbiotic organism and its respective bionts to highly extreme conditions. For interpretation of results the method of modern confocal lasermicroscopy (CLSM) - a novel method in lichenology - is presented.

1089 - Phosphate transporter expression in the soybean AM symbiosis

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We are currently studying genes that are potentially involved in the process of phosphate uptake in the arbuscular mycorrhizal (AM) symbiosis of soybean. We have extracted mRNA from AM colonised and uncolonised soybean roots using biotinylated poly-T probes and streptavidin-coated magnetic particles. Total mRNA extracted by this method was reverse-transcribed into cDNA with poly-T primer and reverse transcriptase. cDNA was next PCR-amplified with primers for a previously published soybean root phosphate transporter gene and amplified sequences were visualized on silver-stained acrylamide gels. In situ hybridization studies were carried out to localise the phosphate transporter in root tissues. Root cryosections were probed with a fluoroscein-conjugated antisense probe and bound targets were visualized either via alkaline phosphatase labeling or with an antifluoroscein texas-red antibody. Results of these investigations are presented here.

1090 - Profiling differences in metabolic activity between isolates of *Penicillium camemberti* using dielectric spectroscopy (impedimetric measurements)

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In this work we characterise *Penicillium camemberti* starter cultures in order to predict the quality of white mould cheeses. Impedimetric changes in the first 100 hours after inoculation are detected using a Bactometer at a 1 kHz frequency. At this low frequency, the capacitance and conductance changes in the medium are measured, thereby indirectly measuring the metabolic activity of the fungi growing in the medium. Traditional impedimetric methods are used for detecting lag phase and growth rate. In the present work we try to use all impedimetric changes in the first 100 hours as a profile of the each isolate and subsequently treat the data using chemometric methods. As the metabolic activity of fungi depends strongly on the environmental conditions, this study is done using a cheese medium developed in our laboratory.

1091 - Intracellular enzymatic activity of the mycelium of *Pleurotus* spp. grown on media containing 2-deoxy-D-glucose

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The strain selection of edible mushrooms has often been done by trial and error because there appear to be no clear correlation between mycelial growth rates evaluated on Petri dishes at laboratory level and productivity under production plant conditions. It has recently been found that the tolerance of some *P. ostreatus* strains to 2-deoxy-D-glucose (DG) at laboratory level, is correlated with improved yield of these strains at mushroom production level. In this work, the effect of DG on the intracellular activity of laccases (L), proteases (P), &beta-1,3-glucanases (G) and endoglucanases (E) of the mycelium of strains of *Pleurotus* was evaluated. Six strains of *P. ostreatus*; 201216, 32783, 58052, 38537, 201218 from ATCC, and 3526 from NRRL, and two strains of *P. pulmonarius*; PPL27 and PPL34 from the Chinese University of Hong Kong Collection were studied. The strains were grown on a starch-based media with 0.01% (SDG) and without addition of DG (S) at 25 °C for 7 days. On S, all the strains presented L, P, G and E activity. However, on SDG, the strains 201216, 32783, 58052, 38537 (previously reported as sensitive to DG) did not present any enzymatic activity. On SDG, the strains 201218, 3526, PPL27 and PPL34 (previously reported as tolerant to DG) presented higher P, G and E activity than those presented on S. It suggests that the DG tolerant strains are derepressed for producing L, P, G and E, which could be importantly related to the mushroom productivity.

1092 - Twin arbuscules in *Linum usitatissimum* L.

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The 'twin' arbuscule development of *Arum*-type mycorrhizal colonization in *Linum usitatissimum* L. were investigated using a time-course study to show formation and senescence of the structures. Roots were freeze-sectioned longitudinally, mycorrhizal structures were visualized using 1) nitroblue tetrazolium as a vital stain to indicate metabolic activity of arbuscules and intercellular hyphae and 2) acid fuchsin counterstaining. Arbuscules occur in pairs in adjacent cortical cells arising from a single, radial intercellular hypha. These 'twin' structures often appeared to be at different stages of metabolic development. Arbuscules and their relationship to

intercellular hyphae and plant cortical cells were imaged using laser scanning confocal microscopy (LSCM). Using the optical xy confocal slices, a 3D reconstruction of a twin structure was produced and the surface area of each arbuscule was measured. The measurement of surface area together with logistic regression calculated over the time course study indicated that there was a delay in development of the second arbuscule. Intercellular spaces within the root cortex appear responsible for determining the structural type of colonization. Crooks (1933) described the unusual cell divisons (and hence intercellular spaces) in *L. usitatissimum* roots which is used to produce a conceptual model of this *Arum*-type mycorrhiza. Crooks DM. 1933. Botanical Gazette 95: 209-239.

1093 - In vitro retention of ^{137}Cs and potassium by the mycelium of mycorrhizal and saprotrophic basidiomycete fungi

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After Chernobyl disaster it has become apparent that natural losses of ^{137}Cs from the European forest soils are proceeding extremely slowly due to the ability of various fungi to accumulate this radionuclide. Large differences were observed in ^{137}Cs levels in fruit bodies of various edible fungal species often independent from the site and time of sampling. This phenomenon has led a number of radioecological modelers to conclude that radionuclides contamination in fungi is very difficult to predict. This paper presents results of the experimental investigation of ^{137}Cs uptake by fungal mycelium versus undisturbed potassium metabolism. A special methodology was employed to study the ability of mycelium of mycorrhizal species - *Suillus variegatus*, *S. grevillei* - and saprotrophic - *Pleurotus ostreatus*, *Stropharia rugosoannulata* - to retain ^{137}Cs and potassium dependent on: (i) genomic factor (strain and species), (ii) cultivation conditions (composition of the medium and the growth rate), (iii) metabolism activity and (iv) in a stream of time. Since high radiocaesium/potassium ratios were demonstrated for ectomycorrhizal fungi only, we postulate the existence of a high-affinity potassium efflux system in cells of mycorrhizal fungi; it is likely that this system promotes the retention of accumulated radiocaesium inside the cell, while potassium can be released back to the medium.

1094 - Mapping of psychro- and/or halotolerance of *Penicillium* spp. and *Aspergillus* spp. and correlations of enzyme profile and habitat

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The extracellular enzymatic activity of psychro- and/or halotolerant *Penicillium* spp. and *Aspergillus* spp. were studied and the correlations to habitat investigated. 170 isolates were screened. As expected, the results showed that the Penicillia tend to be more psychrotolerant and the Aspergilli have better halotolerance. Except for the halophiles, it was observed that the higher the incubation temp., the better the halotolerance. Only mild halophiles were found. Of these many tended to stay halophile at lower temp. Among the Aspergilli only psychrotolerant isolates were found. The Penicillia yielded some psychrofilic isolates. The fastest growing isolates were selected for semi-quantitative enzyme screening. These were inoculated on triglyceride, casein and lignocellulosic liquid media with various amounts of NaCl added. The enzyme activities in the media were tested after 10 to 14 days of growth. Assays were performed for proteinases, hemi- and cellulases and lipases on assay plates. It is expected that the isolates will exhibit habitat specific enzyme profiles, with the enzyme activity dependant on temperature and A_w of the habitat. This hypothesis originates from the fact, that most fungi are highly habitat specific and not metropolitan. It is also expected, that while all (or most of) the isolates will have activity for the substrates screened against, there will be a difference in activity correlated to their habitat.

1095 - A *MAT-2* mating-type gene in the homothallic fungus *Aspergillus nidulans*

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Mating-type (*MAT*) genes have been identified from the pyrenomycete, loculoascomycete and discomycete classes of ascomycete fungi. By using hot-start PCR with degenerate primers, together with thermal asymmetric interlaced (TAIL)-PCR, it has been possible to identify a characteristic *MAT-2*gene from the plectomycete fungus *Aspergillus nidulans*. It includes a conserved high mobility group (HMG)-domain. RACE-PCR analysis has confirmed transcription of the gene during sexual reproduction and the presence of an intron in a conserved position within *MAT-2* genes. Further analysis of the flanking regions of the *MAT-2* gene revealed the presence of a 5S ribosomal-DNA sequence, a putative gene with homology to an element of the anaphase-promoting complex (APC) of *Schizosaccharomyces pombe*, and a putative gene with homology to the transmembrane receptor *SYG1* from *Saccharomyces cerevisiae*. The presence of an *APC* homologue provided evidence of microsyteny around the *MAT* locus. However, no *MAT-1* alpha-domain gene could be detected. This suggests either that a *MAT-1* homologue may be present elsewhere in the genome, not directly adjacent to the *MAT-2* locus, or that *A. nidulans* may contain only a *MAT-2* mating-type gene. This would be a unique situation among homothallic euascomycetes so far analysed, which contain either a *MAT-1* gene or both *MAT-1 /MAT-2* genes.

1096 - Field evaluation of yield and N2-fixation of chickpea (*Cicer arietimum* L.) as affected by phosphorus and biofertilizers

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As a result of our previous work held under green-house conditions, we have decided to develop our experiment under field trial to generalize our results. So, a field experiment was carried out to evaluate the influence of biofertilizers, Mycorrhizae (AM) and / or Rhizobium (Rh), combined with super or rock - on the growth and N2-fixation in chickpea grown in sandy soil. A minimal dose of super and rock-P were added at two doses (15 and 30 kg p/h) as well as 15 kg N /h as ordinary and labeled (15 NH4) 2 SO4. Dry matter yield (DYM) of inoculated plants were higher than control treatments. Combined inoculation (AM + Rh) in case of super or rock-P increased DYM than single inoculation. Also, the results showed that, single inoculation with Rhizobium increased yield in case of 30 kg P/ h than 15 kg P / h application on the other- hand mycorrhizal infection increased chickpea production with 15 kg P / h rather than with 30 kg P / h addition. AM infection increased nodule number, so nitrogen uptake increased in dual inoculation than the single one, at the same time N2 fixation quantities were so much in combined inoculation in case of super-P than with rock-P application. We can conclude that, from an economical and environmental point of view biofertilizers (AM or Rh)) can save a great amounts of P and N fertilizers to increase crop production and this cleared the importance of biofertilizers and rock-P in clean sustainable agriculture systems.

1097 - Identification of essential groups at the active site of inulinase from *Penicillium cyclopium*

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Amino acid residues involved at the active site of inulinase from *Penicillium cyclopium* were determined. DEPC (diethylpyrocarbonate) totally inactivates the enzyme but not in the presence of substrate. The inactivation reaction follows pseudo-first order kinetics with a second-order rate constant of 0.05 mM^{-1} min^{-1}. Reversal of inactivation in the presence of hydroxylamine leads to the inference that histidyl residues are essential for catalysis. EEDQ (2-ethoxy-1-ethoxy-1,2-dihydroquinoline) inactivates the enzyme, but not in the presence of inulin, following pseudo-first order kinetics with a second-order rate constant of 0.02 $mM^{-1}min^{-1}$. This is indicative of the involvement of residues with a carboxyl group in the catalytic activity. Further kinetic analysis of the inactivation caused by EEDQ, strongly implies that modification of a single residue of aspartate or glutamate inactivates the enzyme. Chemical modification of inulinase with tryptophan specific reagent NBS (N-bromosuccinimide) inactivated the enzyme but the enzyme was protected with inulin. The inactivation with NBS indicated that tryptophan residues are essential for the enzyme catalysis. Treatment of the enzyme with pHMB (*p*-hydroxy-mercuribenzoate) and PMSF (phenylmethyl-sulphonyl fluoride) did not influence activity, thus eliminating the possibility that cysteine or serine participate in catalysis. Chemical modification of inulinase with (NAI) N-acetylimidazole and TNM (Tetranitromethane) caused inactivation of the enzyme but in the absence of inulin. These results suggest the necessity of tyrosyl group in the enzyme catalysis.

1098 - *In vitro* element accumulation by macrofungi

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Element accumulation by macrofungi in natural conditions (especially at the level of carpopohores) has been studied since thirty years. Results often showed mycorrhizal species to be better heavy metals accumulators than saprophytes. In vitro studies can be used to find explanations on the phenomenon of element accumulation occuring in natural conditions (e.g. absorption, localization and resistance). Two kinds of research are usually performed: short term experiment without any growth or growth in element enriched medium or substrates. Our *in vitro* study integrate and compare both of these research possibilities, using a selection of five mycorrhizal and ten saprophytic fungi, three heavy metals (copper, iron and zinc) and one semi metal (selenium). Firstly, we analysed under growth condition the absorption (AAS, spectrophotometry) as a function of pH and element concentration. Secondly, we analysed the absorption of those species in short term experiments under the same conditions but without any growth. A comparison of both experiments showed the absorption depending on many factors such as pH, initial element concentration, species and kind of method chosen. As perspectives, we would like to optimize the absorption and localisation of copper, iron and selenium into different parts of mycelium and carpophore. Possible applications, e.g. in the cosmetic industry, where selenium is used as antioxydant or in the alimentary industry (functional food).

1099 - Demonstration of DHN-melanin pathway and genetic analysis of the gene encoding scytalone dehydratase in sapstain fungi

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The presence of the 1,8-dihydroxynaphthalene (DHN) melanin biosynthesis pathway was demonstrated in several sapstain fungi using both chemical inhibitors and molecular techniques. The inhibitor compounds tricyclazole and carpropamid effectively reduced pigmentation at low concentrations in all tested fungal species, but also lead to growth inhibition at higher concentrations. The inhibitor cerulenin prevented fungal growth in all tested fungi at all tested concentrations, likely due to its inhibitory effect on another enzyme, the metabolically critical fatty acid synthase. Partial DNA sequences for the gene encoding scytalone dehydratase (SD) were obtained from species of *Ceratocystis* and *Ophiostoma* and found to have homology with known respective DHN-SD gene sequences. Sequence analysis of the partial SD amino acid sequences showed greater than 80% similarity among the sapstain species, and corresponded well with known phylogenies of sapstain fungi based on rDNA sequences. Aside from the work carried out on the isolate *O. floccosum* 387N, this is the first known documentation of the melanin pigmentation pathway used by species of the sapstain fungi from *Ceratocystis*, *Leptographium* and *Ophiostoma* (Eagen et. al. 2001, Wang et al. 2001, Wang and Breuil submitted). Furthermore, since no fungus has ever been found, to our knowledge, to have more than one melanin synthesis pathway, we can state that these species likely only use the DHN pathway for melanin production.

1100 - Clay effects on the fungal growth morphology and copper sorption ability

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As a prelude to the development of biomineral sorbents for toxic metals, this study assesses the influence of clay minerals (bentonite, kaolinite and palygorskite) on the morphology of mycelial pellets produced by melanin-forming microfungi and on the copper sorption properties of fungal/clay mixtures. In general, a reduction of pellet size, an increase in the length of surface hyphae of the pellets, and a reduction in exopolymer production were observed with increasing clay mineral concentrations up to 5%(w/v). It was found that the clay particles were involved in the development of pellet structure of *Cladosporium cladosporioides* at all stages of growth. A general model of the structure of a fungal pellet grown in clay-containing medium is proposed. The pellets consist of three main layers: a central core with densely packed mycelium aggregated with solid clay minerals or a matrix of clay/polysaccharides; a middle layer with looser mycelium mixed with clay mineral flakes; and an outer, or 'hairy' zone, with loose hyphae surrounded by clay mineral flakes. A study of equilibrium Cu uptake from pH-buffered solutions showed that bentonite addition to the medium increased the sorption of Cu by *Aureobasidium pullulans* and *C. cladosporioides* grown in this medium. The mechanism of changed sorption capacity of the combined biomineral sorbents is suggested to occur by blocking or modification of binding sites on biotic and abiotic components of the 'biomineral' association.

1101 - Effect of nutrient resources on growth and morphology of fungal mycelia penetrating toxic metal domains

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Natural environments contain a heterogeneous distribution of metal concentrations, and the ability of fungi to colonise such metal-contaminated domains will be influenced by the resources available to fungi. The aim of this study was to investigate the growth responses of some common soil fungi (*Trichoderma viride* and *Gliocladium roseum*) towards copper and cadmium under different nutritional conditions and using a system of tessellated agar tiles. The growth parameters recorded in this study demonstrated a decrease in metal toxicity with increasing concentration of available carbon source. It was shown that maximum extension rates and efficacy of carbon substrate utilization of both cultures decreased with increasing concentration of toxic metals. It was observed that in the gap between metal-free tiles and metal-containing tiles, the presence of the toxic metals led to negative chemotropic reactions and stopping of growth, swelling and lysis of some hyphal tips. Penetration of the hyphae into the metal-containing domain was often followed by the formation of very dense mycelium or mycelial 'bushes'. After fungi entered the toxic metal-containing domain, they often produced long sparsely-branched or branchless explorative hyphae. Our data have demonstrated that fungi efficiently use both 'phalanx' and 'guerrilla' states of the mycelial system as a response to toxic metal stress combined with nutritionally-poor conditions.

1102 - Ruderal and combatative strategies in insect pathogenic fungi examined by EST analysis

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Metarhizium anisopliae (Ascomycota) and *Conidiobolus coronatus* (Zygomycota) are facultative saprophytes that are pathogenic to many insect species. About 2000 EST cDNA clones from each species were sequenced to analyze gene expression during growth on host insect cuticle and/or nutrient rich media. Proteases were the commonest transcripts with both species producing multiple subtilisins, as well as trypsin, metalloprotease and aspartyl protease activities capable of degrading host tissues and disabling anti-microbial peptides. However, compared to *M. anisopliae*, *C. coronatus* produced many fewer extracellular hydrolases (e.g., no phospholipases), antimicrobial agents, toxic secondary metabolites and no ESTs with putative roles in the generation of antibiotics. Instead, *C. coronatus* produced a much higher proportion of ESTs encoding ribosomal proteins and enzymes of intermediate metabolism consistent with the rapid growth characteristics of *C. coronatus*. These results are consistent with *M. anisopliae* using a combatative strategy to defend captured resources, while *C. coronatus* has modified the saprophytic ruderal-selected strategy using rapid growth to overwhelm the host and exploit the cadaver before competitors overrun it. Both strategies are consistent with specialization to pathogenicity. Thus, induction of proteases by host cuticles (mucoralean fungi do not produce proteases under these conditions) demonstrates that *C. coronatus* is adapted to entomopathogenicity.

