

Mycological Research News¹

This month *Mycological Research News* reports how elevated carbon dioxide levels affect soil fungi, on cryptic species of macromycetes and species concepts, punching appressoria, and fungi in the Dead Sea. Amongst the topics covered in the 17 research papers included in this issue are ones presenting a new molecular taxonomy for *Metarhizium*, insights into the taxonomy of small-spored *Alternaria* species, sequencing and expression of an enolase gene in *Cunninghamella*, a novel extracellular protease from *Beauveria bassiana*, tests of different mathematical models on the effect on a lichen's growth by neighbouring thalli, how the taenia in *Trichiales* capillitium is formed, histological details of the incompatibility reaction between *Plasmodiophora brassicae* and *Arabidopsis*, and a key to the known *Beltraniopsis* species. The following new species and varieties are described: *Beltraniopsis miconiae*, *Chrysosporium fluoiale*, *Leptographium piceaperdum*, and *Metarhizium anisopilae* vars *acidum*, *lepidotum*, and *majus*, and *M. flavoviride* vars *novazealandicum* and *pemphigum*.

IN THIS ISSUE

The first paper in this issue provides a taxonomic reassessment of *Metarhizium* based on molecular data; three species and eight varieties are accepted, five for the first time, and all described or characterised on the basis of ITS1 and ITS2 sequence differences (pp. 131–151). RAPD molecular analyses have been correlated with morphology to distinguish small-spored *Alternaria* species, i.e. the *A. alternata* group (pp. 152–161). Other molecular papers examine DNA polymorphisms in *Magnaporthe grisea* on rice and finger millet in India which were found to be distinct (pp. 162–168), reveal 14 endophytic fungi in the roots of the ericoid *Woollisia* only one of which was identifiable to genus (pp. 169–175), demonstrate heterogeneity in RAPD patterns and other biological characteristics in *Erynia neoaphidis* (pp. 214–220), the cloning and sequencing of the enolase gene from *Cunninghamella elegans* (pp. 176–180). A new species of *Chrysosporium* able to grow on keratin in river sediments has been discovered and its sequence data compared with that from similar species (pp. 245–251).

A novel protease from *Beauveria bassiana* has been purified and characterized (pp. 181–187), and ways of improving chlamydospore production in *Duddingtonia flagrans* in liquid culture are described (pp. 206–210).

New microcosm studies on the effects of elevated carbon dioxide levels on soil reported here (pp. 188–198) are discussed below, and three mathematical models for testing the effects of neighbours on the growth of lichen thalli have been compared using a sample of 11 246 individuals of *Umblicaria spodothroa* (pp. 199–205).

Elegant histological studies, documented with colour plates, detail the gall-like incompatibility reaction between *Plasmodiophora brassicae* and *Arabidopsis* (pp. 221–226). *Diaporthe viticola* is shown to be teleomorph of *Phomopsis* Taxon 1 attacking grapevines, although not recognized since 1867 (pp. 227–232), and *Sclerotinia nivalis* is reported from central China and its cultural features and protein patterns compared with related fungi (pp. 233–238). *Ophiostoma europhioides* and *Ceratocystis pseudoeurophioides* are shown to be synonyms of *O. piceaperdum* and the name *Leptographium piceaperdum* is introduced for the anamorph of this fungus (pp. 239–244). *Beltraniopsis miconiae* is described as new from Brazil and a key to the seven known species is provided (pp. 252–254).

The taenia on the capillitium of *Trichiales* is hypothesized to develop from polymerization of elater wall material and subsequent osmotic effects (pp. 211–213).

ELEVATED CARBON DIOXIDE AFFECTS SOIL FUNGI

Studies in the Ecotron, a controlled environment facility with specially designed soil microcosms, have indicated that the biomass of cellulose decomposing fungi in soil is increased

when carbon dioxide levels are maintained at 200 $\mu\text{mol mol}^{-1}$ above ambient levels for nine months (Jones *et al.* 1998). Independent studies with constructed microcosms reported in

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this issue (Conway *et al.* 2000) suggested that while reduced litter quality resultant from plant growth at elevated carbon dioxide levels reduced the extent of fungal colonization, some assemblages of fungal decomposers stimulated decomposition with respect to cellulose and nitrogen mineralization. The species spectrum from the different Ecotron treatments also differed, but too few isolations were made to be confident of the significance of that observation. The structure of the collembolan population in the Ecotron was also changed in the period, which was considered as due to the selectivity of these fungus-feeders.

