Mycological Research News[1]

This month *Mycological Research News* features recent work on generic concepts in cantharelloid fungi, and ectomycorrhizal fungi raiding saprotrophic ones for nutrients. Amongst topics covered by the 18 research papers in this issue are molecular systematics of *Craterellus*, variation at the molecular level in *Chondrostereum purpureum* and *Monascus*, genetic exchange between *Metarhizium* strains, β-1,3-glucanase regulation in *Trichoderma harzianum*, specific isozymes in *Botrytis cinerea*, lipid and fat composition of arbuscular mycorrhizal fungi, PCR detection of *Verticillium chlamydosporium*, the storage of *Erynia*, destruxin production by *Metarhizium* strains, ultrastructure of *Entomosporium mespili*, utilization of various substrates by *Aspergillus fumigatus*, *Tuber melanosporum* inoculation and molecular detection, and studies on freshwater ascomycetes from Thailand and *Crepidotus* species in Mexico. New scientific names introduced are: *Botryobasidium musaisporum*, *Deltosperma oblongum*, *Melanochaeta garethjonesii*, *Ophiostoma setosum*, *Unguiculariopsis changbaiensis*, and *C. damingshanica* spp. nov.; and *Craterellus ignicolor* (syn. *Cantharellus ignicolor*) comb. nov.

IN THIS ISSUE

This issue includes the results of molecular studies which reassess the relationships of species placed in *Cantharellus* and *Craterellus* (pp. 388–394; featured below), reveal considerable genetic variation in New Zealand isolates of the silverleaf pathogen *Chondrostereum purpureum* although all were pathogenic to several trees regardless of their original host (pp. 395–402), that *Monascus purpureus* strains used in red rice and sofu production while representing four lineages come from a narrow genetic source (pp. 403–408), suggest genetic recombination between *Metarhizium* strains which pass together through an insect gut (pp. 409–414), enable *Verticillium chlamydosporium* to be detected on roots using a cloned fragment of its β-tubulin gene (pp. 435–439), and to differentiate isolates of seven *Tuber* species (pp. 472–477).

The β-1,3-glucanase system of *Trichoderma harzianum* has been found to be composed of at least five different enzymes, the largest of which was most abundant in the absence of a carbon source (pp. 415–420). Strains of *Botrytis cinerea* from grape leaves and apple soft rot have been found to differ in polygalacturonase isozymes which are suggested to be involved in the soft rot, but the pectin methylesterase isozymes did not match with the pathology (pp. 421–428). While the lipid and fatty acid composition of hyphae and spores of two species of *Glomus* was constant, the phospholipid content of the spores was found to be higher (pp. 429-434). The black truffle *Tuber melanosporum* has been found

experimentally to be capable of associating with five local *Quercus* species in Israel (pp. 472–477).

Two papers focus on aspects of fungal biocontrol agents, addressing the drying and storage of *Erynia neoaphidis* (pp. 440–446), and variation in destruxin production by a range of *Metarhizium* species and strains (pp. 447–452). The ability of *Aspergillus fumigatus*, *Haematonectria haematococca* and *Metarhizium anisopliae* to utilize mucin, lung polymers, plant cell walls and insect cuticle have been compared and depolymerases found to be a key factor in the ability of *A. fumigatus* to utilize human polymers (pp. 463–471). The conidiogenesis and structure of the remarkable appendaged multicellular conidia of *Entomosporium mespili* are fully documented ultrastructurally for the first time (pp. 453–462).

The *Melanochaeta* species occurring in freshwater in Thailand, and their *Sporoschisma* anamorphs, have been reassessed and connections confirmed (pp. 478–485). A new wood-staining species of *Ophiostoma* in the *O. piceae* complex has been discovered in North America (pp. 486–494). Some Mexican species of *Crepidotus* are re-examined and compared with allied taxa (pp. 495–506); and two new species of *Unguiculariopsis* (one with a *Deltosperma* anamorph) are described from the People's Republic of China (pp. 507–509), and one of *Botryobasidium* from Taiwan (pp. 510–512) are described.

CANTHARELLOID FUNGI RECONSIDERED

Just how chanterelles should be classified at the generic level has long been a matter of debate. Some authors have adopted *Craterellus* for dark coloured species such as *C. cornucopioides* (Horn of Plenty) which also lack clamp connexions and have less-developed lamella-like ridges; others have retained the dark-coloured species with the familiar orange ones of *Cantharellus*, including *C. cribarius* (The Chanterelle). Some authors have gone so far as to place the genera in separate families (Pegler, Roberts & Spooner 1997). In addition, *Pseudocraterellus* has been accepted for *P. sinuosus* (Wavy-capped Chanterelle) and its allies, based on the secondary septation of the hyphae and incurved margin of the cap (Pegler *et al.* 1997, Watling & Turnbull 1998). The treatment adopted has depended on the interpretation of particular morphological and anatomical characters. As the groups both include edible species on sale in supermarkets, a clear resolution of the situation was highly desirable to minimize confusions.

