14 Genetic Diversity and Functional Aspects of Ericoid Mycorrhizal Fungi

S. Perotto[1], E. Martino[1], S. Abbà[1], M. Vallino[1]

CONTENTS

I. General Features of Ericoid Mycorrhiza

The aim of this chapter is to review classical and more recent findings on the taxonomic diversity and functional traits of soil fungi forming ericoid mycorrhiza (ERM) with plants in the family Ericaceae. The cladistic relationships within this plant family have been recently investigated by

[1]Dipartimento di Scienze, della Vita e Biologia dei Sistemi dell'Università di Torino and Istituto per la Protezione delle Piante del CNR, v. le Mattioli 25, 10125 Torino, Italy; e-mail: silvia.perotto@unito.it

Kron et al. (2002) using nucleotide sequence data from the nuclear 18S and chloroplast encoded *mat*K and *rbc*L genes. The phylogenetic analysis indicates that Empetraceae and Epacridaceae, previously considered as separate families, are now placed in tribes within the larger family Ericaceae (Kron et al. 2002). The **close relationship between epacrids and ericoid plants** is further supported by the finding of phylogenetically close mycorrhizal fungi associated with these plant groups (Chambers et al. 2000; Sharples et al. 2000a).

Plants in the family **Ericaceae** are **widespread in a diverse range of heathland and open forest communities, both in the Northern and in the Southern hemisphere** (Read 1991; Sokolovski et al. 2002). Although they are commonly found as understorey vegetation, ericaceous shrubs can become dominant in many heathland habitats found at high altitudes and colder altitudes, as well as in Mediterranean climates. These **habitats are characterised by very poor nutrient status and considerable edaphic stress** (Cairney and Meharg 2003), and it is thought that the success of Ericaceae in these habitats is due to their endomycorrhizal association. In addition to being more stress tolerant than non-mycorrhizal plants in harsh environments (see Cairney and Meharg 2003), ericoid mycorrhizal plants have been also found to be better competitors in mixed plant communities (Genney et al. 2000; Van der Wal et al. 2009).

A **common feature of ericoid plants** is the **anatomy of their very fine and delicate roots**, termed **hair roots** (Read 1996). Hair roots (Fig. 14.1) have a very narrow diameter, usually less than 100 μm, and consist of an inner stele surrounded by a two-layered cortex and an
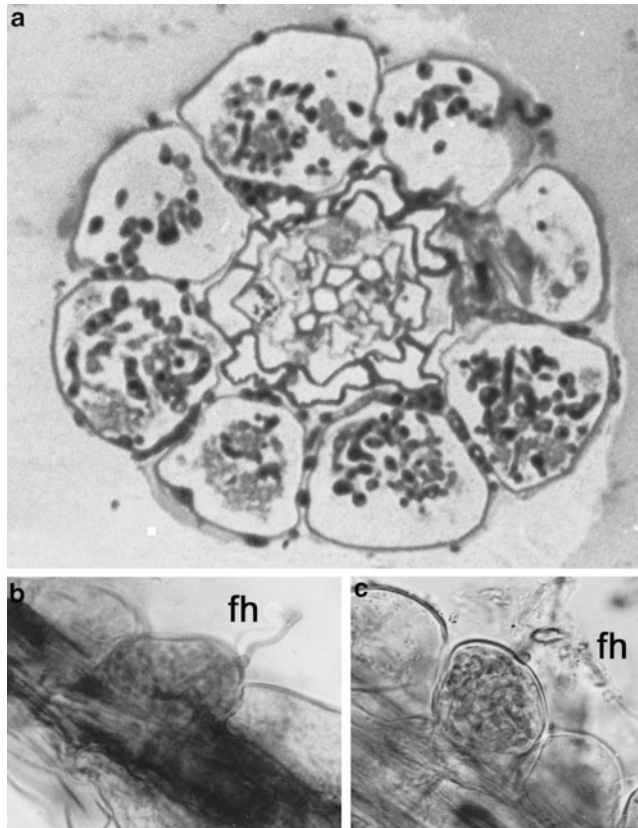
Fig. 14.1. Colonisation of *Calluna vulgaris* hair roots by ericoid mycorrhizal fungi. (**A**) Transverse section of a hair root to show the general structure of the root. Cells of the outer epidermal layer harbour fungal coils formed by a sterile mycorrhizal morphotype. (**B, C**) Fungal hyphae (*fh*) entering the epidermal cells of *C. vulgaris* through the tangential wall. The collapsed cortical cells are visible just underneath the epidermis, surrounding the small central cylinder