1103 - Induction of defense-related enzyme activities in poplar roots inoculated with compatible and non-compatible isolates of *Paxillus involutus*

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Defense-related enzyme activities, especially those of peroxidases, were examined in short roots of control, non-compatible and compatible interactions between hybrid poplar (*Populus tremula* L. x *P. tremuloides* Michx.) and *Paxillus involutus* (Batsch) Fr. Peroxidase activities increased significantly in poplar inoculated with non-compatible isolate of the fungus compared to control, an indication that the plant recognized invasion of the non-compatible isolate and subsequently responded with activation of the defense responses. Elevated levels of the enzymatic activity were maintained after seven weeks of inoculation. On the other hand, accumulation of the same enzyme was not detected in compatible associations. The failure of induction of peroxidase activity in mycorrhizal root tips may suggest low-scale or even lack of plant defense responses in the compatible association between poplar and *P. involutus*. In other experiments, an appreciable amount of hydrogen peroxide (H_2O_2) was surprisingly detected only in compatible tissues, leading to speculation on the presence of stress conditions in the tissues. It has to be noted, however, that the accumulated H_2O_2 in the tissues may have been released by fungal mycelia forming mantle and Hartig net in compatible interactions. It has been recognized that mycelia of some ectomycorrhizal fungi, including *P. involutus*, produce H_2O_2.

1104 - Overflow of organic acids in *Penicillium simplicissimum*

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The efficiency of carbon source utilization by filamentous fungi is strongly influenced by environmental conditions. This is of advantage in biotechnological processes, because it is easier to vary environmental conditions than to change the metabolic properties of a fungus. Understanding how environmental conditions influence a fungal metabolism is therefore important. We used continuous cultivation to study the influence of the specific growth rate and of environmental conditions (glucose-, ammonium-, nitrate-, phosphate- and potassium-limited growth; various pH values, osmolarities and inhibitors) on the excretion of organic acids by *Penicillium simplicissimum*. *P. simplicissimum* is used in heterotrophic metal leaching processes. Citrate was under all conditions the main excreted intermediate. This excretion can be termed an energy spilling process, because it avoids the formation of NADH. The sum of the excreted organic acids was inversely correlated with the biomass yield. Glucose uptake was strongly influenced by the specicfic growth rate, but only weakly by the tested environmental conditions. The highest carbon overflow was observed with phosphate limitation (12% of the total consumed carbon, compared to 1% during glucose limitation). Stress factors (alkaline pH, high osmolarity, addition of benzoic acid) in general increased both glucose uptake and carbon overflow.

1105 - Calcium oxalate and gypsum on the thallial surface of the lichen *Ramalina lacera* exposed to polluted air

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The formation of calcium-containing structures on the thallial surface of the lichen *Ramalina lacera* (With.) J.R. Laund. in response to air pollution and to simulated acid rain, was studied in *in situ* and transplanted thalli. *In situ* thalli were collected from an unpolluted site and transplanted to heavily polluted and to less polluted sites for a ten-months period. Additional thalli were treated either with double distilled water or with simulated acid rain. SEM and infrared spectrometry revealed that thallial surfaces of *in situ R. lacera* samples collected in unpolluted sites were covered with two kinds of calcium oxalate crystals. These aggregates of calcium oxalate crystals appear to disintegrate and provide a crystal layer on the thallial surface. Infrared spectroscopy of powder scrapped from thallial surfaces of transplants, retrieved from non-polluted sites, showed the presence of whewellite and weddelite whereas powders obtained from thalli retrieved from polluted sites contained whewellite, weddelite and gypsum. It is suggested that a certain fraction of the gypsum detected in crater-like structures in transplants from polluted sites and in thalli treated with simulated acid rain is endogenous and should be considered a biomineral.

1106 - Biotechnological aspects of basidiomycetes study

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The study of physiological and biochemical peculiarities of Basidiomycetes opens a wide range of possibilities for their using in biotechnology. The first step of creation of biotechnological process starts with an investigation of physiology of fungal producer. The Collection of LE(BIN), containing a great number of Basidiomycetes provides the opportunity to carry out the study on biology of macromycetes development in culture including the investigation of enzymatic activities of macromycetes. The kinetic regularities (duration of lag-period, specific rate of growth, the velocity of carbohydrate consumption, economical coefficient, the influence of pH on the growth and metabolism) and peculiarities of ligninolytic enzymes biosynthesis under the submerged cultivation of Basidiomycetes belonging to families Polyporaceae, Ganodermataceae and Strophariaceae have been studied. It has been shown that rate of culture growth and enzyme synthesis can be regulated by cultivation conditions and desirable level of enzymes produced can be achieved by screening of appropriated producers. The combination of fast method developed for fungi screening on agar plates and the study of biosynthetic peculiarities of macromycetes under the conditions of submerged cultivation allows to chose the most prominent strains for different biotechnological applications, in particular for obtaining of ligninolytic enzymes, biologically active substances as well as biomass with high protein concentration.

1107 - Identification of transposon-like sequences in the genome of fungi belonging to the Glomales

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Arbuscular mycorrhizal fungi (Glomales) which are important for plant health and survival are ancient organisms which appeared with the first land plants more than 400 million years ago. Their genome is large (108 to 109 bp) and is rich is repeated sequences. A high level of genetic polymorphism exists within their multinucleate spores. The aim of this work was to characterise repetitive DNA elements and provide a greater insight into the structure of the genome in the Glomales. Repeated sequences were isolated by screening lambda ZAPII genomic libraries from *Glomus mosseae* BEG12, *Gigaspora rosea* BEG9 and *Gigaspora margarita* BEG 34 or by genomic self-priming PCR (GSP-PCR). Insert length ranged from 900 to 6000 bp and the copy number of each repeated sequence in the genome was between 70 and 8000. Of 25 sequences that were isolated, all were rich in AT. Most of the sequences had no open reading frame and showed no homology to known sequences. However, short regions in these sequences were identical to elements present in transposons in other organisms. Two sequences showed similarity to reverse transcriptases. The distribution of these sequences in the genome is being determined by Southern blot and FISH. The presence of transposon-like sequences in the genome of the Glomales may have contributed to the appearance of high genetic polymorphism in the large genome of these fungi during evolution. This work is part of the european project GENOMYCA (QLRT-2000-01319).

1108 - Black fungal colonies as units of survival: hyphal mycosporines synthesized by rock dwelling fungi

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Microcolonial fungi (MCF), long living vegetative mycelia frequently occurring on desert rock surfaces, are exposed to strong ultraviolet radiation, desiccation and nutrient scarcity. Fungal rock dwelling microcolonies lack ascospores and all their cells possess a thick melanised cell wall. These simply organised mycelial structures grow by mitotic cell divisions, but frequently are more stress-

tolerant than lichens. Colonies of several randomly selected MCF strains were analyzed by cryo-SEM and TEM electron microscopy and for the presence of mycosporines. This class of UV-absorbing compounds, common in spores and other survival structures, is unknown in vegetative hyphae and was thought to be limited to reproductive morphogenesis. Mycosporine were analysed by LC/MS and LC/MS/MS (using a HPLC with a UV-VIS Detector and a ELSD interfaced with an ion trap MS). Intracellular intrahyphal growth and recolonization of old cells by new ones were observed in all MCF strains investigated. Mycosporines ubiquitous presence in the vegetative MCF cells help explain the high survival potential and longevity of these fungi. Territorial expansion of MCF is limited and mycosporines -known as inhibitors of conidia germination- may also serve as regulators of non-expansive restricted growth inherent for MCF. Also multiple cell functions such as UV-protection and the intracellular signaling for cell division and/or restriction of hyphal branching may be mediated by light-absorbing mycosporines.

1109 - Mutational dissection of morphogenesis in *Neurospora*

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Morphogenesis in filamentous fungi is driven by programmes of tip growth and branching. The main components of current cellular models are the actin cytoskeleton, calcium homeostasis and morphogenes. We are investigating these components using the *Neurospora* model. First, we are characterizing many of the available morphological mutants by examining their responses to calcium and manganese at the macroscopic and cellular levels. This analysis has generated new groupings that could reflect function. Second, we are examining the functional interactions between mutations using epistasis analysis. The results require redefinition of the modes of interaction into full epistasis, partial epistasis, co-epistasis, novelty and cumulative or multiplicative action. This work also generates functional groups that might represent morphogenetic networks. Third, a mutation of the structural gene for actin has been isolated, cloned and sequenced. This clone has permitted more mutations to be isolated by repeat-induced point mutation (RIP). The properties of the mutant strains are being studied at the cell and molecular levels. They show a variety of types of aberrant growth, branching, aerial growth and sexual structures. Attempts at actin staining are in progress. [Supported by a grant from the Natural Sciences and Engineering Research Council of Canada.]

1110 - Natural protease inhibitors from fruit bodies of mushrooms

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Proteolytic enzymes have been firmly established as main regulatory components in a number of cellular, tissue and physiological processes. The most important factors influencing the proteolytic enzymes and pathways of proteolysis are natural protease inhibitors which form complexes with target proteases to inactivate and/or to regulate their activity. There is growing interest in inhibitors of proteases, not only synthetic but also naturally occurring (mushrooms' ones among them), due to their role in various human diseases. Searching for new, bioactive metabolites of basidiomycetous fungi we isolated and characterized some low and high molecular natural protease inhibitors of serine, asparagine, cysteine and metallo-proteases. They were isolated from fruit bodies and mycelia of edible, cultivable and potentially medicinal mushrooms (*Pleurotus ostreatus*, *Lentinus edodes*, *Agaricus bisporus*, *Trametes versicolor*). Isolation of inhibitors was achieved by ion exchange and size exclusion chromatography. Characterization of their inhibitory activity, pH and temperature optima, and molecular mass were analysed. It appeared that low molecular inhibitors of serine proteases were typical for both mycelium and fruit bodies, and high molecular inhibitors ones were characteristic for fruit bodies only. Inhibitors of asparagine proteases were typical for mycelium mainly. And inhibitors of cysteine proteases and metalloproteaes were found mainly in fruit bodies.

1111 - Deliquescing of fruit bodies of *Coprinus comatus*: new insight into proteolysis of the process

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Species of *Coprinus* are well recognized by their deliquescing of fruit bodies. Genus is also mentioned from the point of view of *C. cinereus*, which model organism to study development processes in the Homobasidiomycetes. The species *C. comatus* is characterized by melanized spores and inaequi-hymeniferous gills that tend to deliquesce into black liquid that drips from the disintegrating cap. The autolysis is evidently part of the developmental program of fruit body. The autolysis of the mature cap is caused mainly by chitinases. Process effects cap tissues, but not the outer veil layers and the stipe. Apart chitinases, proteases and glucanases also have been identified. In our work we try to evaluate proteolysis events during deliquescing process. We have been using 'wild' *C.*

comatus model instead of 'laboratory' *C. cinereus* one. During analysis of enzymology of stages of development of fruit body, we were investigating levels of enzymes connected with melanization proces and proteolysis events. We have been searching of also the levels of natural protease inhibitors of various groups of proteases. Comparison of changes in levels of protease activities and of their inhibitors may give us new, interesting insight into relations of various types of proteases and their natural inhibitors, and into a role of proteolysis during development and deliquescing of basidiocarps of genus *Coprinus* mushrooms.

1112 - Characteristics of secondary metabolites from lichen mycobionts

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Secondary metabolites were often obtained from colonies of spore-derived lichen mycobionts cultured on conventional malt-yeast extract media with 10% added sucrose. Some substances not detected in the lichens, were often new, and crystallized on the surface of medium-cultured mycobiont. These substances, which differed among the various lichen mycobionts, included graphenone, graphisquinone, dibenzofurans, xanthones, hybocarpone, isocoumarins and other substances. These substances appear to be often bioactive or toxic to photobionts. In addition to previous work identifying new mycobiont substances, further studies are performed in order to elucidate the biological significance of substances produced by the lichen mycobionts and the origin of lichen symbiosis. In the present study, we have examined the distribution and variation in dibenzofurans, which are common in lichen mycobionts, and distributed over many families, e.g. *Stereocaulon*, *Evernia*, *Usnea*, *Lecanora*. On the other hand, dibenzofurans were not found in lichens. This biosynthetic pathway was thought to dormant in lichenized condition, and was induced in cultured lichen mycobiont. The structure of dibenzofurans varies with species of lichens, and some dibenzofurans are chlorinated derivatives. These substances appear to be toxic to an algal partner. We tried to confirm this hypothesis in experimental procedure. The biological significance of these metabolites is discussed from the viewpoint of lichen symbiosis.

1113 - T-DNA based genetic transformation of the ectomycorrhizal fungi

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The T-DNA of *Agrobacterium tumefaciens* can be transferred to plants, yeasts, fungi and human cells. Using this system, a technique was developed for transforming ectomycorrhiza forming basidiomycetes *Suillus bovinus*, *Hebeloma cylindrosporum*, and *Paxillus involutus*. The selection marker employed was the Shble gene conferring resistance to phleomycin under control of the *Schizophyllum commune* GPD promoter and terminator. Putative transformants were shown by PCR to contain the GPDScP-Shble-GPDScT construct, although the fate of the foreign DNA could not be determined. In order to improve the system, dikaryotic mycelia of *S. bovinus* were transformed with recombinant hygromycin B phsphotransferase (hph) and enhanced green fluorescent protein (EGFP) genes fused with a heterologous fungal promoter and CaMV 35S terminator. Transformation resulted in hygromycin B resistant clones, which were mitotically stable. Putative transformants were analysed for the presence of hph and EGFP genes by PCR and Southern analysis which proved both multiple and single copy integrations of the genes. Several genes encoding small GTPases have been charcterized from *S. bovinus*, including SbCdc42 SbRac1, SbRas1 and SbRas2. The introduction of in vitro mutagenized dominant forms of these genes into *S. bovinus* genome by the transformation system described here will greatly advance our understanding of the function of the actin cytoskeleton and small GTPases in vegetative and symbiotic hyphae of *S. bovinus*.

1114 - Characterization of copper-philic mycobiont from lichen *Tremolecia atrata*

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It is well known that lichens are able to accumulate high amount of heavy metals in their thalli. To clarify the possibility of application of lichens for bio-remediation, we had screened lichen mycobiont cultures for copper-tolerance. As a result of the screening, a mycobiont derived from *Tremolecia atrata* found to be copper-philic rather than copper-tolerant. The copper ion level for optimum growth in liquid medium was between 25 to 30 ppm. The upper limit adequate for growth was around 125 ppm. Analyses by an atomic absorption spectrophotometer revealed an accumulation of copper ion within mycobiont aggregates. To cast further light on molecular mechanisms of this copper-philicity, differential protein expression analyses were performed, resulting in different protein profiles between mycobiont aggregates cultured with and without copper ion.

1115 - Determining the relative role of ligand versus proton-promoted dissolution of metal phosphates by fungi

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Many soil fungi can solubilize sparingly soluble metal phosphates, and are thus important agents in nutrient cycling of phosphate and metal ions. Despite rapid screening methods to identify isolates with proficient phosphate solubilizing activity, there lacks quantitative data on the mechanisms by which dissolution occurs. Two non-mutually exclusive mechanisms involving efflux of (i) protons and/ or (ii) metal complexing ligands (such as organic acid anions) are frequently acknowledged in this role, although their relative importance in the dissolution process is unclear. In this work we used *Aspergillus niger*, *Fusarium sp.* and *Coriolus versicolor*, to develop contrasting models to provide a better mechanistic understanding of phosphate dissolution. We show that the relative role of proton versus ligand-promoted dissolution is influenced by many factors, including nitrogen-source, with marked differences occurring between fungal species. Many fungi subsequently immobilize metals, such as recrystallization as oxalates, while all fungi exhibit surface sorption and/or intracellular accumulation. Similar to the mechanisms of mobilization, these lack proper quantification. A sequential extraction technique has been used to fractionate metal in fungal biomass and agar into soluble, phosphate and oxalate phases, in order to quantify the fate of metal and achieve a mass balance. The functional significance of our findings for fungi in biogeochemical processes will be discussed.

1116 - Production of neutral and alkaline proteases by the thermophilic fungus, *Scytalidium thermophilum*, grown on microcrystalline cellulose

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Scytalidium thermophilum protease production in Avicel-containing media was analysed. Proteases produced by *Scytalidium thermophilum* were found to be most active at neutral and alkaline pH and at a temperature range of 37-45 °C. Highest protease activity was observed at early exponential phase where endoglucanase activity was low. The production of proteases and endoglucanases on different days suggested a possible negative effect of proteases on endoglucanase activity. The effect of protease inhibitors were examined to determine the type of proteases. Those were pCMB, antipain, PMSF, E-64, EDTA and pepstatin A. Antipain, pCMB and PMSF were observed to be effective on proteases produced by the fungus. Two major protease inhibition peaks at day 3 and 5 suggest production of thiol-containing serine protease and serine protease, respectively. The presence of protease inhibitors in culture medium or cell-free supernatants failed to show a significant effect on endoglucanase activity and the ability of endoglucanases to adsorb onto Avicel.

1117 - Development of an *Aspergillus sojae* expression system

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Aspergillus species are known as high-level expression hosts for the production of enzymes or metabolites. In the last two decades for the industrially used species for *Aspergillus niger*, *A. foetidus*, *A. tubigensis*, and *A. oryzae* expression systems have been developed. We describe the development of an expression system based on the koji mold *Aspergillus sojae* for the production of homologous and heterologous proteins. Transformation based on auxotrophic (*pyrG*, *niaD*) and/or dominant markers (*amdS*), was used to introduce the genes of interest. To improve the yield of produced protein, *Aspergillus sojae* mutants with lower protease activity, either by gene disruption or by UV mutagenesis, were isolated. To improve the fermentation yields of *Aspergillus sojae* also morphological and so-called fermentor adapted mutants with lower viscosity, were isolated. As one of the examples for heterologous protein production, the production of human interleukin 6 was used. *References*: Heerikhuisen, M. et al. (2001) World Patent Application WO 01/09352.

1118 - Isolation, structure elucidation and biological activities of novel secondary metabolites from *Pochonia chlamydosporia* (Goddard) Zare & W. Gams

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Extracts from the fungus strain P0297 showed strong activities in two concurrent screenings of natural extracts for new antiviral compounds active against Herpes Simplex Virus 1 (HSV1) and for novel antiparasitic agents active against the protozoan pest *Eimeria tenella*, respectively. Strain P0297 was identified as *Pochonia chlamydosporia* var. *catenulata sensu* Zare et al. [Nova Hedwigia 73 (1-2) 51-86, 2001] by morphological studies and comparison of DNA sequences with data from type strains. Fermentation of the fungus in 10 litre scale and bioassay-guided preparative HPLC of the crude extracts yielded several biologically active metabolites, which were identified by NMR spectroscopy and mass spectrometry. Besides Monorden (Radicicol), several structurally related resorcylic acid lactones, for which the trivial names Pochonins are proposed, were obtained as congeners. In addition, the *spiro*-alkaloid Pseurotin A was isolated. Upon modification of fermentation conditions, a drastic shift in the secondary metabolite profiles of strain P0297 occurred when bromide salts were added to the medium. Interestingly, the fungus now produced the monocillins, which constitute non-halogenated analogues of monorden and pochonins. All compounds were studied on their antiviral and anticoccidial properties in cellular replication assays. Furthermore, their activities against estrogenic receptors were evaluated. Their isolation, structure elucidation and biological activities are discussed.