If these preliminary studies are vindicated and shown to be representative of field situations, increased carbon dioxide levels could affect fungus-driven soil food webs and decomposition processes. The long-term implications of such fundamental changes on a global scale can only be speculated on at this stage, but it is clear they would be significant and could have far-reaching implications for the composition of the soil biota and soil structure. The fungal aspect of increased

carbon dioxide levels merits more attention in the development of global models. However, if, as predicted by Lloyd (1999), large increases in carbon in the soil occurred in tropical forests as a result of elevated carbon dioxide levels, perhaps that could be at least partly ameliorated by increased decomposition rates.

Conway, D. R., Frankland, J. C., Saunders, V. & Wilson, D. R. (2000) Effects of elevated atmospheric CO₂ on fungal competition and decomposition of *Fraxinus excelsior* litter in laboratory microcosms. *Mycological Research* **104**: 188–198.

Jones, T. H., Thompson, L. J., Lawton, J. H., Bezemer, T. M., Bardgett, R. D., Blackburn, T. M., Bruce, K. D., Cannon, P. F., Hall, G. S., Hartley, S. E., Howson, G., Jones, C. G., Kampichler, C., Kandeler, E. & Ritchie, D. A. (1998) Impacts of rising atmospheric carbon dioxide on model terrestrial ecosystems. *Science* **280**: 441–443.

Lloyd, J. (1999) The CO₂ dependence of photosynthesis, plant growth responses to elevated CO₂ concentrations and their interaction with soil nutrient status, II. Temperate and boreal forest productivity and the combined effects of increasing CO₂ concentrations and increased nitrogen deposition on a global scale. *Functional Ecology* **13**: 439–459.

CRYPTIC SPECIES OF MACROMYCETES AND SPECIES CONCEPTS

Hallenberg (1991) asserted that many morphospecies of macromycetes included cryptic species, i.e. reproductively isolated populations behaving as biologically separate species. He suggested that the sporocarps were evolutionarily very old and that subsequent evolution had focused on biological interactions. Evidence that this is a widespread situation is being generated apace. For example, in a recent study of the ectomycorrhizal mushroom *Hebeloma crustuliniforme* (Aanen & Kuyper 1999), incompatibility tests were applied to 110 collections. The crossing experiments between these revealed the existence of not less than a staggering 20 intercompatibility groups (ICGs). The different ICGs were bifactorial heterothallic with multiple mating type alleles.

Most of the ICGs could not be separated morphologically, nor was there a correlation with particular hosts. The material studied was from France, Germany, the Netherlands, Sweden and Switzerland, and associated with a variety of deciduous and coniferous trees. If an even wider range of isolates were studied perhaps even more biologically independent groups would be found.

Almost any fungus studied by cultural methods is showing parallel patterns of behaviour, including those in many micromycete genera. The application of the species concept in fungi in the light of such data was critically reviewed by Brasier (1997) with reference to particular well-studied examples, who refers to morphologically inseparable units as operational species units. Burnett (1983) and Brasier (1987) had previously drawn attention to this problem, and molecular work is confirming that the issue of cryptic species is a general rather than an exceptional case in fungi.

The recognition of operationally separate species within morphologically characterised species of macromycetes inevitably causes problems amongst those wishing to identify or undertake ecological or pathological studies with these fungi. Against this background, in mycology we will have to

accept that in practice we must operate with a ‘pragmatic species concept’ (Hawksworth 1996), that is to allocate species names to units that mycologists wish to communicate about – recognizing that the characters used to separate such units, including those that are biological, will not be the same in all groups. For example, the ability to cause particular diseases may be paramount in plant pathogens, the production of particular toxic metabolites in food spoilage fungi, or the utilization of particular carbohydrates in yeasts.

Rather similar conclusions were arrived at by Petersen & Hughes (1998) as a result of their studies on mating patterns in another macromycete genus, *Omphalotus*. They stated that ‘morphology should be of paramount importance in delineating species or infraspecific nomenclatural entities, with the full knowledge that other character suites may be more natural and/or of equal importance’. These authors subsequently developed their concepts more fully (Petersen & Hughes 1999), suggesting that for the fungi the development of a universal species concept was probably not worthwhile, but that it ‘should be based on a demonstration of phenetic cohesiveness, common evolutionary descent, and reproductive isolation where possible’. However, they considered that ultimately this ‘depends on the best judgement of the experienced investigator based on all the available data’ (*loc. cit.*: 450). This reflects the oft-quoted apocryphal adage that ‘a species is what a good taxonomist recognizes as a species’.