outermost layer of large epidermal cells. These **epidermal cells** represent the interface with the soil environment (Smith and Read 2008) and are the only hair root cells to be **colonised by ERM fungi**. Here, ERM fungi **form hyphal coils** that usually occupy most of the cell volume and are always surrounded by the invaginated plant plasma membrane (Fig. 14.2; Bonfante-Fasolo and Gianinazzi-Pearson 1979; Peterson and Massicotte 2004). Colonisation of epidermal cells by fungi usually occurs directly from the soil through the outer thickened tangential wall (Fig. 14.1), with limited cell to cell hyphal connections (Massicotte et al. 2005) so that **most cells represent individual colonisation units**. Epidermal cells are sloughed off in the older parts of the hair roots, leaving exposed the suberised cortex layer. Thus, ERM cells are ephemeral and symbiotic nutrient exchange is likely restricted to the younger parts of the root, where both partners are viable. Unlike other endomycorrhizal associations, such as arbuscular and orchid mycorrhiza, viable ericoid fungal hyphae have been observed in plant cells showing signs of organelle and cytoplasm degeneration (Bonfante-Fasolo and Gianinazzi-Pearson 1979; Duddridge and Read 1982). Mechanisms of nutrient acquisition by the plant that involve digestion of the fungal hyphae, such as tolypophagy, suggested by Rasmussen and Rasmussen (2009) for orchid mycorrhiza, can be therefore excluded.

Fig. 14.2. Ultrastructure of an epidermal cell of *C. vulgaris* colonised by a typical fungal coil. (**A**) The fungal hyphae form a dense coil, which occupies a central position in the cell. (**B**) Detail of an intracellular hypha showing some fungal compartments (vacuole, mithochondrion) and glycogen deposits. The plant plasma membrane surrounds the hypha. (**C**) A fungal septum labelled with a gold-labelled cellobiohydrolase shows the presence of beta 1,4 glucans (Courtesy of P. Bonfante)

II. Genetic Diversity of Ericoid Mycorrhizal Fungi

To understand the evolution and ecological role of mycorrhizal symbioses, one of the first steps is to unveil the taxonomic position and functional features of the fungal symbionts. The study of ERM is a good example of how the development of molecular tools to investigate fungal diversity has impacted on our view of the specificity of this symbiosis, considered for long time a highly specific interaction restricted to few taxa of plants and fungi (Straker 1996). It has also modified considerably our understanding of ericoid mycorrhizal functioning in ecosystems, revealing potential hyphal networks previously unsuspected (Chambers et al. 1999; Vrålstad 2004; Grelet et al. 2010).

The first attempts to identify ERM fungi were based on the isolation in culture of endosymbiotic fungi from mycorrhizal roots. Several slow growing sterile mycelia, grouped primarily on the basis of cultural morphology

and/or ITS-RFLP profiles, have been isolated world-wide from ericoid plants, and many could re-establish ericoid mycorrhiza when inoculated onto axenic plants in gnotobiotic conditions (e.g. Hutton et al. 1994; Perotto et al. 1996; Hambleton and Currah 1997; Liu et al. 1998; McLean et al. 1999; Monreal et al. 1999; Bergero et al. 2000; Cairney et al. 2000; Chambers et al. 2000; Sharples et al. 2000a; Berch et al. 2002; Bougoure and Cairney 2005a, b).

A. Taxonomic Diversity of Ericoid Mycorrhizal Fungi

The first ERM fungus that could be identified taxonomically was a dark isolate obtained from *Calluna vulgaris* roots by Pearson and Read (1973a) that was eventually induced to form fruiting bodies in pure culture. This isolate formed apothecia and was classified in the genus *Pezizella* as *P. ericae* (Read 1974).