1119 - Heterologous expression of *Phanerochaete* manganese peroxidases in *Pleurotus ostreatus*

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Manganese peroxidases catalyze oxidation of Mn(II) to Mn(III) in the presence of hydrogen peroxide and form a class of extracellular peroxidases of white rot basidiomycetes. The enzymes have been focus of research interests because of their importance in lignin biodegradation and potential for utilization in many industrial processes. Genomic and cDNA sequences encoding manganese peroxidase have been cloned and characterized from variety of white rot basidiomycetes, including *Ceriporiopsis subvermispora*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Pleurotus eringii*. However, overexpression of active ligninolytic peroxidases is difficult with non-basidiomycetous host systems such as Escherichia coli, *Saccharomyces cerevisiae* and *Aspergillus* spp. We have developed a recombinant gene expression system in *P. ostreatus*, using promoter and terminator sequences of sdi1 which encodes iron-sulfur subunit of succinate dehydrogenase. The system was successfully used to overexpress one of its manganese peroxidase genes, mnp3 under the control of homologous sdi1 expression signals. Here we report heterologous expression of wild-type and artificially mutagenized manganese peroxidases from *P. chrysosporium* using the gene expression system in *P. ostreatus*.

1120 - Antifreeze proteins from snow mold fungi

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Many living organisms have biochemical strategies to protect themselves against freezing. Antifreeze protein (AFP) are one of biochemical defense mechanisms by which intracellualr ice formation is inhibited. *Typhula ishikariensis*, one of snow molds secreted a 22kDa protein that only accumulated in culture at a subzero temperature. Reported fish AFPs bound to prism faces of ice crystals that were formed hexagonal bipyramid. The short axes (a-axes) of ice crystals of fish AFPs were limited by the size of the initial ice. However, fungal AFP-bound ice could grow not only in the c-axis direction but also in the a-axis direction. Therefore, fungal AFP formed ice that was 10-fold larger than that of fish AFP-bound ice and became a distorted hexagonal bipyramid shape resembling Stone Age knives. Fish AFPs can cover all ice-growth sites of prism faces and those AFP-bound ice crystals can grow only in the direction of basal faces (c-axis). Our results showed that fungal AFP-bound ice could grow not only in the c-axis direction but also in the a-axis direction. Therefore, it is assumed fungal AFP-bound ice has extra space for crystal growth in prism faces. It is thought that the number of binding fungal AFPs per area of prism face is less than those of fish or plant AFPs. However, the highest measurable value of antifreeze activity of fungal AFPs is higher than average values reported for fish AFPs. It is therefore thought that fungal AFPs inhibit ice growth by another mechanism.

1121 - Repression of *chsB* expression reveals the functional importance of class IV chitin synthase gene *chsD* in *Aspergillus nidulans*

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Chitin is one of the major constituents of the cell wall of *Aspergillus nidulans*. To date, five chitin synthase genes (*chsA-D*, and *csmA*) in this fungus have been isolated and characterized in our laboratory. We have reported previously that a *chsB* single disruptant exhibits severe growth defects, whereas *chsA*, *chsC*, and *chsD* single mutants do not show any obvious growth defects. Here, we constructed conditional *chsB* mutants in which *chsB* expression was controlled under the repressible *alcA* promoter (*alcA*(p)-*chsB*). Under repressing condition, the mutant exhibited similar phenotypes to those of the *chsB* disruptant. To examine the functional relationships of other chitin synthase genes with *chsB*, we constructed three types of double mutants in which the *chsA*, *chsC*, or *chsD* deletion mutation was combined with *alcA*(p)-*chsB*. The *chsD chsB* double mutants grew more slowly than the *chsB* single mutant under high osmolarity conditions. The hyphae of the double mutants appeared to be more disorganized than those of the *chsB* single mutant. These results indicate the importance of the ChsD function in the absence of *chsB* expression. The *chsA chsB* and *chsC chsB* double mutants also showed different phenotypes from the *chsB* single mutant under *alcA*(p)-repressing conditions; the *chsA chsB* double mutant produced less aerial hyphae, and the *chsC chsB* double mutant showed reduced cell mass. These observations suggest that *chsA* and *chsC* each play some different roles in hyphal morphogenesis.

1122 - Effect of the wood degrading basidiomycete *Coniophora puteana* on the chemical and physical properties of pinewood

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Coniophora puteana is a wood destroying basidiomycete, widespread both in nature and construction wood. The fungus produces a brown-rot in soft- and hardwood, primarily degrading its polysaccharides, while lignin is only modified. The fungus is often encountered in the buildings in Latvia, causing considerable damage to wood in service. The aim of this study was to investigate the chemical, physical and ultrastructural changes in the pinewood degraded by *C. puteana* (Schum.:Fr.) Karst. (BAM Ebw.15) isolate. For this, the Scots pine sapwood blocks were subjected to the attack of fungus for 4 months in laboratory conditions. Decayed wood was analyzed by ion exchange chromatography, water vapor sorption and scanning electron microscopy (SEM) methods. At the wood weight loss of 40%, the losses of hemicelluloses forming monosaccharides mannose and galactose were 78% and 56%, while xylose and arabinose were lost by 47% and 39%, respectively. The losses of cellulose forming monosaccharide, glucose, comprised 32%. Based on the water sorption isotherms, value of the accessible specific surface A, and constant am, characterizing the mass hydrophilicity, were determined. Correlation between the value A and wood weight losses was obtained. The hydrophilicity am tended to decrease as the share of lignin in decayed wood increased. SEM micrographs illustrated the pattern of thinning and cracking of the wood cell wall, confirming the considerable loss of wood carbohydrates.

1123 - Solubilization of metal phosphates by fungi as a consequence of carbon translocation

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The spatial distribution of nutrients in most soils is heterogeneous due to factors that include soil type and structure. Pollutants, such as toxic metal-contaminated wastes, and soil amendments such as fertilizers, also increase the heterogeneity of the edaphic environment. The ability of fungi to solubilize insoluble metal phosphates, by the production of organic acids, is important for both plant and microbial nutrition. Whilst filamentous fungi are well adapted for growth in heterogeneous conditions, experimental information in relation to the functional consequences of such phenomena is sparse. A model system consisting of concentric rings of discrete agar droplets was developed which allowed study of fungal growth *in vitro* in nutritionally-heterogeneous conditions. Droplets containing different combinations of glucose and calcium phosphate were used to study the consequences of spatially separating these components in relation to metal phosphate solubilization by *Rhizoctonia solani*. In the presence of the fungus, solubilization of calcium phosphate only occurred when glucose was present in the underlying medium. However, solubilization occurred in droplets containing calcium phosphate, but no glucose, when glucose was present in other droplets within the tessellation and where fungal hyphae spanned the droplets. This demonstrates that substrate was transported via mycelia from glucose-containing domains, with the functional consequence of metal phosphate solubilization.

1124 - Modulation of phospholipid metabolism in cAMP-dependent protein kinase mutants of *Aspergillus niger*

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Cyclic AMP-dependent protein kinase (PKA) plays a crucial role in the regulation of metabolic pathways by means of enzyme phosphorylation and therefore in changes of the activities of key cellular enzymes. Genes encoding PKA catalytic and regulatory subunits in *Aspergillus niger* were cloned, characterized, overexpressed and/or deleted. The correlation between cAMP-dependent protein kinase activity and phospholipid biosynthesis in wild type *A. niger* strain and strains with overexpressed and deleted genes for regulatory *pka*R and/or catalytic *pka*C subunit was followed. Isolated mutants displayed different lipid, especially phospholipid metabolism. In mutant strains with abolished PKA activity amount of total lipids was 1.6 times higher, with much more neutral lipids than in other mutant strains. Relative as well as absolute amounts of phospholipids and glycolipids were 1.3 and 4.9 times higher in mutants with regulatory subunit deleted than in the control strain. Differences in individual phospholipids were most distinctive. Mutant strains with increased *pka*C and disrupted *pka*R genes showed several times lower amount of phosphatidylcholine with concomitant increase in phosphatidylethanolamine and lysophosphatidylethanolamine, as well as differences in phospatidylinositol in relation to other followed strains. Therefore consequences of mutations of PKA on phospholipid synthesis could be interpreted through cAMP signaling.

1125 - Copper ions might have a significant role in redirecting metabolic fluxes in *Aspergillus niger*

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In commercially important micro-organisms, such as *Aspergillus niger*, lipid synthesis was often regarded as an unwanted process since it diverts metabolic fluxes from final bio-product synthesis - citric acid. The presence of copper ions was found to induce the citric acid overflow, while concomitantly lower levels of total lipids were detected in the cells. Its effect was more obvious in the medium with magnesium ions as a sole divalent metal ions, while in the substrate with magnesium and manganese the addition of copper had less pronounced effect. Since the malic enzyme was recognised as a supplier of reducing power in the form of NADPH for lipid bio-synthesis, its kinetic parameters in regard to different concentrations of copper ions were investigated and a strong competitive inhibition of enzyme by Cu2+ ions proposed. It seemed that copper ions compete with Mg2+ and Mn2+ ions for the same binding site on the protein, since the deactivation of malic enzyme after the addition of metal ions was a time dependent process. Sesamol, another substance reported to reduce the lipid content in some fungi, had no significant effect in *A. niger* cells. The data illustrated that copper ions were capable of inhibiting the *A. niger* malic enzyme, which might lead to redirection of metabolic fluxes from lipid synthesis toward citric acid overflow.

1126 - Ammonium assimilating GS/GOGAT genes of the ectomycorrhizal basidiomycete *Suillus bovinus*

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Nitrogen is a major plant growth limiting nutrient in boreal forests where the bulk of soil nitrogen is bound as organic complexes inaccessible to uptake by plants. Root symbiotic ectomycorrhizal fungi are able to mineralize organic soil nitrogen to ammonium and subsequently translocate nitrogen as amino acids to mycorrhizal roots. Glutamine synthetase (GS) and gluamate synthase (GOGAT) are key enzymes responsible for ammonium incorporation into host plant and fungal cellular amino acid pools. Genomic and cDNA libraries were constructed for the ectomycorrhizal basidiomycete *Suillus bovinus*, which is commonly found in Scots pine forest soils. Full genomic GS and GOGAT sequences, several GS cDNAs and one GOGAT cDNA were isolated and sequenced. The complete GOGAT cDNA sequence was determined using RT-PCR. Transcription initiation sites were identified using primer extension and confirmed by utilising 5'-RACE methods. Fungal transcription levels in the Scots pine mycorrhizosphere were assessed by Northern analysis. Although present as single copy genes, the amount of GS transcripts was much higher than that of GOGAT. The majority of GS transcripts seemed to be non-translatable, due to loss of mRNA 5'-ends, making the actual amounts of functional GS and GOGAT transcripts comparable. Total fungal GS transcript levels seem to be high throughout the mycorrhizosphere.

1127 - Surface structure and composition of *Aspergillus nidulans* walls

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Wildtype *Aspergillus nidulans* has highly polarized growth producing narrow tubular hyphae, but at 42 °C the conditional non-lethal *hypA1* mutation leads to non-polarized growth producing wide, tapered cells. TEM shows that 42 °C *hypA1* tip walls are four-fold thicker than those produced at 28 °C, with wall thickness increasing subapically. Subapical walls of wildtype hyphae are inextensible, however, the 42 °C; *hypA1* growth pattern implies they can remain plastic, consistent with Bartnicki-Garcia's model that wall softening is an integral part of deposition. Can differences between 28 °C and 42 °C walls of *hypA1* strains be detected at the surface? *hypA1* colonies were prepared for cryo-SEM after growth at 28 °C, 42 °C or a shift from 42 °C to 28 °C. Surprisingly, the wall surface was similar for all growth regimes: a finely pebbled texture, and no evidence of stretching or insertion of material even at tips. Atomic force microscopy (AFM) can create a detailed surface image, and in some modes can also provide chemical information, potentially monitoring changes as a hypha grows past a fixed point. Here we show that AFM images reveal the same wall textures in fixed chemically *hypA1* cells as are seen with cryo-SEM. Nevertheless, despite the similarity between these surface images, infrared spectroscopy of wildtype hyphae showed that tips were relatively enriched for protein compared to subapical regions, which were enriched for carbohydrate.

1128 - Nuclear NAD metabolism in fungi and yeast

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Activity of NAD metabolism enzymes in nuclei at stimulation of NAD production by *F. sambucinum* and *S. cerevisiae* selected earlier as NAD-producers has been studied. Cultivation of these fungi and yeast in the medium containing 1 mM nicotinamide was accompanied by up to 3-fold increasing of intracellular NAD concentration in steady state growth phase. The increasing of intracellular NAD concentration accompanied by falling of its intranuclear pool caused by acceleration of coenzyme turnover due by growing up of nuclear NAD-pyrophosphorylase and NAD-ase activites. Also we found that the decreasing of nuclear concentration of NAD during stimulation of its synthesis in cells by nicotinamide correlates with its utilization at ADP-riboslylation of nuclear proteins. It was detected that increasing of basal and total ADP-ribosyl transferase activity attendant with appropriate changes in the level of modification of nuclear proreins as determined by the incorporation of ^{14}C -adenine from NAD into fungi and yeast nuclei. Moreover, as it was established by fractionation of nuclear proteins, although 80% radiolabel is localized in the fraction of histones, the NAD content fluctuations mostly influences on the level of ADP-ribosylation of nonhistone proteins. The conclusion was made that ADP-rybosylation of nuclear proteins might be one of the possible mechanisms of NAD oversynthesis regulation in fungi and yeast.

1129 - Alkaline-tolerant fungi from Thailand: a source for alkaline enzymes?

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A collection of 490 isolates of alkaline-tolerant fungi was established using PDA buffered at pH11. This was from sand, soil, tree-holes and assorted plant material collected from southern, central and northern Thailand. If possible the sample pH was recorded and was between pH4 and 9. Although alkaline habitats were good sources of alkaline-tolerant fungi it was significant that alkaline-tolerant fungi could be isolated from samples that were pH4. Strains were screened for their ability to hydrolyze arabinan, amylose, potato-galactan and skimmed milk at pH10. Eleven genera had activity against at least one of these substrates. Of these 11, 7 were members of the Hypocreales. Furthermore, of 57 positives, 51 belonged to the Hypocreales and 38 were *Acremonium*/*Stilbella* spp. *Acremonium* was therefore selected for further study. Alkaline enzyme production was tested using 9 substrates. Four different groups of *Acremonium* were separated based on their enzyme production profiles. From these, isolate WK276 (neutral rock-hole, south of Thailand) was selected for further study. Of the nine enzymes, the α-amylase properties of isolate WK276 were characterised. The N-terminal of WK276 α-amylase was studied for developing a specific primer. This was used to determine the complete sequence coding for the α-amylase.

1130 - A time course study of functional and structural differences between two *Glomus mosseae* isolates

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A time course study was conducted with symbioses between *Cucumis sativus* and two *Glomus mosseae* isolates BEG161 and BEG29 previously shown to differ in hyphal growth pattern. Four consecutive harvests were employed to investigate plant growth and P uptake through time as well as the development of intra- and extraradical mycelium of the two fungi. Intraradical fungal structures were visualised by light microscopy and laser scanning confocal microscopy of NBT- and acid fuchsin-stained sections. Mycorrhizal cucumber plants grew better and reached higher P contents than non-mycorrhizal plants within 29 days. However, the magnitude of the mycorrhizal growth response depended on the fungal isolate. The rate of intraradical development differed between the two isolates, but reached the same final level. The most rapid colonisation occurred in the time intervals 14-23 days and 23-29 days for BEG161 and BEG29, respectively. The structure of the intraradical mycelium was similar, but arbuscular branching patterns generally seemed very variable. Finally, the present data show that cross walls, as reported by Dickson & Smith (2001), are also formed in arbuscular trunk hyphae of *G. mosseae* colonising *C. sativus*.

1131 - The pecularieties of laccases biosynthesis by co-cultivated white-rot fungi *Cerrena maxima* and *Coriolus hirsutus*

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Laccase (EC 1.10.3.2) belongs to a group of enzymes called blue copper oxidases which uses oxygen as electron acceptor to remove hydrogen from phenolic hydroxyl groups. This reaction leads to the formation of free radicals that can undergo rearrangements leading to alkyl-aryl cleavage, oxidation of benzyl alcohols, and cleavage of side chains and aromatic rings. Presently of great interest is the examination of laccase in relation to the possibility of enzyme applications in biotechnology in particular for pulp biobleaching. The main limitation of laccase-mediator system application is very high dosage of laccase and redox mediator needed for pulp beableaching or detoxification processes. In order to overcome this factor it is necessary to investigate the mechanism of lignin degradation, the interaction between lignolytic enzymes and especially their synergism during lignin degradation. The two strains *Cerrena maxima* and *Coriolus hirsutus* producing high yield of extracellular Mn-peroxidase and laccase under co-cultivation have been found. It was shown that both *C. maxima* and *C. hirsutus* produced three isoenzymes pI 3.53, 3.91, 4.25 and 3.6, 4.0, 4.5, respectively. However, during co-cultivation of these strains, only one laccase isoenzyme pI 4.0 has been produced. The physico-chemical properties and N-terminal amino acid sequence of this isoenzyme have been studied in comparison with isoenymes produced by pure fungal cultures of *C. maxima* and *C. hirsutus*.

1132 - Aquatic hyphomycetes living under heavy metal stress

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In a former copper shale mining district in Central Germany, weathering of slag heaps and dumps resulted in groundwater, lakes and streams with extremely high heavy metal and metalloid concentrations. Communities of aquatic hyphomycetes (AQH) in such streams are clearly impoverished but still surprisingly diverse, and leaf decomposition and conditioning proceeds even [1, 2]. Crystalline components form a crust over the developing fungal biofilms. Two strains of *Heliscus lugdunensis* isolated from a harsh and a moderately polluted habitat respectively, had clearly adapted to these conditions. Conidia of both strains are significantly distinct in shape and size. Their capacity for biosorption and accumulation of Cd(II) and Cu(II) in liquide culture was quite different. Under Cd stress both strains showed specific response in their production and turnover of glutathione (GSH) and sulfur-rich peptides. Both strains synthesized significantly more GSH in the presence of Cd ranging from 25 µM to 100 µM, but quantitiy and time course were different. It seems likely that resistant AQH have undergone considerable physiological and genetic changes. [1] KRAUSS, G. et al. (2001) Aquatic hyphomycetes occur in hyperpolluted waters in Central Germany. Nova Hedwigia 72: 419-428 [2] SRIDHAR, K.R. et al. (2001) Decomposition of alder leaves in two heavy metal polluted streams in Central Germany. Aquatic Microbial Ecology, 26: 73-80.