In this issue, Dahlman, Danell & Spatafora (2000) studied this problem using nuclear large subunit rDNA sequence data from a wide range of species from both North America and Europe. The robust cladistic analysis that resulted, provides convincing evidence that *Cantharellus cibarius* and its close relatives constitute a genus distinct from *Craterellus*, to which *C. cornucopioides* belongs. However, *C. tubaeformis* (Autumn Chanterelle; sometimes incorrectly as '*tubiformis*') was found to belong to *Craterellus*, and also to be distinct from *C. lutescens* which has been subsumed with it by British authors. The type species of *Pseudocraterellius*, *C. sinuosus*, came out well within the *Craterellus* clade. The authors predict that all species previously placed in *Cantharellus* subgen. *Lepto-cantharellus* will eventually be proved to belong to *Craterellus*.

Independently, Pine, Hibbett & Donoghue (1999) included six cantharelloid species in an investigation of the relationships of the cantharelloid and clavarioid fungi. These authors used mitochondrial mtDNA and small subunit rDNA. The cladogram from the small subunit tree in particular, showed *Cantharellus cibarius* in a separate clade from the other five species included, amongst them *C. lutescens*, *C. cornucopioides*, and *C. tubaeformis*. Although different genes were sequenced, these results were entirely concordant with those of Dahlman *et al.* (2000). Further, Pine *et al.* found that the *Cantharellaceae* (incl. *Craterellaceae*) were closely allied to the *Hydnaceae*, and even more surprisingly also to *Clavulina* and *Stichoclavaria*. It is therefore clear that undue emphasis should not be placed on the arrangement of the hymenial layer in the classification of these fungi.

As a result of these two molecular studies, it is evident that *Cantharellus* should be retained as a small genus around *C. cibarius* for species, which have clamp connexions, a well-developed stipe, and are often brightly pigmented. Many species formerly placed in *Cantharellus* merit separate recognition in the genus *Craterellus*, characterized by the absence of clamp connexions, a tubular or funnel-like habit, and varying from bright to dark colours. *Craterellus* now includes *C. cornucopioides* and *C. tubaeformis*, amongst other species. Field mycologists and mycophagists will be pleased that these uncertainties have now been resolved, but will have to become acustomed to using a less familiar generic name.

Dahlman, M., Danell, E. & Spatafora, J. W. (2000) Molecular systematics of *Craterellus*: cladistic analysis of nuclear LSU rDNA sequence data. *Mycological Research* **104**: 388–394.

Pegler, D. N., Roberts, P. J. & Spooner, B. M. (1997) *British Chanterelles and Tooth Fungi*. Royal Botanic Gardens, Kew.

Pine, E. M., Hibbett, D. S. & Donoghue, M. J. (1999) Phylogenetic relationships of cantharelloid and clavarioid *Homobasidiomycetes* based on mitochondrial and and nuclear rDNA sequences. *Mycologia* **91**: 944–963.

Watling, R. & Turnbull, E. (1998) *Cantharellaceae, Gomphaceae and amyloid-spored and xeruloid members of the Tricholomataceae (excl. Mycena)*. [British Fungus Flora, Agarics and Boleti No. 8.] Royal Botanic Garden, Edinburgh.

ECTOMYCORRHIZAL FUNGI RAID SAPROTROPHIC ONES

The nutritional interactions between mycorrhizal and saprotrophic fungi have now been described using a non-destructive electronic autoradiography technique (Lindahl *et al.* 1999). The study sheds new light on the types of organic nutrient sources which may be available to ectomycorrhizal fungi.

It is now well established that many ectomycorrhizal fungi are able to use organic forms of nutrients. Proteases produced by the fungi enable ectomycorrhizal plants to grow on proteins as a sole source of nitrogen (Abuzinadah, Finlay & Read 1986). Phosphatases are commonly produced by ectomycorrhizal fungi, and the capacity to produce enzymes that can degrade complex recalcitrant polymers like chitin and lignin has also been demonstrated (Leake & Read 1997). The extent to which different organic substrates are used as nutrient sources by ectomycorrhizat fungi in the field is still poorly understood. Bending & Read (1995) demonstrated the mobilisation of organic nutrients from patches of forest floor material introduced into microcosm systems with ectomycorrhizal plants. It is, however, unclear which components of the patches were actually degraded and utilized. The mycelium of soil fungi is rich in nutrients with a nitrogen content of 4–9 % (Gebauer & Taylor 1999). This compares to recently shed plant leaves which have a nitrogen content of between 0.4 and 3 % (Aber & Melillo 1980). Access to nutrients contained within fungal hyphae is not hindered by lignocellulose cell walls and fungal rnycelia have a much larger surface area to volume ratio compared with plant litter, making them more vulnerable to enzymatic attack. Fungal mycelium could thus constitute a high quality nutritional substrate for ectomycorrhizal fungi. As significant amounts of the total forest soil nitrogen and phosphorus are incorporated into fungal mycelium (Bååth & Söderström 1979), this nutrient pool could be very important for the nutrition of ectomycorrhizal plants.