The taxonomic position of *P. ericae* has since been revised: the new name *Hymenoscyphus ericae* was proposed by Kernan and Finocchio (1983), but a phylogenetic revision of the *Hymenoscyphus* genus by Zhang and Zhuang (2004) showed that *H. ericae* was outside this genus, and the new name *Rhyzoscyphus ericae* was thus proposed. For several years *R. ericae*, with its anamorph *Scytalidium vaccinii* (Dalpé et al. 1989; Egger and Sigler 1993), was the only identified fungal symbiont of Ericaceae. The ERM was thus described as a highly specific association that at that time contrasted with the apparent lack of specificity of other endomycorrhizae, such as arbuscular mycorrhiza.

Most **slow-growing mycelia isolated from ERM** remained unidentified until molecular tools were developed to investigate their phylogenetic affinities. It turned out that a high proportion of these slow-growing isolates, together with fungi isolated from plants with different mycorrhizal status, formed an aggregate of closely related species in the order Helotiales. This assemblage, named "**R. ericae aggregate**" by Vrålstad et al. (2000, 2002a) because it included *R. ericae,* contained **four main clades**. The clades were further refined by Hambleton and Sigler (2005), who also proposed three new species in the anamorphic genus *Meliniomyces* as members of the "*R. ericae* aggregate" (Fig. 14.3). Clade 3 contains most of the proven ERM isolates, including type cultures of *R. ericae*

and *S. vaccinii.* Clade 1 (*Meliniomyces variabilis*) contains sequences of ericoid root-associated fungal isolates, previously described as "variable white taxon" (Hambleton and Currah 1997), as well as sequences of root endophytes from other host plants from cold-temperate soils of the Northern hemisphere. Some of these fungi can colonise the roots of both ERM and ECM hosts, but although they formed typical ERM structures in hair root hosts, no true mantle was observed in ECM hosts (Piercey et al. 2002; Vohník et al. 2007). Clade 2 (*Meliniomyces vraolstadiae*) is a small clade containing sequences from fungi so far only found to form ECM, or to be non-mycorrhizal (Vrålstad et al. 2002b).

Other ERM fungi have been isolated in culture and were identified as **ascomycetes in the genus *Oidiodendron*** (Dalpé 1986, 1989). Resynthesis experiments (e.g. Couture et al. 1983; Dalpé 1986) have demonstrated the ERM nature of *Oidiodendron*, and their occurrence as ERM symbionts has been confirmed in several Ericaceae (Read 1996; Straker 1996; Xiao and Berch 1996; Allen et al. 2003; Bougoure and Cairney 2005a, b). A comparison of rDNA ITS region sequences suggests that many *Oidiodendron* isolates may have been misidentified in older studies, and that only *O. maius*, or phylogenetically close species in the Onygenales, forms mycorrhizal associations with Ericaceae in the field (Hambleton et al. 1998; Lacourt et al. 2002).