1133 - Diversity of genome size in zygotic meiosis of *Pleurotus* studied by flow cytometry

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For quantitative evaluation of nuclear DNA and protein contents of spores, the flow cytometer (PAS) with staining DAPI SR101 was employed. The spore print of *P. ostreatus* (TAA 142824) is applicable as a standard (24Mb). The spore nuclei of *P. ostreatus* are unreplicated (Kullman, 2000). Two spore prints collected from wild (PV and PU) fruitbodes and one from a commercial (PM) fruitbody were studied. Two subpopulations were discovered in the spore print of PM and in PU and one in PV. The diversity of a spore print reflect the fate of hybrid genomes during meiosis. The spores of PM are divided in to two equally represented groups. One of them has the smallest DNA and protein content per spore compared with wild mushrooms. At the same time, these characters, especially protein content, are more variable. It can be suggested that in meiosis two hybrid genomes be divided. They differ 1.5 times in DNA content and 4.1 times in protein content, which corresponds to the loss of many chromosomes. Different chromosome numbers and genome sizes reported by several authors for one and the same species may also refer to the existence of a true aneuploidy indicating the plasticity of the fungal genome. Meiosis can occur even in the case of low density of homology between chromosomes (CLP and aneuploidy) and may ensure distribution of highly different strains. If in the zygote two nuclei are only a little conjugated than their mitotic haploidisation may results in dimorphism of spores.

1134 - Isolation and characterization of thermophilous fungi from temperate soil of north India

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Thermophilic fungi are now a well-recognised group for their temperature relationships, taxonomic characterization, industrial application and for wide occurrence. In the present study 19 species of thermophilic and thermotolerant fungi belonging to 14 genera were isolated and identified from temperate soils of varying altitudes of North India. The isolation of *Chaetomium senegalense* belonging to Ascomycetes, *Myceliophthora* fergusii and *Synnmukerjiomyces thermophile* belonging to mitosporic fungi are reported for the first time from India. The later species is a new synnematous hyphomycetous fungus. Detailed studies on importance value index (IVI) and numerical values for population number were conducted. Soil pH, moisture and altitude of their habitat were recored. In the present work, an attempt was also made to evaluate the thermostable enzymes especially, amylase, cellulase, lipase and xylanase employing *Chaetomium thermophile*, *Thermomyces lanuginosus*, *Malbranchea sulfurea* and *Torula thermophila* species. In the course of present investigation, it was further realised that the exploration of the biodiversity of thermophilic fungi has immense potential for the future.

1135 - Gene expression in a whitish truffle during the phase transition from mycelium to fruitbodies

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The transition from vegetative mycelium to the fruit body in truffles requires differentiation processes which lead to edible ascomata consisting of different cell and tissue types. The identification of genes differentially expressed during these developmental processes can contribute greatly to a better understanding of truffle morphogenesis. A cDNA library was constructed from vegetative mycelium RNAs of the white truffle Tuber borchii and 214 cDNAs were sequenced. Up to 58% of the ESTs were coding for known genes. The majority of the identified sequences represented 'housekeeping' proteins, i.e., proteins involved in gene/protein expression, cell wall formation, primary and secondary metabolism and components of signaling pathways. We screened 171 arrayed cDNAs by using cDNA probes constructed from mRNAs of vegetative mycelium and ascomata to identify fruit body-regulated genes. Comparisons of signals from vegetative mycelium and fruit body, bearing 15% or 70% mature spores, revealed significant differences in the expression levels for up to 33% of the investigated genes. The expression level of six highly regulated genes was confirmed by RNA blot analysis. Expression of glutamine synthetase, 5-aminolevulinic acid synthetase, isocitrate lyase, thioredoxin, glucan 1,3-ß-glucosidase and UDP-glucurosyl transferase were highly up-regulated suggesting that amino acid biosynthesis, glyoxylate cycle pathway and cell wall synthesis are strikingly altered during morphogenesis.

1136 - Methyl radical derived from non-enzymatic coordinated-copper system and its potential relation to the selective delignification by white-rot fungi

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White-rot fungi are able to degrade lignin more or less selectively. A non-enzymatic catalytic system consisting of a Cu-coordinating compound and hydroperoxides was proposed to play a role in the selective delignification of wood. White-rot fungi produce hydroperoxide containing a pyridine nucleus. Cu is present in wood. Hydroperoxide could be either obtained from lipid contained in resin or derived by fungi. Due to this hypothesis the radicals produced oxidatively by coordinated-Cu system could be the compound mediating the depolymerization of lignin. EPR measurement is the method of choice to elucidate the radicals produced from organic peroxides by coordinated transition metals. Methyl-, hydroxyl-, methoxyl-, and acyl radicals were generated and trapped with DEPMPO. The strongest signal was found for the methyl radical when Cu was coordinated with 4-AP. In uncoordinated reactions as well as when Fe or Mn was used, the hydroxyl radical was predominated. When these systems were applied on wood sections, fast delignification without major visual damage of the cellulose part of wood cell wall was only found with the coordinated Cu system. Faster delignification occurred on hardwood than softwood. These correlated to the preference of white-rot fungi on native lignin. Therefore, we propose that the methyl radical formation from coordinated-Cu and organic peroxide is the key step leading to selective delignification in native wood probably involve in selective delignify fungi.

1137 - Genome characterization of the ectomycorrhizal fungus *Paxillus involutus*

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The basidiomycete *Paxillus involutus* is forming ectomycorrhizal (ECM) symbiosis with a broad range of forest trees. Reassociation kinetics and genomic reconstruction analyses on nuclear DNA indicated that *P. involutus* has a haploid genome size of 21.5 Mb including 11% of repetitive DNA. Analyses of a 33-kb genomic sequence indicated the presence of twelve potential ORFs, and by extrapolation the haploid genome of *P. involutus* was estimated to contain approximately 7,700 genes. In order to obtain information on genes specifically expressed during the symbiotic interactions with forest trees, 3,555 Expressed Sequence Tags (ESTs) have been analyzed in a cDNA library constructed from an ECM formed between *P. involutus* and birch (*Betula pendula*). In parallel, cDNA libraries from saprophytically growing fungus (3,964 ESTs) and from axenic plants (2,532 ESTs), respectively, have been analyzed. By assembly of all ESTs, 2,284 unique transcripts have been identified either of fungal or plant origin. Approximately, 51-75% of the ESTs displayed significant homology to sequence information found in the GenBank (nr) protein database and these have been annotated to various functional and metabolic groups. The expression levels of the identified transcripts are examined using RNA blots and microarray analyses.

1138 - Pathogenicity tests on *Heterobasidion annosum* hybrids from a S-type/P-type crossing

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Little is known about the pathogenicity of *Heterobasidion annosum* on a molecular level. It is however crucial to develop an understanding of the genes involved in order to fully understand the infection process. Knowledge of this process would be drastically enhanced if the genes of pathogenicity could be cloned and analysed, e.g. what proteins are involved, what singal pathways are used etc. The aim of this project is to study the pathogenicity of *Heterobasidion annosum* on pine at a molecular level. This goal will be obtained by the construction of a genomic map, using AFLP markers, and defining regions of the genome which contain genes of interest for the pathogenicity. These regions will be fished out of a genomic library and transformed into Heterobasidion annosum, using Agrobacterium tumefaciens or particle bombardment technique. In this first step, a S- and a P-type of the fungus has mated in laboratory conditions. The dikaryotic hybrid has been isolated and allowed to form fruitbodies. From these, 100 single spore isolates has been isolated. The pathogenicity of these isolates were tested on pine saplings, 2-3 weeks old. The results were measured in percentage of dead pines every two days for a 25 days period. These results will be correlated to the genomic map, in order to find regions of interest common for all the highly virulent isolates and not present in those of lower virulence.

1139 - Efficient production by *Aspergillus awamori* of a Llama antibody fragment fused to a peroxidase

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The development of fusion proteins consisting of antibody fragments and enzymes is of great medical and industrial importance. Previously, our group has demonstrated that single-chain Fv antibody fragments (scFv) could be efficiently produced by *A. awamori* (Frenken et al. 1998). Recently we have studied the production of Llama variable heavy-chain antibody fragments (V_{HH}) by *A. awamori*. The advantage of V_{HH} over scFv fragments is that V_{HH}s are devoid of light chains. Furthermore, V_{HH}s lack the hydrophobic regions that are normally facing the variable domain of the light chain and are therefore suggested to be better secreted than scFv fragments. A suitable enzyme for industrial applications is *Arthromyces ramosus* peroxidase (ARP). This 41 kDa monomeric glycoprotein has a broad specificity for phenolic and anilinic hydrogen donors. In previous studies we have demonstrated that there is no heme limitation during overproduction of ARP in *A. awamori*. Under control of the endoxylanase promoter secretion of active ARP was achieved up to 0.8 g/L in shake flask cultures (Lokman et al. submitted). Fusions between enzymes and V_{HH}s permit interesting applications due to the fact that V_{HH}s can direct enzymes to the place where they should act. This study shows the production of N-terminal and C-terminal fusions of ARP with a V_{HH} fragment against the azo-dye RR6. RNA and protein analyses were performed to investigate possible limitations in production of the fusion proteins by *A. awamori*.

1140 - Water stress effects on water/turgor potentials of mycelium of *Agaricus bisporus* and relationship with polyol accumulation/translocation of nutrients

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The mechanisms by which *A. bisporus* is able to translocate water and nutrients under dynamic and changing environmental conditions is little understood. We have studied the growth of *A. bisporus* strains from wet to dry conditions and vice versa to examine the changes in internal mycelial osmotic and turgor potentials and correlated this with accumulation of sugars and sugar alcohols for the first time. These studies demonstrated that matric and solute potential had makedly different effects on this relationship. Studies using radio-labelled nutrients showed that accumulation of phospohate varied with regard to water potential, growth rate of strain and with water potential.

1141 - Effect of concentration of Tris-HCl buffer (hydroxymethyl aminomethane) on sporulation of arbuscular mycorrhizal fungi (AMF)

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Organic buffers can stimulate growth of arbuscular mycorrhizal fungi through maintenance of stable hydrogen ion concentration, and by the stimulatory capability of the molecule, being an alternative for increasing inoculum production of AMF. In order to determine the effect of concentrations of Tris-HCl buffer on sporulation of AMF, fifth spores of: *Glomus etunicatum* Becker & Gerdemann, *Gigaspora albida* Schenck & Smith, *Scutellospora heterogama* (Nicol. & Gerdemann) Walker & Sanders, and *Acaulospora longula* Spain & Schenck were inoculated, separately, in *Panicum miliaceum* L. seedlings and five replicates of each treatment were maintained for 85 days at the greenhouse (27°C ±4°C and 45 - 81% relative humidity). The pots were irrigated every other day with nutrient solution supplemented with 0, 10, 25, 50, and 75 mM de Tris-HCl and, once a week, the plants were irrigated with distilled water. Different results on spore production were related with the buffer concentration. Growth and sporulation of *S. heterogama* were not benefited by addition of buffer in the nutrient solution. Conversely, comparing with the control, 10 mM and 25 mM of Tris-HCl were enough to increase significantly the sporulation, respectively, of *G. etunicatum* and *G. albida*. For *A. longula*, production of spores was significantly stimulated when the nutrient solution was supplemented with 50 or 75 mM of buffer. The results indicate that Tris-HCl can be used for improving sporulation of some AMF.

1142 - Physiological and molecular variability in strains of a white rot fungus *Physisporinus rivulosus*

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An isolate of *Physisporinus rivulosus* has been shown to have excellent properties for the use as a pretreatment step for mechanical pulping. In this study, several strains from type culture collections and the wild strain T241i were compared for the cultural morphology, growth characteristics and ability to decolorise Poly R dye and oxidise ABTS. Fungal growth in spruce wood was studied by light and confocal laser scanning microscopy using conventional anatomical stains, chitin binding lectin Alexa Fluor 660 conjugate, lipid staining Nile red as well as ethidium bromide and DAPI. Another basidiomycete *Ceriporiopsis subvermispora* was used in comparison. Extracellular enzyme activities of lignin degrading manganese peroxidase (MnP) and laccase were recorded. Strain variation at molecular level was investigated using sequence data from MnP encoding genes. Growth habit and speed on malt agar, in and on wood blocks revealed differences among *Physisporinus* strains. Variation was noted in their ability to oxidise the dyes used in the study. All *Physisporinus* strains produced MnP, although heterogeneity was found in the MnP isoenzymes secreted during fungal growth in wood chips. The strain T241i was the only one capable to lower the pH value of the surrounding growth medium during the growth. Morphological, physiological and molecular data is pointing to the conclusion that *P. rivulosus* is heterogenous and may be in need of further systematic study.

1143 - Impact of ectomycorrhiza formation on the expression of water channel proteins (aquaporins) in fine roots of Norway spruce

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Root fungus symbiosis (mycorrhiza) improves the nutrient, and also the water supply of the host plant. Water flow across membranes is facilitated by water channel proteins, aquaporins. In order to detect a possible effect of mycorrhiza formation on root aquaporins, we used a sterile Petri dish system to obtain ectomycorrhiza between spruce (*Picea abies*) seedlings and fly agaric (*Amanita muscaria*). Two transcripts were detected with sequence homologies to plant plasma membrane aquaporins (PaPip1-1 and PaPip2-1). Translation of full length cRNA in Xenopus oocytes identified the respective proteins as functional aquaporins. Oocyte swelling studies showed that one of them, PaPip2-1, supported very high rates of water flow (Pf values about 200), up to now only known for aquaporins from motor cells of leaf pulvini. An organ-specific Northern blot analysis showed that the expression of PaPip2-1 was mainly confined to fine roots, and that the amount of the transcript was decreased to about 25% of the control upon mycorrhization. In contrast, PaPip1-1 dominated in hypocotyl tissue, facilitated only low rates of swelling in oocytes (Pf values around 25), and its expression was not affected by mycorrhization. We suggest, that PaPip2-1 is

mainly responsible for facilitating water uptake via fine roots of spruce, and that the down-regulation of gene expression is an adaptation to the improved water supply mediated by the symbiotic fungus.

1144 - Characterisation of a phosphate transporter in *Candida albicans*

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The human pathogen *Candida albicans* is a dimorphic fungus that can reversibly alter its mode of growth from yeast-like cells to mycelial cells, depending upon its environment. As the mycelial form is usually associated with candidiasis, genes involved in the control of dimorphism represent potential targets for the development of antifungal drugs. Using the differential display technique, we have identified a gene preferentially expressed in the mycelial cells. We have cloned and sequenced that gene. Sequence analysis revealed that this gene displays 70% homology with the *Saccharomyces cerevisiae* Pho89 gene, encoding a phosphate transporter. The *C. albicans CaPho89* gene was cloned into a *S. cerevisiae* expression vector and transformed into a S. cerevisiae pho89 null mutant strain. Phosphate transport in the transformants was evaluated using [^{32}P]-orthophosphate. The results obtained showed that *CaPho89* could complement the *S. cerevisiae* pho89 mutation, suggesting that *CaPho89* encodes a functionnal phosphate transporter.

1145 - Mitochondrial group-I intron in a higher basidiomycete, *Pleurotus ostreatus*, is mobile in sexual crosses

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Inter-parental recombination between mitochondrial (mt) genomes of *Pleurotus ostreatus* occurred at high frequency in sexual crosses. To reveal the process leading to this recombination, we estimated a certain recombination site in each parental mitochondrial genome contributing to the generation of the new restriction fragments which characterized a RFLP pattern of recombinant mtDNA, and determined their nucleotide sequences. It was found that the regions of recombination within parental mtDNAs possessed consensus sequences in common, which were divided by some regions of non-consensus sequences. Meanwhile, the nucleotide sequences in the new restriction fragments and their surrounding regions in recombinant mtDNA were harbored all consensus and non-consensus sequences recognized in both parental mtDNA, suggesting that this structure was caused by the integration of regions with non-consensus sequences that exist in only one parental mtDNA to the other. All non-consensus sequences were homologous to the sequences of the group I intron reported in fungi. They also included the ORF sequences that encode the LAGLIDADG motif, a characteristic of endonuclease involved in intron mobility. These results indicate that the manner of recombination for generating the new restriction fragment is elucidated by gene conversion, achieved by the mobility of the introns. This suggests that, in *P. ostreatus*, intron mobility is playing a large role for the increase of diversity of mtDNA.

1146 - Effect of carminic acid and other mediators on lignosulfonates degradation by extracellular enzymes of *Trametes versicolor*

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Many low-molecular mass compounds have been suggested as possibly mediating mobile factors during biodegradation of lignin. Degradation of lignosulfonates by ligninolytic enzymes produced by the white-rot fungus *Trametes versicolor* was studied. The 29,6 kDa fraction of Peritan Na obtained by a gel filtration method using a Sephadex G-100 column was used as a substrate. The substrate was incubated with extracellular idiophasic enzymes of *Trametes versicolor* and with mediators. HBT (1-hydroxybenzotriazole), ABTS (2,2'azinobis-(3-ethylbenzenthiazoline-6-sulfonic acid) and carminic acid were used as mediators. Following the incubation products were separated using gel filtration on a Sephadex G-50 column and capillary electrophoresis. When the mediators were added to the incubation mixture, depolymerization was more extensive than in the experiment with no mediators. In the presence of carminic acid the depolymerization was the most extensive and as it's result a fraction of about 2 kDa appeared. Similar results were observed when HBT or ABTS were used. However, products of higher molecular weight besides the 2 kDa fraction were also obtained. Experiments with the reaction mixtures supplemented with H_2O_2 were also performed. The above experiments revealed that only in the case of HBT used as a mediator H_2O_2 addition resulted in the increased depolymerization of lignosulfonates. Supported by the EC Contract ICA 2-CT-2000-10050, and by KBN grant 139/E-SPUB-M-5PR-UE/DZ 280/2000.

1147 - Comparative biochemical analysis of enzyme electrophoretic spectra from mycorrhizal roots

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Metabolic changes induced by mycorrhizal colonization can be evaluated by modifications appear in enzyme activities. We have investigated the potential use of electrophoretic analysis for detection the specific isozyme that presents differences in metabolic pathway in maize colonized roots. Staining protocols for esterases, alkaline phosphatases (ALP) and malate dehydrogenases (MDH) provided the enzymes for the mycorrhizas. We found differences between spectra of these enzymes at various time of cultivation. We used a starter inoculum of *Glomus mossae* (SI) and a current granular inoculum of *Glomus* sp (CGI). For esterases, the electrophoretic spectrum revealed 5 bands for mycorrhizal roots comparative with 3 bands of non-mycorrhizal roots using SI. After 42 days of colonization on the gel appeared 2 electrophoretic bands. For MDH the electrophoretic spectrum showed 6 bands at 12, 34 days and 2 bands at 42 days of symbiosis. We found no differences between electrophoretic spectra of SI and CGI. We used also various concentrations of inocula. The electrophoresis spectra emphasized the following: 9 bands for SI 5% and 10 bands for SI 10% comparative with 8 bands for control and 9 bands for both concentrations 5% and 10% CGI for esterases. 8 electrophoretic bands using 5% and 10% concentrations of SI and CGI, and also 8 bands at control for detection isozymes of MDH. 3 electrophoretic bands of isozymes of ALP were revealed comparative 2 bands for control.