Aanen, D. K. & Kuyper, T. W. (1999) Intercompatibility tests in the *Hebeloma crustuliniforme* complex in northwestern Europe. *Mycologia* **91**: 783–795.

Brasier, C. M. (1987) The dynamics of fungal speciation. In *Evolutionary Biology of the Fungi* (A. D. M. Rayner, C. M. Brasier & D. Moore, eds): 231–260. Cambridge University Press, Cambridge, UK.

Brasier, C. M. (1997) Fungal species in practice: identifying species units in fungi. In *Species: the units of biodiversity* (M. F. Claridge, H. A. Dawah & M. R. Wilson, eds): 135–170. [Systematics Association Special Volume No. 54.] Chapman & Hall, London.

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PUNCHING APPRESSORIA

Plant cell contents are a valuable food source for any fungus, but cell walls pose a formidable barrier. The cuticle poses a front line, but may be bypassed by fungi using pre-existing openings such as stomata, hydathodes (on leaves), lenticels, or wounds (Manners 1993). However, some fungi have a specialised structure, the appressorium, enabling them to penetrate the cuticle and cell wall of the intact leaf surface. A novel technique described by Bechinger *et al.* (1999) provides new light on how this penetration is achieved.

When mature, a hypha of narrower than usual diameter grows down from the base of the appressorium through the plant's cuticle and epidermal cell walls. Once through these barriers, this specialised hypha, referred to as a *ëpenetrationí* hypha or peg, increases in diameter (Howard & Valent 1996, Mendgen *et al.* 1996). Cutinases and cell wall degrading enzymes are produced at the locality of some appressoria (Parbery 1981), and high concentrations of osmolytes accumulate inside appressoria, especially glycerol (De Jong 1997). Appressoria usually develop inside water drops on leaf surfaces, so considerable turgor pressure can build up inside the appressorium. But can this pressure alone penetrate the host cell, or must enzymes also soften the cell wall? The turgor pressure inside appressoria of *Magnaporthe grisea*, the causal agent of rice blast, has been indirectly measured by Howard *et al.* (1991) at around 6–8 megapascals; that is 60–80 bar, about 10^3 pounds per square inch (psi) around 30–40 times the average car tyre pressure. Appressoria of that fungus can penetrate artificial plastic membranes (Howard *et al.* 1991).

Bechinger and his coworkers directly measured the force generated by individual appressoria and their penetration hyphae for the first time. They allowed conidia of *Colletotrichum graminicola* to germinate and form appressoria on a waveguide. As the penetration hypha deformed the waveguide, its optical properties changed. These changes were recorded and used to determine the length of the penetration hypha. By measuring the mechanical pressure needed to deform the waveguide by the same amount, the pressure exerted by the appressorium was determined. The waveguide used was of the planer type, a core of high refractive index (in this case polydimethylsiloxane, PDMS, also used in breast implants), sandwiched between two layers of lower refractive index (in this case aluminium). The upper aluminium layer was 50 nm thick and the lower 13 nm, while the PDMS film between was 1 μ m thick. The waveguide was formed on a glass slide attached to an optically matched prism. This allowed the waveguide to be illuminated from below by a 3 mW laser which produced light at a wavelength of 670 nm. The beam diameter was adjusted to 5 mm. If the angle of incidence (q) of this beam to the internal reflective surfaces of the

waveguide meets the resonance condition of the waveguide, the light is propagated laterally and so there is a marked reduction in the intensity of the reflected light. The angle of resonance is highly dependant on the local thickness of the waveguide. If q is held constant any change in thickness of the waveguide will result in increased reflection.