Ericoid fungi also encompass so far unculturable members of the **Basidiomycota** that could be precisely identified only by molecular phylogeny. Early ultrastructural studies provided evidence of basidiomycetes forming ERM in roots of *Rhododendron*, *Calluna* and *Vaccinium* (Bonfante-Fasolo 1980; Englander and Hull 1980; Peterson et al. 1980). PCR amplification of the fungal rDNA from mycorrhizal roots of *Gaultheria shallon* (Berch et al. 2002; Allen et al. 2003) for the first time revealed sequences of Sebacinales that corresponded to more than half of the cloned ITS sequences. Selosse et al. (2007) recently investigated, by direct PCR amplification of ERM samples and ultrastructural observations, the occurrence of Sebacinales in the Ericaceae and found it to be
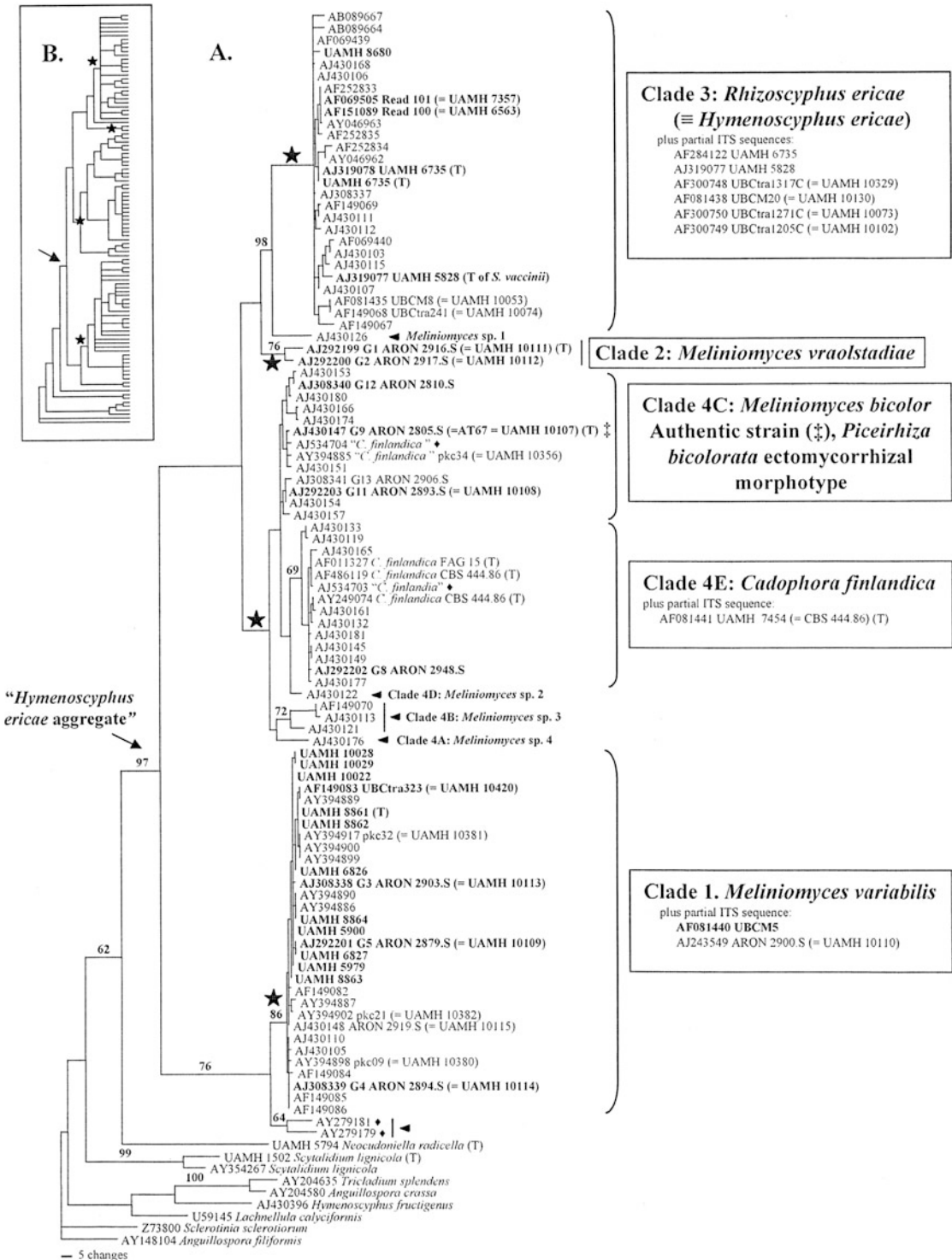
Fig. 14.3. (A) One of 5,000 MPTs from an aborted parsimony analysis of the ITS-B data matrix. Sequences AJ534704 and AY394885 were deposited under the name "*Cadophora finlandica*" (as "*C. finlandia*") based on ITS sequence similarity scores. *Arrowheads* indicate additional species that could be described if

a common symbiont. All sequences of Sebacinales recovered from ERM plants, together with sequences derived from the newly described cavendishioid mycorrhizal type (Setaro et al. 2006) belonged to clade B identified by Weiss (2004) in the phylogeny of *Sebacina*.

The frequent finding of unculturable fungi in ERM roots is in contrast with the general observation that ericoid symbionts have good saprotrophic abilities and are able to grow on common culture media (Leake and Read 1991). The use of PCR techniques to amplify fungal DNA directly from mycorrhizal roots has made it possible to identify these unculturable fungi, although their role as mutualistic symbionts in the mycorrhizal association remains unclear.