1148 - Early molecular communication among plant and fungus: detection of specifically expressed genes during pre-symbiotic interaction

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Tuber borchii Vittad. is an ascomycetous fungus which forms ectomycorrhizae on the roots of angiosperms and gymnosperms. After the achievement of this symbiotic association, the mycelium can form the hypogeous fruitbody, commonly known as truffle. Ectomycorrhizae formation is a highly regulated process that is accompanied by molecular reorganization of both partners during symbiosis. An analogous molecular mechanism also takes place during the pre-symbiotic phase, when the partners exchange molecular signals, in order to take their stand and prepare both organisms for symbiosis instauration. We studied this latter genetic reorganization in *T. borchii* during the interaction with its symbiotic plant *Tilia americana*. For this purpose we set up a culture system where the mycelium interacts with the plant, but avoiding physical contact between the two organisms. By suppressive subtractive hybridization, we identified several specifically expressed genes. All selected clones were further analysed by Northern blot hybridization comparing transcript levels of control RNA (mycelium grown alone) and tester RNA (mycelium grown in presence of *T. americana*), both obtained from a tested in vitro ectomycorrhizae synthesis system. The same analysis was conducted on RNA of *T. borchii* extracted at different stage of its life cycle. Some differentially expressed genes are involved in nuclear rearrangement, while some others seem to be involved in extra cellular signal transduction.

1149 - Molecular karyotype of *Thraustochytrium striatum* Schneider

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The thraustochytrids comprise a group of marine protists that are important ecologically and commercially. Strains of thraustochytrids can produce commercially significant quantities of docosahexaenoic acid (DHA), a medically important long chain polyunsaturated fatty acid. Ecologically, thraustochytrids have been identified as important degradative organisms, breaking-down naturally occurring recalcitrant carbon-containing polymers, and an important food source for picoplankton. Despite the rising importance of thraustochytrids, little is known about their life cycle or their molecular biology. This study presents preliminary findings on the genome of *Thraustochytrium striatum* Schneider, a member of the marine protistan group the thraustochytrids. Pulsed field gel electrophoresis studies identified 18 presumptive chromosomes from the zoospores and thalli of *T. striatum*. The sizes of these molecules varied from 0.4 to 2 Mbp and gave a genome size of 18.77 Mbp. The chromosome locations of the D15/n-3 desaturase gene, 18S rRNA genes and thymidine kinase gene were determined. Results indicated that there were at least four copies of the desaturase gene on separate chromosomes; multiple copies of the 18S rRNA gene present on all chromosomes and two copies of the thymidine kinase gene. The unusual gene distribution and unequal distribution of chromosome bands suggests that these organisms might be processing their chromosomes.

1150 - Ecophysiological manipulation of fermentation improves viability of the biocontrol yeast *Pichia anomala*

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For effective biocontrol to be achieved it is important that cheap and economic substrates can be used to produce ecologically competent inocula. To this end we have examined and compared the production of the biocontrol yeast *P. anomala* on rich defined media (NYDB) and on molasses. Manipulation of the physiology of the yeast by modification of water stress [water activity (a_w) of 0.98 and 0.96] using different compatible solutes/sugars and NaCl markedly affected yield and quality of the cells. Endogenous water potential of cells, and sugar/sugar alcohol contents were significantly modified.In general, accumulation/synthesis of trehalose or sugar alcohols was affected by the solute used in media. For example, use of proline, glucose and sorbitol in molasses-based media resulted in accumulation of the desiccation protectant trehalose, while proline, NaCl, glucose, sorbitol and glycerol in media resulted in an accumulation/synthesis of glycerol and varying amounts of arabitol. Ecological competence of the yeast treatments was examined by plating on non-stressed (0.995 a_w) and water stressed media (0.96 a_w). Viability was significantly improved by the use of some solutes in molasses-based media. Such studies have implications for improving shelf-life and perhaps the production of ecologically stable biocontrol agents.

1151 - Identification of mating type sequences in toxigenic *Fusarium* species known as asexual fungi

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Fusarium species currently known as asexual fungi showed to possess mating type (MT) sequences similar to those of species in which sexual recombination regularly occurs. Two pairs of degenerated oligonucleotide primers based on known fungal MT sequences were designed. Each of the two conserved MT sequences, ALPHA- and HMG-boxes, could be detected by PCR in *F. camptoceras*, *F. cerealis*, *F. culmorum*, *F. poae*, *F. langsethiae*, *F. semitectum*, *F. sporotrichioides*, *F. redolens* and *F. oxysporum*. Sizes of MAT-1 and MAT-2 amplicons were 200 bp, and 260 bp long. Both MAT-1 and MAT-2 sequences were identified for all species, but *F. camptoceras* (MAT-2) and *F. langsethiae* (MAT-1) for which just one MT was detected. As the sexual behaviour of *F. avenaceum* is not clear, this fungus was also studied. No strain of *F. avenaceum* was found to harbour both MT sequences, although it has been reported as weak homothallic species. On the other hand both MAT-1 and MAT-2 sequences occurred in single strain of *F. graminearum*, a typical homothallic species. RT-PCR experiment was set up to find the transcription of MAT genes in *F. avenaceum*, *F. culmorum*, *F. poae* and *F. sporotrichioides*. By using the primers pairs, fusALPHAfor/fusALPHArev and fusHMGfor/fusHMGrev, one characteristic PCR amplicon was produced in all samples, indicating that MT genes are transcribed in all asexual *Fusarium* strains included in these experiments. Support of EU Fifth Framework (Detox fungi QLK1-CT-1999-001380).

1152 - Analysis of *CHS8* - a fourth chitin synthase isoenzyme of *Candida albicans*

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All fungi studied to date have multigene families of chitin synthase enzymes. In the dimorphic pathogen *Candida albicans*, three genes encoding chitin synthases have previously been identified. *CHS1* encodes a chitin synthase involved in primary septum formation and is essential for viability. *CHS2* is not essential for viability, but is responsible for the majority of chitin synthase activity detectable *in vitro*. *CHS3* is responsible for producing most of the chitin present in both yeast and hyphal cell walls, and deletion of the gene results in attenuation of virulence. Recently a fourth chitin synthase gene *CHS8* encoding a second class I enzyme was identified in the genome sequence. The *chs8* null mutant was more sensitive to certain agents disruptive to cell wall integrity, had a significant reduction in the *in vitro* hyphal chitin synthase activity and a slight reduction in hyphal chitin content. Microsomal preparations of *chs2/chs8* double mutant had very low chitin synthase activity but the organism had normal morphology. Class I enzymes are therefore dispensable for normal growth of this organism.

1153 - Cloning of chitin deacetylase gene from *Phycomyces blakesleeanus* and its expression in *Escherichia coli*

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Chitosan is a biopolymer with unique properties favorable for a broad variety of industrial, biomedical, and agricultural application. The chitosan is produced from crab shell through the chemical procedure which leaves a lot of industrial waste at present. Chitin is converted to chitosan by chitin deacetylase. To develop an enzymatic process for chitosan production that reduces the waste, we have planned to isolate a clone of chitin deacetylase gene from *Phycomyces blakesleeanus* cDNA library which we made in the _ZAP vector. The 218bp-DNA fragment amplified from genomic DNA of *P. blakesleeanus* using PCR (polymerase chain reaction) primers to two highly conserved sequences within chitin deacetylase gene of *Mucor rouxii* was the probe for isolation the clone from the library. A positive cDNA clone which is composed of 1,613bp was isolated. The deduced protein encoded by this clone is composed of 459 amino acids. The protein sequence comparison revealed that the protein is significantly similar to chitin deacetylase of *M. rouxii*. After ligation of the clone to the expression vector pET32 Ek/LIC, chitin deacetylase activity was detected in the purified His-tagged protein fraction of the crude extract from a *E. coli* transformant. These results show that the chitin deacetylase gene has been successfully cloned from *P. blakesleeanus*. The cloned gene was named PbCD and the nucleotide sequence of PbCD was registered to GenBank (accession #AB046690).

1154 - Genome analyses of *Micromucor ramanniana* and *Micromucor isabellina*

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The *Micromucor* species (genus *Mortierella*) belong among the most common saprophytic soil fungi. Some members of the genus (*Mortierella* species) are potent producers of the long-chain polyunsaturated fatty acids (e.g. arachidonic acid). These compounds are important both nutritionally and pharmacologically. These fungi are biotechnologically important, but the organisation of their nuclear genome has not been described so far. Electrophoretic karyotype analysis using different approaches of pulsed field gel electrophoresis (PFGE) has led to significant progress in fungal genetics: the physical karyotypes of numerous previously genetically uncharacterised fungal species have been established. In the present study, orthogonal field alternation gel electrophoresis (OFAGE) and the contour clamped homogeneous electric field (CHEF) technique were applied to obtain preliminary information on the organisation and intrageneric variability of the nuclear genome in three *Mortierella* (*Micromucor*) strains of 2 different species (*M. isabellina* and *M. ramanniana*). Conditions for the preparation of highly-intact chromosome-size DNA molecules and for the separation of DNA molecules were established. Furthermore, through the use of homologous and heterologous gene probes (e.g. 3-hydroxy-3-methylglutatyl coenzyme A reductase) gene assignment experiments have been performed. This research was supported financially by the Hungarian Scientific Research Fund (OTKA) D29113 and T 032738.

1155 - Is the interfacial pH a measure of the efficiency of nutrient transfer in ectomycorrhizas?

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Interfacial pH-conditions in *Pinus nigra* ectomycorrhizas were determined using the pH-sensitive ratio-dye Cl-NERF and a confocal laser scanning microscope (CLSM). A pseudo-ratiometric method is commonly used for determining apoplastic pH-values. In the present study, a non-invasive ratiometric method relying on a single fluorescence dye was devised and tested. Results show homogeneous pH-values near 3 in non-mycorrhizal roots from different plants and heterogeneous interfacial pH-conditions between 3.1 and 4 in various pine ectomycorrhizas. In mycorrhizas, reduced sucrose and phosphate leads to decreasing interfacial pH-conditions, whereas external applied glucose induced an increase of the interhyphal pH-values in the mantle. Based on the results we developed a model for the interfacial transfer processes in ectomycorrhizas. Transfer processes on the ectomycorrhizal interface are not pH-regulated. The processes generate an interfacial pH-value to maintain the symbiotic balance. In balance you find optimal pH-conditions for the nutrient transfer. The lack of only one of the transfer goods induces a decrease of the interfacial pH-conditions. A decreasing pH-value in the interface of ectomycorrhizae leads to reduce transfer processes and the mutualistic balance in the symbiosis can be maintained.

1156 - Effect of ferulic acid on guaiacylglycerol-β-guaiacyl ether degradation by extracellular enzymes of *Trametes versicolor*

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Trametes versicolor, the effective lignin decomposer, produces a set of enzymes acting on bonds in lignin polymer, especially on most abundant in lignin ether β-O-4 bond. In our studies the most active enzymes are secreted into the medium in idiophasic cultures of *Trametes* as a result of carbon starvation. During experiments destaining of Remazol Brilliant Blue and/or carminic acid were used as an indirect method and degradation of guaiacylglycerol-β-guaiacyl ether as a specific method for the measurement of ligninolytic activity. Secreted enzymes were prepared from media of carbon-starved, ferulic acid supplemented cultures of *Trametes*. Capability to degradation of β-O-4 bond was measured in reaction mixtures containing enzymes, the dimer substrate, hydrogen peroxide and ferulic or p-coumaric acid. Reaction products were analysed by capillary electrophoresis. Presence either ferulic or p-coumaric acid in reaction mixture resulted in significant decrease of dimer degradation. Both hydroxycinnamates are parts of lignocellulosic polymer, forming bridges between hemicelluloses and lignin, and both are known as natural antioxidants. When released by relevant esterases during lignocellulosics degradation, they stimulates production of ligninolytic enzymes and simultaneosly diminish enzymatic activities splitting the ether bond β-O-4. This paradoxical effect deserves further reasearch. Supported by the EC Contract ICA 2-CT-2000-10050 and by KBN grant 139/E-SPUB-M-5PR-UE/DZ 280/2000.

1157 - Can wooddecay fungi use chitosan as a N-source at extreme C/N-ratios?

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Chitosan has been proposed as an alternative, environmentally safe wood preservative. *In vitro* tests of wood preservatives are often carried out on media with moderate C/N-ratios in contrast to the high C/N-ratio in wood. Chitosan contains nitrogen and fungi could therefore be suspected to use this as an N-source under extreme C/N-ratios. In this study, we investigated the relationship between the inhibiting effect of chitosan and the C/N-ratio of the media. The fungi used were *Trametes versicolor*, *Heterobasidion annosum*, *Gloeophyllum trabeum* and *Oligoporus placenta*. The experimental set-up was a factorial design with 5 concentrations of chitosan and 6 C/N-ratios in a solid caboxymethylcellulose medium. The growth was measured as colony diameter. In general, the growth of the fungi was much more affected by chitosan than by the C/N-ratio. The C/N-ratio had only limited effect on *Trametes versicolor*, *Heterobasidion annosum* and *Gloeophyllum trabeum*, whereas *Oligoporus placenta* was clearly stressed by high C/N-ratios. However *Oligoporus placenta* was also inhibited by the chitosan. The effect of chitosan was independent of the C/N-ratios, indicating that wooddecay fungi are not able to use chitosan as an alternative N-source at extreme C/N-ratios.

1158 - Environmental signals associated to the transcriptional activation of the mycoparasitism related gene prb1 in *Trichoderma atroviride*

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Trichoderma atroviride parasites a large variety of phytopathogenic fungi. This characteristic has allowed its use as a biological control agent. The production of hydrolytic enzymes appears to be a key element in the parasitic process. Among the enzymes released by *Trichoderma*, the proteinase Prb1 plays a major role. Herewith we show that the corresponding gene (prb1) is subjected to Nitrogen Catabolic Repression. Accordingly, induction of prb1 transcription by *Rhizoctonia solani* cell walls and by osmotic stress requires the release from a represed condition which is determined by nitrogen availability. Furthermore, the transcription pattern of the prb1 gene was not affected when a p38-Hog1 inhibitor was used. In contrast, a MEK1/2 inhibitor blocked prb1 transcription in response to nitrogen limitation, indicating that the pathway employed in the nitrogen response involves proteins similar to p42-p44. Fusions of the prb1 promoter with the gfp reporter gene allowed the detection of a novel regulatory element and represent the first insight into relevant sites that control prb1 expression.

1159 - Heavy metal accumulation and distribution in mycorrhizal and nonmycorrhizal roots of *Plantago lanceolata*, AAS and micro-PIXE analysis

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Mycorrhizal and nonmycorrhizal *Plantago lanceolata* were grown for six weeks under greenhouse conditions in rhizo-boxes filled with industrial waste. According to atomic absorption spectroscopy (AAS) the shoots of plants inoculated with *Glomus* spp. contained less Zn and Pb than nonmycorrhizal ones; their roots did not differ statistically. Selected nonmycorrhizal and mycorrhizal roots were studied using proton microscopy accompanied by PIXE microanalysis. Significant differences in element distribution were found between investigated roots. Especially a strong accumulation of Zn within the cortex of mycorrhizal roots was visible. The present results do not allow judgements on whether the metals are located exclusively in fungal structures, or both by the fungus and the colonised plant cells. In mycorrhizal roots more Zn and Pb were found within the vascular tissues than in nonmycorrhizal ones. Therefore, mycorrhiza is not effective as a filtering mechanism; still, its role in the transformation of heavy metals into non-toxic compounds cannot be excluded. According to AAS these compounds are retained in roots, as a decrease of these elements was observed in shoots. This could also suggest the presence of additional mechanisms depending on the plant, which enable it to cope with metals transferred by the fungus.

These findings confirm our previous observations of mycorrhizas under heavy metal stress.

1160 - Metabolism in ectomycorrhizal preinfection: Expression of malate synthase and acetylCoA acetyltransferase genes from *Laccaria bicolor*

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The ectomycorrhizas are beneficial fungal associations with temperate tree roots. Ectomycorrhizal fungi improve survivability, pathogen resistance, drought tolerance and provide mobilized mineral nutrient in exchange for fixed carbon from the plant. Molecular level analysis suggests that, in response to the perception of the plant root, there is a directional shift in fungal cell physiology as early as four hours into the interaction. During the interaction between *Laccaria bicolor* and *Pinus resinosa*, we have observed the upregulation of metabolic genes such as Lb-MS coding for a malate synthase and Lb-AAT that codes for an acetylCoA acetyltransferase. The former, one of the two unique enzymes of the glyoxylate pathway and the latter the terminal thiolase of beta oxidation of fatty acids. The expression of these two genes in conjunction with others (LB-AUT7) suggests that recycling of cell contents and breakdown of reserve food and channeling towards growth occur early in the preinfection stage of the symbiosis. Regulation of Lb-MS and Lb-AAT under interaction conditions and under alternate carbon sources have been studied. Lb-MS has been analyzed at the protein level and gene structure of Lb-AATg has been elucidated. We present our findings on these two metabolic genes and their significance in the symbiosis via the agency of the peroxisome.

1161 - Pre-infection and nutrient starvation expression profiling of interaction related ESTs from the ectomycorrhizal fungus *Laccaria bicolor*

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In this study, a 384 member macroarray was created consisting of 340 ESTs derived from mRNA of *Laccaria bicolor* during early interaction with *Pinus resinosa*. The macroarray also contained several positive and negative controls as internal standards and to confirm reproducibility and reduce experiment-to-experiment variability. This array was used to study multiple factors of interaction, including expression patterns at various time points of early interaction (<96 hours) and fungal reaction to limiting amounts of nutrients (ammonium, phosphate, and glucose) known to be fixed and transported between the symbionts. Our results from this pilot study led to a better understanding of signaling pathways and metabolic shifts occurring after host recognition during preinfection stage of symbiosis. Ammonium and phosphate starvations yield insight to fungal response in nutrient deprived soil conditions. Whereas carbon source limitation generates a model of fungal transcription when in need of a host. In all these conditions tested, there was significant increase in expression of genes that regulate transcription of other genes. These results suggest common regulatory circuits of many genes involved in various pathways during ectomycorrhizal symbiosis.