In the experimental set up of Bechinger *et al.*, the angle of incidence (q) was set to produce a uniform low-intensity reflected light image. The reflected light was projected onto a charge-coupled device (CCD) camera chip and stored on computer. As the penetration hyphae of individual appressoria tried to force their way through the upper aluminium layer of the waveguide, the deformation they caused changed the thickness of the waveguide and therefore the amount of light reflected. Having previously calibrated the apparatus by allowing a test structure to deform the waveguide by known amounts, the vertical deformation caused by the penetration hyphae was determined and found to be around 10 nm. The diameter of the penetration hyphae was around 2 μ m. To find out what force was needed to produce this amount of deformation, a thin tapered glass capillary with an external diameter of 5 μ m and a known spring constant was used. When mounted on a micromanipulator this could be used to exert known forces on the same waveguides used to measure the appressoria. The deformations produced when these forces were applied were also measured. The forces generated by the appressoria were found to average 16.8 μ N with a range of 8–25 μ N. This should be sufficient to punch through and penetrate the cuticle and epidermal cell walls of most plants (Howard *et al.* 1991). If a similar force were exerted over the surface of a human hand it could support a school bus (Money 1999).

As well as being useful for individual measurements, this technique offers exciting prospects of measurement of other dynamic processes. The waveguides have a relaxation time of less than 1 second, and the sensitivity of the measurements could be increased by using waveguides of different elasticities (Bechinger *et al.* 1999).

The forces appressoria are capable of generating are remarkable, but it must be remembered that an equal opposite force is generated which could push the appressorium off the surface were it not securely attached. How the fungus adheres itself securely enough to prevent this is another question to which we have only partial answers. Indeed there is no evidence for a common adhesion compound or mechanism (Dean 1997). A hemicellulose of unspecified constitution was found to bind appressoria of *C. graminicola* on glass (Lapp & Skoropad 1978). Hydrophobic interactions are believed to be important for adhesion in many leaf pathogens (Mendgen *et*

al. 1996). On leaves adhesion can additionally be assisted by reconstituted cuticular components brought into solution by cutinases released from the appressoria (Morgan & Parbery 1977). Appressoria of *C. gloeosporioides* have been found buried in the cuticular wax of avocado (Binyamini & Schiffman-Nadel 1972), but that cannot help adhesion onto glass (Morgan & Parbery 1977), plastic membranes (Howard *et al.* 1991), or the aluminium used in this study.

Whether all appressoria are able to exert such pressures remains to be ascertained. Nobody has yet reported the pressure inside *C. graminicola* appressoria (Money, pers. comm.). These structures are produced by many plant pathogenic fungi, (Emmett & Parbery 1975, Dean 1997), but may be at the end of germ tubes, on vegetative hyphae, or from clusters of hyphae. Further studies on invasive forces developed by fungi with different kinds of appressoria are clearly needed.

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FUNGI DISCOVERED IN THE DEAD SEA

Reports of fungi from extremely hypersaline environments are very few, but lack of representation in the literature may reflect not the inability of fungi to colonize these extreme environments but rather the little effort that has been spent to identify them (Javor 1989). The only previous report on the isolation of a halophilic filamentous fungus from a hypersaline lake is that of an unnamed *Cladosporium* on a submerged piece of pine wood in the Great Salt Lake, Utah, USA (290–360 g l⁻¹-salinity) (Cronin & Post 1977). In the same lake, non-filamentous fungi belonging to *Thraustochytrium* were also found (Amon 1978, Brown 1990).

We have now discovered a variety of filamentous fungi in The Dead Sea. This hypersaline lake is located in the Syrian-African rift valley, on the border between Israel and Jordan. It is one of the most saline lakes on earth, with a salinity of about 340 g l⁻¹. The lake differs from other hypersaline lakes in the unique ionic composition of its waters, with high concentrations of divalent cations (Mg 40.7 g l⁻¹; Ca 17 g l⁻¹) exceeding those of monovalent cations (Na 39.2 g l⁻¹; K 7 g l⁻¹). The major anions are Cl (212 g l⁻¹) and Br (5 g l⁻¹). The water activity in undiluted Dead Sea water was reported to be about 0.669 in 1979 (Krumgalz & Millero 1982), and today is probably even lower.