Fungi, growing under conditions of poor nutrient avail-

ability, tend to reallocate their cytoplasm from senescing parts of the mycelium to the actively growing hyphal tips, leaving vacuolated or dead hyphae behind (Cook & Rayner 1983). It is therefore important, not only to study the ability of ectomycorrhizal fungi to degrade dead mycelium, but to investigate the potential of ectomycorrhizal fungi to interact antagonistically with other fungi in order to capture nutrients from the actively growing parts of their mycelia.

In the recent microcosm study (Lindahl *et al.* 1999), intact mycelia of the wood degrading fungus *Hypholoma fasciculare*, extending into soil from wood blocks, was confronted with the rnycelium of an ectomycorrhizal fungus, either *Suillus variegatus* or *Paxillus involutus*, extending from the roots of pine seedlings. In half of the microcosms, the wood decomposing fungus was labelled with ^{32}P, supplied as orthophosphate in a small water droplet on top of the wood block. In the rest of the microcosms, the mycorrhizal fungus was labelled with ^{32}P supplied in a small plastic bowl beneath a mycorrhizal root tip. The tracer isotope was added at the time of physical contact between the saprotrophic and mycorrhizal mycelia. Distribution and movement of radioactivity in the microcosms was studied non-destructively with an electronic autoradiography system (Instant Imager, Packard Instrument Co, Meriden, CT) which allowed repeated scanning of the microcosms.

When the mycorrhizal and saprotrophic mycelia met in the soil, the mycorrhizal fungi formed patches of dense mycelium over the saprotrophic mycelial fronts, where the hyphae are most densely cytoplasmic. Similar patches of dense mycorrhizal mycelium have been observed in other experiments in connection with enrichment of the substrate with organic nutrients (Unestam 1991, Bending & Read 1995). In the systems where ^{32}P was supplied to the wood decomposing fungus, the tracer isotope was rapidly translocated throughout the saprotrophic mycelium and concentrated at the mycelial front. Ten days after labelling, activity could be found in the mycorrhizal root tips, and after 20 days activity was also found in the shoots of the host seedlings. After 30 days, when the experiment was harvested, 12 ± 4 % of the activity outside the wood blocks was found in the plant (including mycorrhizal mantles) in systems with *Paxillus involutus*. The corresponding figure for systems with *Suillus variegalus* was 14 ± 4 %. When the mycorrhizal mycelium was labelled, very little activity was captured by the wood decomposing fungus. At harvest 0.11 ± 0.3 % of the activity outside the root zone was found in the wood block in systems with *P. involutus*. The corresponding figure for systems with *S. variegalus* was 0.6 ± 0.2%.

This is the first published microcosm study to show net transfer of nutrients between interacting fungi from these two important ecological groups. With this experimental design, the mycorrhizal fungi were dominant over the wood decomposing fungus and were able to take up phosphorus from the saprotrophic mycelium. In other microcosm studies, *Hypholoma fasciculare* grown under similar conditions, has been shown to take up phosphorus from the soil and translocate a major part of the acquired phosphorus to the wood block (Wells & Boddy 1995). The transfer of phosphorus to the mycorrhizal fungi is thus not likely to be dependent on exudation of phosphorus by the wood decomposing fungus,

but suggests antagonistic interactions, lysis of saprotrophic mycelium followed by degradation and uptake of phosphorus containing compounds (nucleotides, phospholipids, polyphosphates etc.) by the mycorrhizal fungi. Other experiments in our research group have demonstrated drastically reduced growth of saprotrophic mycelium when in contact with ectomycorrhizal fungi compared to growth in controlled systems without ectomycorrhizal fungi, indicating an antagonistic effect. It is important, however, to consider that the described study represents only one possible outcome of this type of interaction. Changes in size or quality of the resources available to the fungi (wood block and host plant), as well as the use of different species and isolates, could lead to different results.

The ability of ectomycorrhizal plants to compete with saprotrophic fungi and to use nutrients in saprotrophic mycelia represents a shortcut in traditional models of nutrient cycling. In boreal ecosystems, where the availability of inorganic forms of nutrients is low and saprotrophic organisms may be limited by their access to nutrients, plants and their associated symbiotic fungi must be able to compete with the saprotrophs in order to acquire nutrients (Kaye & Hart 1997). In these ecosystems, a major part of the nutrients taken up by mycorrhizal fungi and transferred to their host trees is likely to be in an organic form. These organic nutrients may be acquired from a range of sources. The results described above suggest that fungal mycelium can be an attractive and rapidly degradable nutrient source available to mycorrhizal fungi.

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