1162 - Coal ash dependency and subsequent heavy metal uptake by some selected ectomycorrhizal fungi in vitro

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Six isolates of ectomycorrhizal fungi *Laccaria fraterna* (EM-1083), *Pisolithus tinctorius* (EM-1081), *Pisolithus tinctorius* (EM-1290), *Pisolithus tinctorius* (EM-1293), *Scleroderma verucosum* (EM-1283), *Scleroderma cepa* (EM-1233), were grown on three types of coal ash moistened with MMN medium invitro. The colony diameter reflected the growth of the isolates on the coal ash. Heavy metal accumulation in the mycelia was assayed by atomic absorption spectrophotometry. Six heavy metals namely Al, Ni, Pb, Cr, Cd, Fe were selected on basis of their abundance in coal ash and toxicity potential and assayed for the present work. The heavy metal assay indicated that *Pisolithus tinctorius* (EM-1290) was the most tolerant isolate for most of the metals. Since this isolate is known to be mycorrhizal with *Eucalyptus*, it could be used for the reclamation of coal ash over burdened sites.

1163 - Effect of neem leaf extract on aflatoxin formation,glutathione S-transferase activity and microscopic morphology in *Aspergillus parasiticus*

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Aflatoxins are secondary fungal metabolites produced by toxigenic strains of *Aspergillus flavus* and *A. parasiticus*. The relationship between aflatoxin synthesis with glutathione S-transferase (GST) activity and microscopic morphology in *A. parasiticus* grown under different culture conditions has been investigated in order to find out the regulatory mechanism(s) of aflatoxin biosynthesis at cellular level. Aflatoxin formation was inhibited in *A. parasiticus* grown in the presence of various concentrations(1.56-50%, v/v) of aqueous neem leaf extract with a maximum about >90% at 50% concentration ($P<0.05$). The specific activity of cytosolic GST in aflatoxigenic *A. parasiticus* was significantly higher (i.e., 7-8 folds) than that non-toxigenic examined strains, when aflatoxin biosynthesis reached to its maximum level at the time of 96h ($P<0.05$). GST activity was inhibited as 67% in mycelia fed with 1.56% (v/v) neem leaf extract with a maximum of 80% at 6.25-12.5% (v/v) concentration. Morphological changes as attenuation of the cell wall and herniation and vacuolation of the cytoplasm were observed in *A. parasiticus* mycelia and vesicles in the presence of 50% (v/v) neem leaf extract in semithin sections (x 1000) under the light microscope. These results indicate that neem components primarily cause fungal structural damages as a result of which transportation of aflatoxins is impaired leading to irreversible inhibition in aflatoxin biosynthesis.

1164 - Study on the role of metabolic pathways in formation of aflatoxins in *Aspergillus flavus* and *Aspergillus parasiticus*

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Aflatoxins are secondary metabolites produced by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus*. Possible correlation between activities of certain enzymes with aflatoxin synthesis in toxigenic and non-toxigenic *A. flavus* group isolates has been investigated in order to find out the exact role of primary and secondary metabolic pathways in aflatoxin biosynthesis. The presence of fatty acid synthetase(FAS), isocitrate dehydrogenase(IDH) and glutathione S-transferase (GST) activities was established in the cytosolic fractions of both aflatoxigenic and non-aflatoxigenic strains of *A. flavus* and *A. parasiticus*. The age related elevation in the production of aflatoxins B1, B2, G1, and G2 was correlated with increasing in cytosolic FAS and GST activities and decreasing in IDH activity($P<0.05$). Also, against the IDH, the activity of FAS and GST in toxigenic strains was higher in sucrose low-salts medium which support aflatoxin synthesis as compared with non-supporting medium known as glucose ammonium nitrate ($P<0.05$). In toxigenic strains, unlike the IDH, the FAS and GST activities were significantly higher (4-11 folds) than that of non-toxigenic strains, when the rate of aflatoxin biosynthesis reached to its maximum level at 96h cultures ($P<0.05$). These results show that secondary metabolism i.e., aflatoxin biosynthesis is induced in fungal mycelia immediately after the cease of primary metabolic pathways.

1165 - *Xanthoria elegans* (Link) Th. Fr. - Photosynthetic features of a lichen growing on an alpine/nival habitat

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Xanthoria elegans (Link) Th. Fr is a common lichen in the Austrian Alps. On the summit of the Hoher Sonnblick at a height of 3100 m above sea level, this lichen grows on nutrient rich acid rocks as well as on man-made habitats. Experiments were conducted in the laboratory under controlled conditions to measure CO_2 gas exchange of the lichen at optimal thallus water content and various combinations of PPFD (Photsynthetic photon flux density) and temperature. The results indicate that *X. elegans* occurs in an environment exposed to high PPFD. With sufficient light, this lichen was able to maintain CO_2 uptake at high and subzero temperatures. In spite of the broad temperature range of lichen activity the optimal temperature was relatively small at all PPFD which is typical for lichens from cold habitats. Measurements to determine the response of CO_2-exchange to thallus water content, light, temperature and moisture were also made on lichens growing at 3100 m during the summer (July and August 2001) over a period of 235 hours. The pattern of net photosynthesis was determined by the thallus water content with the thalli mainly being hydrated by rainfall. Net photosynthesis occurred for 35% of the total research period and respiration for 20% so that the lichen was hydrated more than half of the time. Long term measurements of lichen performance are being made using a chlorophyll fluorescence system in order to obtain a full picture of the annual activity.

1166 - Molecular cloning and electrophysiological characterisation of a K+ channel from *Neurospora crassa*

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In contrast to animal and plant cells, very little is known of ion channel function in fungal physiology. Indeed, no ion channels have been cloned from filamentous fungi and only a few recordings of ion channel activity have been made from hyphal cells. In an attempt to gain an insight into the role of ion channels in fungal hyphal physiology, NcTOKA was cloned from the filamentous fungus, *Neurospora crassa*. Hydropathy analysis showed NcTOKA to be a member of the two-pore domain K+ channel family. The patch clamp technique was used to investigate the biophysical properties of NcTOKA after heterologously expression of NcTOKA in yeast. NcTOKA mediated mainly time-dependent outward whole cell currents which represented K+ efflux. However, expression of NcTOKA was able to overcome the K+ auxotrophy of a yeast mutant missing the K+ uptake transporters, TRK1 and TRK2, suggesting that NcTOKA mediated K+ influx. Consistent with this, close inspection of NcTOKA-mediated currents revealed small inward K+ currents at potentials negative of EK. NcTOKA single channel activity was characterised by rapid flicking between the open and closed states with a unitary conductance of 14 pS. NcTOKA was effectively blocked by extracellular Ca2+, verapamil, quinine and TEA but insensitive to Cs+, 4-aminopyridine and glibenclamide. The physiological significance of NcTOKA is discussed in the context of its biophysical properties.

1167 - Identification of fungal enzymes in faecal droplets from leaf-cutter ants

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The enzymatic capability of the symbiotic fungus is crucial for the ability of leaf-cutter ants to use fresh leaves as a substrate. The ants place their faeces as droplets on the plant material before it is incorporated into the fungus-garden, and these faecal droplets contain enzymes of which some are of fungal origin. The aim of the present study was to identify specific fungal enzymes in faecal droplets from the leaf-cutter ants *Acromyrmex echinatior* and *Atta colombica* to clarify the role of these enzymes in degradation of leaves in the fungus-garden. CMCases, laccase, proteases and pectinases were identified in faecal droplets by isoelectric focusing and staining in overlays. The amount of fungal pectin esterase in faecal droplets was comparable to that in pure mycelium indicating that the enzymes pass the ant-gut without loosing activity. The importance of the fungal enzymes for the evolutionary transition of the ants from feeding on dead organic material to be able to exploit fresh leaves is discussed.

1168 - ABC transporters in *Trichoderma harzianum*

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ATP-binding cassette (ABC) transporters are membrane glycoproteins that utilize the energy derived from the hydrolysis of ATP to drive transport of endogenous metabolites and toxic xenobiotics through biological membranes. Overexpression of some ABC's determines simultaneous resistance to many chemically unrelated compounds whereas disruption of genes encoding ABC transporters is associated with increased sensitivity to the same compounds. We hypothesize that ABC transporters of *Trichoderma harzianum* have an important role in a number of processes such as resistance to environmental toxicants produced either by soil microflora or introduced by human activity (e.g. fungicides, heavy metal pollutants), secretion of factors (mycotoxins and cell wall degrading enzymes) necessary for the establishment of a compatible interaction with a host fungus. Several oligonucleotides were designed from conserved regions of the sequences of many ABC transporters from different filamentous fungi, and used to clone and partially sequence several genes encoding putative ABC transporters from genomic DNA of *Trichoderma harzianum* P1. Functional analysis to investigate their role during mycoparasitic events are in progress. We are using gene disruption and overexpression in *Trichoderma* to determine the involvement of these transporters in biocontrol and fitness.

1169 - Influences of temperature and light on morphological changes during fruit body formation in *Flammulina velutipes*

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Influences of light and temperature on morphological changes during fruit body formation in *Flammulina velutipes* were investigated. Fruit bodies of *F. velutipes* were induced after temperature reduction in complete darkness. However, fruit bodies formed in complete darkness had a long stipe with undeveloped pileus on the top (pinhead fruit body), thinner and whiter than normal fruit bodies formed under light illumination. This suggests that *F. velutipes* can be induced fruit bodies, but cannot mature in complete darkness. We subsequently irradiated light to pinhead fruit bodies formed in complete darkness. It is revealed that pinhead fruit bodies formed in complete darkness can be induced pileus after light stimulation. We could observe hymenium formation immediately after light stimulation. We also observed thickening and pigmentation after light stimulation, suggesting that light can directly induce fruit body maturation in *F. velutipes*. Protein expression patterns were investigated by two-dimensional electrophoresis, and 22 protein spots were newly expressed in the mycelia and fruit bodies after temperature reduction in complete darkness. We also revealed proteins spots that were newly expressed after light stimulation and localized in pileus. These suggests that many of proteins involved in fruit body induction in *F. velutipes* are expressed after temperature reduction, and involved in pileus induction are expressed after light stimulation.

1170 - Ultrastructure and biochemistry of hyphae of different zone of growth in a fruiting colony of *Pleurotus pulmonarius*

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In a fungus colony, hyphae from the peripheral zone (i.e. young hyphae) are responsible for colony expansion. On the other hand, hyphae from the central zone (i.e. mature hyphae) may differentiate into reproductive structures. It has previously been observed that when *P. pulmonarius* cultures were stained by using conventional histological stains the peripheral zone of the colony (PZC) and the fruiting structures were stained, but the central zone of the colony (CZC) was unstained. In this research, the ultrastructure and biochemistry of hyphae from the PZC and CZC of a fruiting colony of *P. pulmonarius*, developed on potato dextrose agar were studied. The wall thickness of hyphae from the CZC was approx. twice that of hyphae from the PZC (0.15 and 0.07 µm, respectively). Hyphae from the PZC had about twice glycogen content and 50% higher protein content (501 and 18 mg/g dry biomass (dX), respectively) than those from the CZC (237 and 11 mg/g dX, respectively). The cell wall of hyphae from the CZC had about twice soluble and insoluble glucans content (7.7 and 6.4 mg/g dX, respectively) that of hyphae from the PZC (3.8 and 3.4 mg/g dX, respectively). The histological differences between young and mature hyphae of a colony of *P. pulmonarius*, particularly in the cell wall and the amount of cytoplasmic material present in the hyphae, suggest that the wall is used as a temporary nutrient reserve during development of *Pleurotus*.

1171 - Stability of some commercial mushrooms in Thailand after continuous subcultures of mycelia for production

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The decrease in fruiting body yields commonly occurs in the mushroom production in Thailand when mycelia obtained from continuous subcultures are used. The stability of commercial mushrooms: *Auricularia auricula*, *Agrocybe cylindracea*, *Lentinula edodes*, *Lentinus polychrous*, *L. squarrossulus*, *Pleurotus ostreatus*, *Pleurotus* species under names *P. cystidiosus* and *P. sajor-caju*, and *Tricholoma crassum*, after continuous subcultures of mycelia for production twenty times was investigated. Mycelial growth rates and fruiting body yields were determined. The PCR-RFLP technique was also tried to be employed for the preliminary detection of fungal genetic stability. After the twentieth subculture, growth rates and fruiting body yields of all mushrooms were found to be rather consistent except *Auricularia auricula* giving the decrease in both its growth and yield after the third subculture then reversing to be stable through the subsequent subcultures. For the detection of genetic stability, all fungal species gave consistent DNA patterns (600-800bp PCR products from ITS4 and ITS5 primers, and the unique RFLP pattern of each species when the DNA was digested with either AluI, TaqI, MboI, or HinfI) except *A. auricula* giving the different pattern of HinfI digest after the third subculture which was corresponding to its growth and yield reduction. The genetic stability of mushrooms should be examined after continuous subcultures of mycelia prior to application in the large scale production.

1172 - Long-term exposure of lichens to high atmospheric CO_2 under controlled conditions: effects on morphology and chemistry

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The lichens *Usnea strigosa* and *Parmotrema hypotropum* were collected at the FACE (Free Air CO_2 Enrichment) site in operation near Duke University. FACE technology is able to monitor and maintain open-air sites with atmospheric CO_2 levels kept either at ambient (^500 ppm) or enriched (^700 ppm) values. Flora is grown under natural conditions realistically simulating future concentrations of atmospheric CO_2. This setup, in operation for 15 years, is ideal for monitoring long-term effects of CO_2 enrichment throughout the lichens' lifespan, with a degree of control not possible in earlier studies. Both species of lichens were collected almost exclusively from the bark of Sweetgum (*Liquidambar styraciflua*) trees from both CO_2-enriched and control sites. Each specimen was photographed, sectioned, and observed by standard and fluorescence microscopy. The secondary compounds were extracted to near completion (^95%) and quantified (in reference to thallus dry weight) by digital analysis of Thin Layer Chromatography data. Significant differences in micro-anatomy and chemistry were found between the lichens collected in the high CO_2 and control sites.

1173 - Correlation of parasitism and gradual selectivity between lichen bionts - molecular data and culture experiments verify morphological observations

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The mutualistic interactions between lichen species range from different steps of symbiosis to parasitism. Intra- and interspecific interactions characterise the lichen species within the close association on the gravel alvar of Gotland, Sweden. Concerning these complex interactions the degree of selectivity of the lichen bionts is of great importance. For lichens only reproducing sexually the availability of the suitable photobiont is a crucial prerequisite for relichenisation. The ITS rDNA sequences of the photobionts demonstrate the diversity of the photobiontal pool within the lichen community. Different species of *Trebouxia* belonging to Clade I which is divided in two subgroups as well as species of the genus *Asterochloris* has been found. The molecular data verify and support the morphological observations of the parasitic contact between *Fulgensia bracteata* and *Toninia sedifolia*. Both lichens share the same photobiont. In culture experiments with the isolated photobionts of five lichen species in combination with the mycobiont of *F. bracteata* gradual stages of the first steps of the relichenisation process get obvious. These observations indicate to a reduced selectivity of the mycobiont of *F. bracteata* in its photobiontal choice. The mycobiont of *F. bracteata* forms a temporary aggregate with different ? maybe less - compatible bionts. This enables the mycobiont to survive until the association with a compatible photobiont is established and thallus formation is initiated.

1174 - Mating reactions, sexual morphogenesis, and trisporic acid in zygomycetes

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It is understood that mating reactions in zygomycetes are mediated by trisporic acid. But this generalization was only valid for mucoralean fungi, as no other orders had been examined in that respect. We present data on several species of the order Mortierellales covering physiological, biochemical, and molecular biological approaches. We found that in Mortierellales, too, the sexual responses are mediated by trisporoids. So in zygomycetes, the same signalling system is used not only beyond family limits but also crossing order barriers, a feature unique in fungi. Trisporoids are derivatives of beta-carotene that are synthesized in a cooperative process by the different mating types. Some trisporoids act as pheromones, others show hormone-like activities. Although specific trisporoid isoform patterns are typical for certain species, interspecific reactions occur quite frequently and we have also observed inter-order reactions. Interspecific reactions are especially interesting in parasitism. A few species, e.g. *Parasitella parasitica* are parasites to other zygomycetes in a mating type-dependent manner: interactions occur only if the partners belong to opposing mating types. The susceptibility to parasitism by *Parasitella* can therefore be used as a marker for studying the relationship between species. Using in-situ techniques, the involvement of the same key enzymes for trisporoid metabolism could be shown in sexual as well as in parasitical interactions.

1175 - Regulation of sex-pheromone biosynthesis and its role in mating and parasitic interactions of mucoralean fungi

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Successful infection of *Absidia glauca* (Mucorales, Mucoraceae) by *Parasitella parasitica* (Mucorales, Mucoraceae) requires that the partners belong to complementary mating types. These observations point towards a physiological relationship between parasitism and the sexual pathway. In all mucoralean fungi analyzed, sexual differentiation depends on the synthesis of the sex pheromone trisporic acid. Trisporic acid is synthesized via the complementary biosynthetic action of both mating types. One of the genes for an enzyme of the trisporic acid biosynthesis pathway was cloned from host and parasite. Expression studies at the transcriptional level show transcripts only after stimulation by mating and infection. DNA sequence analysis of the genomic surrounding detects a second gene in close neighborhood of the first one, with an unknown function. Nevertheless, this gene is transcribed in *P. parasitica*. Recently, vectors were constructed containing the gene for green fluorescent protein (GFP) for transformation of *A. glauca* strains. Now, also in vivo expression can be studies to obtain more insights in the interaction of parasite and host.

1176 - Screening of environmental and clinical *Cladosporium* isolates for cellobiose dehydrogenase production

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Cellobiose dehydrogenase(CDH)is an extracellular enzyme produced by wood-degrading and also other fungi. In this communication, CDH activity of 24 *Cladosporium* isolates was measured using a zymogram procedure for determining the possible role of the CDH in the fungal pathogenesis. 2,6-Dichlorophenolindophenol (DCPIP) was added into czapek agar medium containing Avicel (1% w/v) and cellobiose (20mM). The blue coloured agar plates were inoculated with fungal spores and incubated for 5 days at 25C. Production of CDH was assessed by the measuring zones of clarification in the agar around the growing colonies. This clarification was higher for 10 clinical *Cladosporium* isolates (5-6 mm) as compared with 14 environmental ones (0.5-1.4 mm)($P<0.05$). Also, clinical fungal isolates were gelatinase negative, whereas environmental isolates were gelatinase positive. With respect to this fact that higher proteolytic activity of environmental (non-pathogenic) isolates of *Cladosporium* as compared with clinical (pathogenic) ones resulted in the break down and subsequently decreasing in CDH activity, and regarding to the key role of CDH in electron transport chains reactions in fungi, it is concluded that CDH may be involved in the pathogenesis of *Cladosporium* isolates in human beings.