The Dead Sea is a naturally stressful laboratory for the evolution of adaptation to extremely hypersaline environments. Since the discovery of life in the Dead Sea by Wilkansky (1936), the lake has been found to be inhabited by

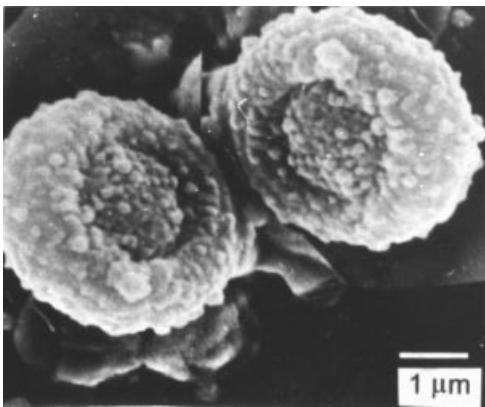
several types of microorganisms, including archaeal and eubacterial prokaryotes, the unicellular green alga *Dunaliella parva*, and possibly even protozoa (Elazari-Volcani 1940, Volcani 1944). Quantitative studies performed since 1980 have shown the lake to be a dynamic ecosystem. There are periods of dense blooms of algae (*D. parva*) and red halophilic archaea, triggered by dilution of the upper water layers by freshwater floods during rainy winters. Blooms alternate with long periods characterized by an almost total absence of life-forms (Oren 1988, 1992, 1993, 1999). The paucity in biodiversity of the Dead Sea biota is probably determined by the unique composition of the Dead Sea waters, with high concentrations of magnesium and calcium.

No fungi had been recorded in the hypersaline water of the Dead Sea prior to our discovery (Buchalo *et al.* 1998). Three species of filamentous fungi were isolated from surface water samples: *Gymnascella marismortui* described as new to science, *Ulocladium chlamydosporum* and *Penicillium westlingii*. The first two species grew on media containing up to 50% Dead Sea water. *G. marismortui* was shown to be an obligate halophile growing optimally in the presence of 0.5–2 M NaCl or 10–30% (by volume) of Dead Sea water. Isolated cultures did not grow on agar media without salt, but grew on agar prepared with up to 50% Dead Sea water.

We have since discovered more species of filamentous fungi from the Dead Sea (Buchalo *et al.* 1999, 2000). Twenty six species representing 13 genera of *Zygomycota*, *Ascomycota*,

Table 1. Filamentous fungi isolated from the Dead Sea

<i>Zygomycota</i>
<i>Absidia glauca</i>
<i>Ascomycota</i> (some as anamorphs)
<i>Aspergillus caespitosus</i>
<i>A. carneus</i>
<i>A. fumigatus</i>
<i>A. niger</i>
<i>A. phoenicis</i>
<i>A. terreus</i>
<i>A. ustus</i>
<i>Chaetomium aureum</i>
<i>C. flavigenum</i>
<i>C. funicola</i>
<i>C. nigricolor</i>
<i>Cladosporium cladosporioides</i>
<i>C. macrocarpum</i>
<i>Emericella nidulans</i>
<i>Eurotium amstelodami</i>
<i>E. herbariorum</i>
<i>Gymnascella marismortui</i>
<i>Paecilomyces farinosus</i>
<i>Penicillium variabile</i>
<i>P. westlingii</i>
<i>Thielavia terricola</i>
<i>Mitosporic fungi</i>
<i>Acremonium</i> sp.
<i>A. persicinum</i>
<i>Stachybotrys chartarum</i>
<i>Ulocladium chlamyosporum</i>

**Fig. 1.** *Gymnascella marismortui*, ascospores (SEM).

and mitosporic fungi were isolated from the Dead Sea (Table 1). Only one zygomycete (*Absidia glauca*) was registered. Mitosporic fungi were represented by four species belonging to the genera *Acremonium*, *Stachybotrys* and *Ulocladium*. Species of ascomycetes (some as anamorphs only) belonging to *Aspergillus* and *Chaetomium* were the most numerous, and *Penicillium*, *Cladosporium* and *Eurotium* were each represented by two species respectively. The other five genera of ascomycetes found in the Dead Sea were represented by one species each.

A new species of the genus *Gymnascella*, named *G. marismortui*, was also found (Buchalo *et al.* 1998), differing from the known species of the genus, including *G. punctata*, in the shape of its ascospores, which have a very broad rim

(Fig. 1). Ascospore shape is an important taxonomic character in *Gymnascella* and the new species also differs from *G. punctata* in the presence of arthroconidia and chlamyospores, the occurrence of nodulose hyphae, and the colour and smaller size of the ascomata (c. 250 µm diam in *G. punctata* and 40–70 µm diam in *G. marismortui*), as well as in the obligate halophily.

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