1177 - Molecular architecture of growing and non-growing regions of *Achlya bisexualis* cell wall

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We approached the problem of hyphal tip growth by comparing the cell wall composition of elongating and non-elongating regions of the oomycete *Achlya bisexualis*. Light microscope observations were used to determine the growth rate and to distinguish elongating and non-elongating hyphae for further EM studies, since we had found non-elongating hyphae among growing mycelia. We found that hyphal growth is a discontinuous irregular process with periods of elongation and no elongation. Both TEM and SEM were used with a variety of cytochemical labels, and several fixation techniques. Cellulose, the microfibrillar component of the *Achlya* wall, was identified with cellulase enzyme-gold affinity labeling. Elongating hyphae have cellulose in mature and subapical regions, but not at the apex. In non-elongating hyphae, cellulose was found in all the regions including the apex. These results suggest that the apices of elongating hyphae lack cellulose. This contradicts the long-standing hypothesis that the microfibrilar component is present in the elongating hyphal apex. β-(1->3)-glucan, the major matrix wall component, was immuno-localized in all regions of elongating and non-elongating hyphae. A number of cytochemical, biochemical and physiological controls were performed to assure the reliability of these findings. We suggest that in elongating regions, the matrix is synthesized first and synthesis of microfibrilar component follows. Another explanation for our results is that localized apical cellulose hydrolysis by endoglucanase creates plastic wall regions consisting mainly of β-(1-3)-glucans, which expand under turgor and/or cytoskeleton pressure. Cellulose deposition quickly follows.

1178 - *Aspergillus nidulans* polarity genes *swoA*, a protein mannosyl transferase, and *swoF*, an N-myristoyl transferase

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Hyphal growth in filamentous fungi requires first establishment and then maintenance of polarity and is necessary for disease in many fungal pathosystems. Germination of *Aspergillus nidulans* conidia involves isotropic (nonpolar) expansion of the cell through the first two nuclear divisions. Subsequent development is polar with production of a germ tube and establishment of vegetative growth. The *A. nidulans* swo mutants are temperature sensitive mutants defective in one or both stages of polar growth during the process of spore germination. *A. nidulans* swoAp is a protein mannosyl transferase, a protein responsible for the first stage of O-glycosylation. A glycosylated substrate of swoAp may be a cell wall associated protein important for polar growth. swoFp is an N-myristoyl transferase, a protein responsible for the co-translational addition of a small fatty acid to the N-terminus of its substrate thereby giving it higher affinity for association with the membrane. In *Saccharomyces cerevisiae* N-myristoyl transferase substrates are commonly signaling proteins. The temperature-sensitive allele changes a conserved aspartic acid to a tyrosine. The wild type D appears to stabilize a Beta strand bend through two hydrogen bonds and an ionic interaction. A myristoylated substrate of swoFp may be a signaling molecule important for polar growth. A proteomics approach is being taken to characterize the substrates of these proteins.

1179 - Effect of bacteria on germination and mycelial growth of arbuscular mycorrizal fungi, in vitro

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The effect of plant growth promoted rhizobacteria (PGPR) on germination and mycelial growth of *Gigaspora albida*, *Glomus etunicatum* and *Scutellospora heterogama* was studied. Aqueos suspensions of *Bacillus cereus* (C210), *Bacillus* sp. (RAB9), *B. thuringiensis* subvar. *kurstakii* (ENF10) and *Pseudomonas chlororaphis* (GN1212) were adjusted to 2 × 10^7 ufc mL^{-1} and 50 mL inoculated into Petri dishes with 8% water agar; six superficially disinfected (NaOCl) AMF spores/plate were then placed on the medium and incubated in the dark, at room temperature (28 °C). Experiments combining PGPR and AMF, with five replicates, were performed and evaluated at the 21th and 28th days. The PGPR promoted different effects on germination and mycelial growth of the AMF. Spore germination of *G. albida* was stimulated by RAB9 and GN212 and the mycelial growth stimulated by the latter, but inhibited by the other bacterial strains. Neither the incubation period nor the PGPR affected germination of *S. heterogama*. However, the mycelial growth was stimulated since the 21th day by GN1212, and later by the other bacteria. Germination of *G. etunicatum* was stimulated by the PGPR, but at the 28th day did not differ from the control, while mycelial growth was benefited by ENF10 and C210. In general, among the AMF, higher germination and mycelial formation were obtained with *G. albida*. Financial support: CAPES and CNPq.

1180 - Effects of photosynthetically active radiation (PAR) and carbohydrate source on UV-B induced parietin synthesis in *Xanthoria parietina*

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Lichen secondary compunds can be removed by rinsing in 100% acetone without affecting thalli viability. Acetone-rinsed parietin-free *Xanthoria parietina* thalli were cultivated in two growth chamber experiments. Thalli were soaked in water or carbohydrate solution once a day during both seven days experimental periods. In the first experiment a factorial design with ±UV-B (0.75 Wm-2), ±PAR (200 µmol photons m-2s-1) and 0, 2 or 5 g ribitol per liter water was used. No resynthesis of parietin took place in the abcence of UV-B. For thalli exposed to UV-B both PAR and ribitol contributed to the parietin resynthesis. Thalli exposed to PAR and the highest concentration of ribitol resynthesized more than 20% of the original content during the seven days experimental period. In the second experiment thalli were exposed to UV-B in the dark and moistened with various carbohydrate solutions. Thalli moistened with ribitol or sucrose resynthesized about 14% of their original parietin content, those moistened with mannitol or glucose resynthesized about 8%, while moistening with sorbitol or water resulted in resynthesis of 2-4% parietin only. The results are discussed in relation to the role the different carbohydrates may play in the lichen symbiosis e.g. that ribitol is the carbohydrate that the photobiont *Trebouxia* export to the fungal partner in the lichen *X. parietina*.

1181 - Eucalypt wood pretreatment with *Phanerochaete crassa* and *Peniophora lycii* decreased pitch content after laboratory kraft cooking

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During last years eucalypt wood treatment with basidiomycetes is being studied as an alternative to traditional methods for pitch control. In this way, *Phanerochaete crassa* and *Peniophora lycii*, isolated from the Uruguay forestry, have been selected by their ability to remove both sterols, which are responsible for pitch deposition, and lignin from eucalypt wood. Here we confirm the effectiveness of *Eucalyptus globulus* wood pretreatment with these two fungi to reduce the amount of free and esterified sterols from pulp and black liquors after laboratory kraft pulping, and the distribution of lignin and free sterols in the pretreated wood is analyzed. *P. lycii* and *P. crassa* decreased lignin content after two weeks of industrial chips treatment (24 and 16% respectively). An extensive alteration of lignin was observed by confocal laser scanning microscopy of wood sections. Filipin staining (a selective method to localize free sterols) showed that most of sitosterol was degraded after wood pretreatment, although some deposits remained in the lumen of parenchymatic cells. Chemical analysis after laboratory kraft cooking showed that both fungi decreased the content of free sterols (32 and 31%, respectively) and sterol esters (39 and 45%, respectively) in brown kraft pulps, and the free sterol content in black liquors (87 and 73%, respectively). The optical and papermaking properties of the bio-pulps obtained are being analyzed.

1182 - A gene cluster associated with production of insecticidal loline alkaloids in the fungal endophyte *Neotyphodium uncinatum*

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Grass plants can acquire chemical defenses against insect and vertebrate herbivores by living in symbiosis with fungal endophytes of the genus *Epichloë* (anamorphs, *Neotyphodium*). In several *Epichloë/Neotyphodium* - grass associations, insecticidal 1-aminopyrrolizidine (loline) alkaloids are produced, which hold promise as natural plant protectants. The objective of this study was to identify the genes involved in loline production. *N. uncinatum* can produce lolines under certain culture conditions, and a culture system was developed for regulated loline expression in this fungus. With this system, genes differentially expressed during loline accumulation were isolated by subtractive hybridization. Two genes isolated, *lolA* and *lolC*, were similar to genes encoding aspartate kinases and homocysteine synthase, respectively, which are enzymes in methionine biosynthesis. *lolA* and *lolC* were highly expressed during loline production in culture, and the two genes were present only in endophytes with loline-producing phenotypes. Close linkage of *lolA* and *lolC* in *N. uncinatum* was found by PCR, corroborated by data from a genomic library survey of the closely related endophyte *E. festucae*. At least five putative genes were found clustered with *lolA* and *lolC*. These putative genes have similarity to polyamine biosynthesis/interconversion genes, and genes for monooxygenases, oxidoreductases, demethylases and aminotransferases, which are frequently found in secondary-metabolite gene clusters.

1183 - Vacuolar and non-vacuolar protein degradation routes in the white-rot fungus *Phlebia radiata*: their influence on ligninolytic enzymes

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Continuous protein turnover is involved in basic cellular functions such as the modulation of the levels of proteins, adjustment to stress, as well as preferential removal of defective proteins. Eukaryotic cells contain two major systems for protein degradation: lysosomal (vacuolar) and non-lysosomal (non-vacuolar). The lysosome was long believed to be the only site for protein breakdown in cells, but it is now clear that intracellular proteolysis is largely accomplished by a highly selective non-lysosomal pathway that requires a large multicatalytic proteinase complex known as the proteasome. The 26S eukaryotic proteasome catalyses the ATP-dependent degradation of polyubiquitinated proteins. Lignin-modifying enzymes of white-rot fungi are mainly expressed during the secondary phase of growth (idiophase), when the limitation of carbon and nitrogen occurs. It has been demonstrated for many eukaryotic organisms, that both the vacuolar and non-vacuolar proteolytic systems are activated by nutrient starvation. A major goal of the present study was to examine whether the specific agent (CbzLLLal, MG 132) that blocks the function of yeast and mammalian 26S proteasome and the lysosomotropic agent inhibiting intralysosomal degradation of proteins (chloroquine) can affect the levels of ligninolytic enzymes of the white-rot fungus *Phlebia radiata* during the shift to idiophase triggered by nitrogen or carbon starvation. Supported by the EC Contract ICA2-CT-2000-10050, 5PR-UE/DZ 280/2000.

1184 - Degradation of natural and artificial humic acids by the litter-decomposing basidiomycete *Collybia dryophila*

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The litter decomposing fungus *Collybia dryophila* is a common basidiomycete colonizing different types of forest soils. It decomposed in our experiments a natural humic acid isolated from pine-forest litter (LHA) and a synthetic ^{14}C-labeled humic acid (^{14}C-HA) prepared from [UL-^{14}C]catechol in liquid culture. Polar, lower-molecular mass fulvic acid (FA) and carbon dioxide were formed during the degradation. Manganese (200 µn; M Mn^{2+}) enhanced considerably the decomposition of HA leading to 75% conversion of LHA and 50% mineralization of ^{14}C-HA compared to 60% and 20%, respectively, in the absence of Mn^{2+}. These findings strongly indicated an involvement of the ligninolytic enzyme manganese peroxidase (MnP), the production of which was noticeably increased in Mn^{2+}-supplemented cultures and which was found to be capable of converting LHA *in vitro*. The enzyme was produced as a single protein with a p*I* of 4.7 and a molecular weight of 44 kDa. In solid-state culture (pine-forest litter), *C. dryophila* mineralized ^{14}C-HA as well and released substantial amounts of water-soluble FA from the insoluble litter material. The results indicate that litter-decomposing basidiomycetes such as *C. dryophila* are involved in humus turnover by recycling high-molecular mass humic substances.

1185 - Ultrastructural analysis of sterile elements of basidiomes and fruitbodies in holobasidiomycetes

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Comparative ultrastructural analysis of morphogenesis of sterile elements of basidiomes (B) in 6 species of the Aphyllophorales and fruitbodies in 17 of Agaricales and 1 Gasteromycetes showed that the most specialised are the cells of subhymenium, having the features of meristematic cells. During the grows of B and FB the cells of sterile elements are vacuolised, reserve substances, accumulated in many species, disappear, cytosol and number of organelles are reduced.For all studied species (expect the species of Coprinaceae) cells of sterile elements has ultrasructure indicated of low level of its metabolism. It was shown, that mass senescence and death of the cells of sterile elements occur before the formation of mature basidium. The process of cell senescence and death in B (Aphyllophorales) occur in the direction from the trama of pileus to trama hymenophore and subhymenium. In FB of Agaricales and Gasteromycetes this processes occur in direction, from the base of stipe to its upper part, later they occupied the trama of pileus, trama pf hymenophore and subhymenium. It was supposed that during the morphogenesis of B and FB in Holobasidiomycetes they are early transforming in to the autonomous from mycelium way of morphogenesis.

1186 - Construction of a gene-trap vector, pPTR-EGFP1 and pUsCdelPA2, for *Aspergillus oryzae*

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A promoter-trap vector that uses GFP as a reporter for *Aspergillus oryzae* gene expression and a polyA-trap vector using auxotrophic selection marker were constructed for the first time. The promoter-trap vector, pPTR-EGFP1, and polyA-trap vector, pUsCdelPA2 can be used to investigate unknown gene function. These gene-trap vector were integrated into a host genome randomly and transformants were selected by drug resistans or auxotrophic marker gene on the vector. The endogenous promoter activity trapped by the pPTR-EGFP1 vector was used to express the reporter gene *egfp*. The endogenous polyA addtion signal trapped by the pUsCdelPA2 vector was used to express the auxotrophic marker gene *sC* on the vector. By the transformation using pPTR-EGFP, approximately 300 drug resistant transformants were obtained, one of which showed the same strong fluorescence as GFP. The expression of *egfp* gene was confirmed by Northern blot analysis. The flanking genome regions of the integrate site was isolated by plasmid rescue method. Our resuls show that this newly constructed vector, pPTR-EGFP1, can trap endogenous promoter activity. The transformation using pUsCdelPA2 is under continuation now.

1187 - Production of CsmA, a class V chitin synthase with a myosin motor-like domain of *Aspergillus nidulans*, is regulated in multiple ways

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Chitin, a β-1,4-linked homopolymer of *N*-acetylglucosamine (GlcNAc), is one of the major structural components of the fungal cell wall. Chitin synthases are membrane-bound proteins and have been classified into five groups, classes I to V, on the basis of the structures in their conserved region. *csmA* gene of *Aspergillus nidulans* encodes a class V chitin synthase with a myosin motor-like domain at its N-terminus. *csmA* null mutants showed remarkable abnormalities in polarized growth, hyphal wall integrity, and conidiophore development. In this study, to investigate the intracellular behavior of the *csmA* product (CsmA) and the regulation of its production, we constructed strains that produced CsmA tagged with nine repeats of hemagglutinin A (HA) epitope at its C-terminus, CsmA-HA, instead of CsmA. Western blot analysis with anti-HA antibody proved that the entire coding region of *csmA* was translated as a single polypeptide with an approximate molecular mass of 210 kDa. CsmA-HA was found in the membrane fraction of the CsmA-HA producing strain. CsmA-HA was produced during vegetative growth, and its amount increased under low osmotic conditions. A discrepancy in the amounts of CsmA-HA protein and *csmA* transcript as determined by northern analysis under those conditions suggests that the production of CsmA is regulated at both the transcriptional and post-transcriptional levels.

1188 - Media optimization for manganese peroxidase production by response surface methodology in a newly isolated white-rot fungus *Trichophyton rubrum* LSK-27

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Response surface methodology (RSM) is a useful technique for media optimization, which is a crucial step for maximizing enzyme production in fungal cultures. In current study, RSM was applied for media optimization in a newly isolated white rot-fungus *T. rubrum* LSK-27, for manganese peroxidase (MnP) production. Carbon, nitrogen and also phosphorus are known to have significant impact in the production of fungal enzymes. Manganese stimulates MnP production in most fungi. In the first optimization step, glucose (x_1), mycological peptone (x_2), $MnSO_4$ (x_3) and KH_2PO_4 (x_4) were studied to investigate their effect on MnP production by fractional factorial design, 2^{4-1}. The first order equation with the regression coefficients were computed to be $y=0.7175+0.0625x_1+0.4318x_2-0.1201x_3-0.0364x_4$ with a probability level of 91% for MnP production. Although glucose and KH_2PO_4 positively affected the cell growth, they did not increase the enzyme levels. Among the response curves obtained for four media components, the highest response was obtained for $MnSO_4$-peptone pair, which was chosen as main factors for central composite design. Effect of each media ingredient on fungal morphology was also followed. The optimum $MnSO_4$-peptone concentrations were found for maximum MnP production.

1189 - Expression studies of plant genes differentially expressed in leaf and root tissue of arbuscular mycorrhizal (AM) fungal colonised tomato plants

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Arbuscular mycorrhizal (AM) fungi of the unique phylum Glomeromycetes are a multifaceted group of mutualistic symbionts that are common to terrestrial ecosystems. The interaction between AM fungi and plant root systems is of environmental and agronomic importance. Understanding the molecular changes within the host plant upon AM fungal colonisation is a pre-requisite to a greater understanding of the mechanisms underlying the interaction. Differential mRNA display was conducted on leaf tissue of tomato plants colonised and non-colonised by the AM fungus *Glomus mosseae* and five putative differentially regulated cDNAs were identified. All cDNAs isolated shared high sequence similarity to known plant genes. Semi-quantitative RT-PCR was used to establish gene expression patterns for all five clones within leaf and root tissue of mycorrhizal and non-mycorrhizal colonised tomato plants. Differential regulation was observed for all five cDNAs. Down-regulation within the leaf tissue of mycorrhizal plants was observed for 4 out of the 5 cDNAs with an up-regulation observed only for one. Tissue specific regulation was observed for several cDNAs, with down-regulation observed in mycorrhizal leaf tissue and up-regulation observed within mycorrhizal root tissue as compared to non-mycorrhizal tissue. We thank the Scottish Executive Environmental Rural Affairs Division (SEERAD) for financial support.

1190 - Application of AFLP markers to genetic study of an edible mushroom, *Lentinula edodes*: strain typing, genetic diversity, and genetic linkage map

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Lentinula edodes (shiitake) is one of the most popular edible mushrooms in east Asia (Japan, Korea and China). It is desirable to establish efficient breeding systems of this mushroom using molecular markers. In this study, we evaluated the usefulness of amplified fragment length polymorphism (AFLP) as genetic marker in *L. edodes*. Strain typing and genetic diversity: Six AFLP primer pairs reproducibly detected 179 polymorphic DNA fragments among 15 strains of *L. edodes* currently used for cultivation in Japan. These markers could differentiate all of the strains. Cluster analysis and principle coordinates analysis based on AFLP data revealed two groups which corresponded to those categorized on their fruiting season types. Genetic linkage map: A medium-dense genetic linkage map of *L. edodes* was constructed based on 203 AFLP markers and two mating type factors. The segregation of these markers was generated from 95 progeny of a single cross of two distantly related *L. edodes* strains. Segregation analysis showed that the map consisted of 11 linkage groups, and the total genetic distance of the map was 1956.7cM. The average rate of physical size to genetic distance could be roughly estimated to be less than 18.4 kb/cM, which is low compared to the values obtained for other filamentous fungi. Seventeen of the AFLP markers showed highly distorted segregation ratios (χ^2 values ≥ 6.63; $P \leq 0.01$), and many of these were located in LG II (6 markers) and IV (6 markers).

1191 - pH optimization of AM symbiosis with *Glomus intraradices* and carrot transformed roots in vitro

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AM fungi *Glomus intraradices* was introduced on M media with different pH regimes ranging from pH 4 to pH 13 in vitro. The symbiosis was found to express differently over the acid and alkaline range. The investigation was directed to study the difference in expression of AM fungi in terms of inter and intraradical spread, spore formation, mycelial and root biomass along with nutritional uptake in roots. pH 8 was observed to be more supportive to maximum AMF symbiosis, also supporting maximum root biomass. pH 9 supported more vegetative proliferation of the fungus. An increasing trend was observed in mycorrhizal colonization percentage (MCP) from pH 4 to 13. The study is significant in optimizing parameters responsible for optimum symbiosis and exploit its potential in mass inoculum production.

1192 - Comparative symbiotic events of various coal ash amendments in vitro on AM fungi *Glomus intraradices* and Ri T-DNA transformed carrot roots

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In vitro mycorrhizal behaviour in the presence of three types of coal ash was studied. Minimal media was amended with 5% of three types of coal ashes with different physico-chemical properties i.e., ESP ash, pond ash and bottom ash obtained from the Korba Super ThermalPower Station located in Chattisgarh, India. A comprehensive elemental analysis was conducted to evaluate the absorption, translocation and tolerance of macro, microelements and heavy metals (Ca, Na, Mg, K, Zn, Fe, Cu, Mn, Cd, Co, Pb, Cr, Mo, As, Al, & B) in roots and extramatrical mycelial biomass. The effect of ashes on the mycorrhizal expression of *Glomus intraradices* was compared in terms of spore production, mycelial biomass, root biomass and extent of root colonization. Mycorrhizal expression measured by spread and internal and extramatrical proliferation was maximum in ESP ash and minimum in bottom ash. This is the first report of direct ash amendment with multiple heavy metal accumulation in AM fungal biomass and roots in vitro.

1193 - Conidiation in *Penicillium cyclopium* is triggered by conidiogenone: an endogenous diterpene

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The filamentous fungus *Penicillium cyclopium* conidiates in submerged culture without nutrient limitation, in the presence of calcium ions according to a precisely timed programme. Conidiation could be prematurely induced in a supplemented medium which had previously supported growth, suggesting that a metabolite which influenced the process was produced. A diterpenoid compound with conidiation inducing activity, conidiogenone, was purified from the culture medium, along with conidiogenol, a derivative with delayed activity. Contrary to previous thought, the presence of calcium was demonstrated to only decrease the requirement of accumulated conidiogenone for the induction to proceed. In the light of the results a mechanism of conidiation induction is presented, according to which the mycelium constitutively produces a conidiation inducer (conidiogenone) that accumulates extracellularly. When a threshold concentration is reached induction takes place by the likely interaction with a specific cellular receptor. The results indicate that conidiogenone is both sufficient and necessary to induce conidiation.

1194 - Isolation and characterization of the glyceraldehyde-3-phosphate dehydrogenase gene from *Rhizomucor miehei*

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Fungi belonging in the *Rhizomucor* genus has medical and biotechnological importance. *Rhizomucor miehei* isolates are well known as producers of rennin, which enzyme is used in cheese-industry. Until now little is known about the genetics of *Rhizomucor* strains. The aim of the present study was to clone the *gpd* gene from *Rhizomucor miehei* and characterize the structural gene and its adjacent regulatory elements (promoter and terminator regions) at sequence level. Glyceraldehyde-3-phosphate dehydrogenase is an important enzyme in both glycolysis and gluconeogenesis. It catalyses the oxidation and phosphorylation of the glyceraldehyde-3-phosphate to 1,3-bis-phosphoglycerate. As a main housekeeping enzyme its gene shows a strong structural conservation by different prokaryotic and eukaryotic organisms. The *Rhizomucor miehei* gene encoding glyceraldehyde-3-phosphate dehydrogenase (*gpd*) was isolated from a genomic library by hybridization of the library clones with *gpd*-specific gene probe. The complete nucleotide sequence encodes a polypeptide chain of 337 amino acids interrupted by 5 introns. The putative protein product of this gene shows a high degree of sequence identity with the corresponding gene products of various fungi. The promoter region, containing a consensus TATA box and a 246 nucleotid long termination region were also determined. This study was supported in by OTKA grant T 032738.

1195 - *Chrysosporium lucknowense*, a new fungal host for protein production

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Chrysosporium lucknowense, an ascomycetous fungus, not closely related to *Aspergillus* or *Trichoderma*, is being developed as a new fungal host for homologous and heterologous protein production. This thermophilic fungus is able to secrete large amounts of (hemi)cellulases. Mutant strains of *C. lucknowense* have been isolated with improved protein yields (per gram biomass) compared to available *Aspergillus* and *Trichoderma* strains. Large-scale fermentations were developed up to 150,000 litre cultures. Growth conditions of *Chrysosporium* strains are very versatile, with growth from acid to alkaline pH and at temperatures of 25-43 °C. In contrast to other industrial fungi, *Chrysosporium* has growth characteristics that make it amenable to microtiter plate based culture methods. The development of a versatile gene expression and High Throughput gene discovery system for this fungus will be discussed.

1196 - Quantitative transcript analysis and heterologous expression of copper-radical oxidases in the white rot basidiomycete *Phanerochaete chrysosporium*

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The white rot basidiomycete *Phanerochaete chrysosporium* is able to degrade all major components of wood: cellulose, lignin and hemicellulose. The oxidative enzymes thought to be involved in lignin degradation by this model system include lignin peroxidases (LiP), manganese peroxidases (MnP), and the peroxide-generating enzyme glyoxal oxidase (GLOX). Recently, a draft *P. chrysosporium* genome sequence has been made publicly available (http://www.jgi.doe.gov/programs/whiterot.htm). Blast searches of this database unexpectedly revealed additional GLOX-like sequences. Comparisons of these sequences to GLOX and to related copper-radical oxidases showed close structural similarities around the active site. At least three of the GLOX-like sequences have a putative eukaryotic secretion signal as predicted by SignalP (http://www.cbs.dtu.dk/services/SignalP/). Transcripts were detected in various defined culture media and from *P. chrysosporium*-colonized wood. Quantitative transcript analyses and heterologous expression of these genes will be presented.

1197 - Evidence for carbon source regulated PKA and PKC signaling in the duplication cycle, polarization and septum formation in *Aspergillus nidulans*

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The effects of glucose and of a pectic substrate in the duplication cycle, spore polarization and septation of *Aspergillus nidulans* were tested in poor and rich media. Growth on poor conditions and on sodium polypectate slowed nuclear duplication and reduced the coupling of polarization to mitosis. Coupling of septation to the third mitosis was also reduced by changing growth conditions. When PKA and PKC activators were added to the media the results suggested a role for PKA in slowing the duplication cycle, while allowing polarization. Addition of a PKC activator to poor media uncoupled the first septum formation from the third mitosis in a carbon source-regulated manner, suggesting a role for PKC in coordinating cell cycle signals, growth and cytokinesis.

1198 - Ultrastructural study of partial symbiont delichenisation process in lichen *Peltigera aphthosa*

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The thalli of the foliose lichen *Peltigera aphthosa* were kept in the different conditions. The influence of unfavorable conditions on lichen thalli resulted in their degradation associated with the ultrastructural changes, including destructive alterations of cell organells, thickening of cell walls of both phycobiont and mycobiont and loosening of contacts between the symbionts. These changes indicated some degree of delichenisation of lichen components. Within certain time limits the degradation was reversible: the symbiont cell structure and accordingly their functions and interaction could be reestablished by transferring to the favorable conditions. The phycobiont recovered more quickly than the mycobiont, whose nutrition depended sufficiently on the algal partner, demonstrating the dominant role of the photobiont in this situation. However, the prolonged action of unfaborable conditions led to the irreversible ultrastructural alterations: to the cell destruction and the disjunction of symbionts and accordingly to the pronounced delichenisation, to the degradation of the whole thalli and finally to their death. The cell ultrastructure and the viability of such thalli could not be restored. The reversible destruction of the lichen symbionts could be one of the mechanisms of the reversible delichenization. It may be considered as a constituent of normal lichen physiology ensuring survival of these organisms in the extreme environmental conditions.

1199 - Comparative karyology of *Agaricus bisporus* (Lange) Imbach strains

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There was made the comparison of basidial morphogenesis, sporogenesis and nuclear behavior in vegetative mycelium in several strains of *A. bisporus*, which have different types of life cycle, by light microscopy (Feulgen method and DAPI) and electron microscopy. The next cultivated and wild strains were investigated: pseudohomothallic Bs26 (*Agaricus bisporus* var. *bisporus*), heterothallic Bs94 (*A. bisporus* var. *burnettii* Kerrigan et Callac), and primary homothallic Bs423 (*A. bisporus* var. *eurotetrasporus* Callac &

Guinberteau). Nuclear number in vegetative mycelium varied from 1 to 8 per cell and most of the cells contained 4 nuclei. We observed amitotic nuclear divisions and nuclear migration through dolipore septum. Morphometric measuring of basidia and nuclear apparatus were made. Some preliminary results were obtained for ploidy level and place of meiosis in examined strains. We have described in details basidium morphogenesis in all the strains by using light and electron microscopy. We've observed: asynchronous 2-nd meiotic division; asynchronous migration of postmeiotic nuclei into sterigmata; postmeiotic mitosis in basidium. Electron microscopy of basidial nuclei showed the presence of synaptonemal complexes (SC) in all the strains; in heterothallic and primary homothallic ones we revealed SC for the first time. This fact confirms the processing of meiotic division in basidia of all the strains. We've reconstructed life cycles of all investigated strains.

1200 - Biochemical and molecular characterization of arginase from *Agaricus bisporus*: Expression study in sporophore formation and postharvest development

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An extensive survey of higher fungi reveals that members of the family Agaricaceae, including *Agaricus bisporus*, accumulate substantial amounts of urea in their fruit bodies. During postharvest storage the amount of urea even increases substantially. Such a large increase in urea content may affect the quality of the stored mushroom, e.g. by the formation of ammonia from urea through urease action. Production of urea is not limited to fruit bodies, but also occurs in the mycelium. Despite the abundance of urea in the edible mushroom *A. bisporus* little is known about its physiological role, although it was proposed that in fruit bodies urea is an end product of catabolic pathways. Two major mechanisms for urea formation can be envisaged: the ornithine cycle and nucleic acid degradation. Arginase is the ornithine cycle enzyme that catalyzes the hydrolysis of arginine to urea and ornithine. In the scope of the study on urea metabolism, the work on arginase was initiated with the isolation of the arginase cDNA, gDNA and promoter region. Sequence analysis revealed the cDNA encodes a 311 aa protein which is probably expressed in the cytosol. Expression of the cDNA in *E. coli* has been established as a His-tagged protein. The purified recombinant protein was used to determine enzyme kinetics. The characterized gene and enzyme are used as molecular markers to study expression and regulation during sporophore formation and postharvest development.

1201 - Effect of *Glomus intraradices* inoculation on phosphate uptake in lettuce with two different P sources

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The effect of *Glomus intraradices* inoculation on phosphate uptake in *Lactuca sativa* L. (lettuce) cultivated in sand was examined. Soluble KH_2PO_4 (SP) and sparingly soluble $Ca_5(PO_4)_3OH$ (RP) were applied to the potting medium at the rates of 0, 25, 125 mgP/kg sand. The harvest at week 6 shown that among all the treatments, the non-mycorrhizal (NM) plants with 25 mg SP/kg sand had the highest yield in dry matter. However, within the RP treatments, the dry matter for the mycorrhizal (M) plants with 25 mg and 125 mg RP/kg sand were significantly higher ($P<0.05$) than the NM plants with the same rates of RP application. The results also shown a general trend that all the inoculated treatments with both SP and RP have higher shoot phosphate levels than the NM treatments. For the intensity of root colonization, both the 25 and 125 mgRP/kg sand treatments gave rise to significantly higher ($P<0.001$) values compared with the SP treatments. The results suggested that the M plants were more efficient in utilizing phosphate from the RP source. It has been shown by other researchers that intense root colonization by arbuscular mycorrhiza could result in enhanced uptake of other mineral elements apart from P and alleviation in some toxicity effects. [The work was partly supported by the EU project MYCHINTEC (ICA4-CT-2000-30014).]

1202 - A search for proteins related to freezing resistance of basidiomycetes

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The investigation was targeted at frost-resistance and protective proteins of mycelium and fruit bodies of xylotrophic Basidiomycetes (*Pleurotus* and *Flammulina genii*) to low positive and negative temperatures. Immunoblotting were used to identify the proteins homologous to plant dehydrins, uncoupling proteins PUMP and CSP 310. It was shown that proteins homologous to dehydrins are present in mycelium and fruit bodies. Dehydrin related polypeptides with molecular weights 70 and 71 kDa demonstrated cold induction. Proteins immunochemically related to PUMP and CSP 310 were found only in mycelium. The quantity of CSP 310 was the same regardless of temperature treatment. PUMP from mycelium cooled at 2Ïó accumulated in the highest amount. In the experiments in vitro under the effect of proteins from

cooled and non-cooled mycelium *P. ostreatus* on isolated plant mitochondria (*T. aestivum* L.) was observed uncoupling of oxidative phosphorilation. Uncoupling of mitochondria oxidation and phosphorilation processes contribute to heat generation and reduce oxidative stress that may be important to survival of mycelium. The results acquired confirm a certain degree of similarity in mechanisms of plant and fungi cells protection at low-temperature stress. However, unlike plants, mycelium of the fungi of boreal zone is able to survive at low negative temperatures without preliminary acclimation, and contains protection proteins in the conditions of 'comfortable' temperature.

1203 - Alpha-galactosidase gene cloning from *Aspergillus fumigatus*

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In this study, molecular cloning studies were performed on the α-galactosidase gene of *Aspergillus fumigatus* IMI 385708. Degenerate primers were designed based on amino acid sequence of the purified enzyme and conserved sequences among α-galactosidases of *Aspergillus* and *Penicillium* species. These primers were used in PCR to amplify α-galactosidase gene fragments. A 900 bp PCR product was selected as a probe for further studies. RNA dot blot analysis showed that the highest expression of α-galactosidase mRNA was on the fifth day of growth on locust bean gum. This information was employed in cDNA synthesis. The synthesized cDNA was ligated onto Lambda ZAP Express vector to construct the primary cDNA library. After amplification of the primary library, screening studies were performed. At the end of primary and secondary screening, a number of putative positive plaques were obtained for further characterization studies.

1204 - Transformation of *Neotyphodium lolii* with plasmids containing a native promoter disturbs the symbiotic interaction with its host

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Neotyphodium lolii is an endosymbiont of perennial ryegrass. It produces alkaloids neurotoxic to herbivores. We wanted to determine *in planta* expression patterns of the *N.lolii* 3-hydroxy-3-methylglutaryl-CoA reductase (HMG CoA reductase) gene, believed to be involved in neurotoxin synthesis. We transformed the endophyte with plasmids, in which DNA fragments upstream of the open reading frame of the HMG CoA reductase gene controlled expression of the GUS reporter gene. In laboratory cultures, >400 bp were required for expression and >=1100 bp for maximum expression. Transformants often showed abnormal hyphal morphology when growing within ryegrass. This occurred significantly more often in transformants which carried additional functional HMG CoA reductase promoter sequences compared to transformants with HMG CoA reductase gene upstream sequences of <=400 bp and transformants in which expression of the GUS gene was regulated by a constitutive heterologous promoter. There was no correlation between abnormalities and levels of *in planta* GUS activity or hygromycin resistance (the selective marker used in transformation). Therefore introduction of additional copies of native promoter sequences seems to be a major cause of these abnormalities. This suggests limits to the usefulness of reporter genes in endophytic fungi but also a potential to identify genes important for plant-fungus interaction by transformation with additional copies of their promoters.

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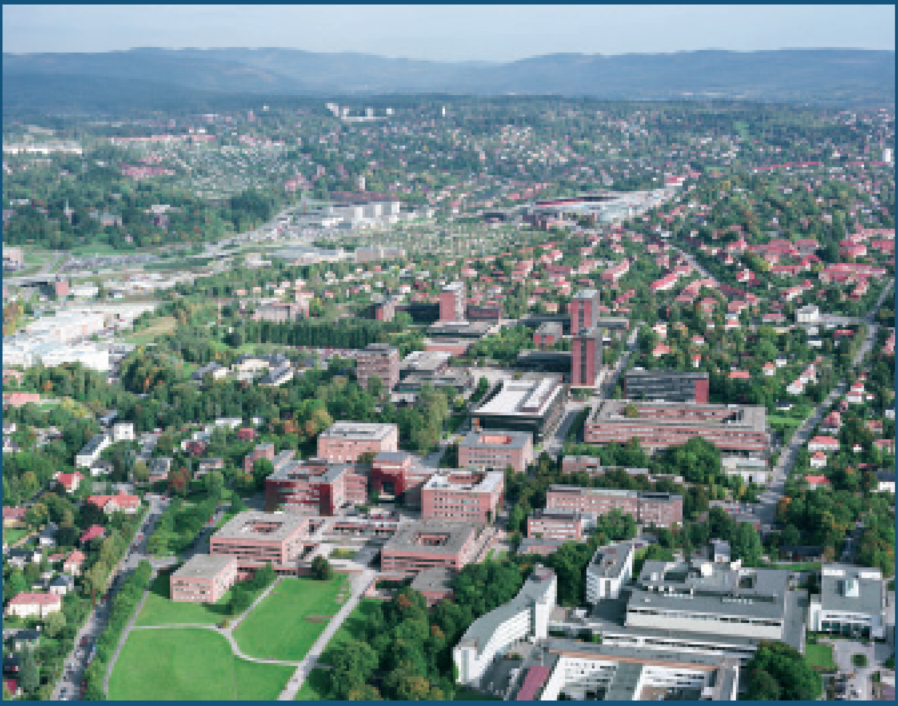

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