B. Multiple Fungal Occupancy in Ericoid Mycorrhizal Roots

The morphology of mycorrhizal roots in the Ericaceae indicates that epidermal root cells could potentially function as separate units, challenged and colonised by a variety of fungi resident in the rhizosphere. ITS-RFLP analyses of ericoid fungal isolates from different mycorrhizal plant species has demonstrated that **multiple occupancy is a common phenomenon in ericaceous roots** (e.g. Perotto et al. 1996; Hambleton and Currah 1997; Monreal et al. 1999; Bergero et al. 2000; Chambers et al. 2000; Midgley et al. 2004c; Bougoure and Cairney 2005a, b). This is revealed by the simultaneous presence of fungi with different ITS-RFLP profiles in the same root system. In addition, more sensitive fingerprinting methods (RAPD and ISS-PCR) showed that distinct genotypes of ERM fungi sharing the same ITS-RFLP type can occur in individuals of *C. vulgaris* (Perotto et al. 1996) and *Epacris pulchella* (Curlevski et al. 2009). These data indicate that the **roots of ericaceous plants are a complex mosaic where different populations of mycorrhizal fungi coexist**, each represented by a variable number of genotypes.

The simultaneous association with many and diverse symbiotic fungi may represent an important **strategy to broaden the array of functions in the colonisation of difficult substrates**. This hypothesis would require that genetic diversity was mirrored by functional diversity, and that different species of ERM fungi were able to perform different physiological functions. This has been demonstrated for some ERM fungi. For example, as discussed in Sect. III.B, ERM can hydrolyse complex substrates, and they do so to a different extent (e.g. Cairney and Burke 1998; Leake and Read 1991; Varma and Bonfante 1994; Midgley et al. 2004a). Even fungal strains within the same species can perform differently. For example, Cairney et al. (2000) found that isolates of *R. ericae* display different abilities to utilise inorganic and organic nitrogen sources, and Grelet et al. (2009b) demonstrated that functional differences in N transfer to the host plant shoot are maintained among closely related ERM fungi in symbiosis with their hosts.

Better knowledge of the true ERM diversity in ericaceous plants has changed our perception on the specificity in this association, with a wider number of fungal taxa being involved than once thought, mainly in the ascomycetes but also in the basidiomycetes. However, although this wider number and taxonomic position of potential ERM partners, there is evidence of specificity in the composition of the ERM fungal communities associated with co-existing plant species and different environments.

Bougoure et al. (2007) have investigated the diversity of fungi associated with plants of *C. vulgaris* and *Vaccinium myrtillus* along a heathland—forest gradient in Scotland. The assemblages of fungi associated with the two plant species were different, even though plants were co-occurring in the forest understorey. In addition, the

---

Fig. 14.3. (continued) isolates are available for examination. All ingroup sequences were derived from cultures isolated from roots, except for four (◆) derived from DNA extracted directly from roots. A majority of ingroup taxa were resolved into four clades, indicated by *stars* adjacent to the relevant nodes, corresponding to *Meliniomyces variabilis*, *Meliniomyces vraolstadiae*, *Cadophora finlandica*/*Meliniomyces bicolor*, and *Rhizoscyphus ericae*. (**B**) Strict consensus of all 5,000 MPTs with taxon names removed. *Stars* indicate the same four clades as in (**A**) (From Hambleton and Sigler (2005), with permission)

community of fungi associated with *C. vulgaris* hair roots was different for samples collected from the forest, open heathland and a transition zone between the two (Bougoure et al. 2007). Part of the differences were due to the amplification, in the forest samples, of typical ECM ascomycetes and basidiomycetes sequences.

In a more homogeneous environment such as the subarctic heaths, however, Kjøller et al. (2010) investigated the fungal communities associated with the roots of four co-existing ericaceous plants (*Empetrum hermaphroditum*, *Andromeda polifolia*, *Vaccinium uliginosum*, *V. vitis-idaea*) and could not find significant differences in relationship with the host plants, whereas significant differences in spatial distributions were observed. Thus, it seems that, similarly to what happens in other endo- and ectomycorrhizal symbioses, environmental factors as well as the host plant species may influence, at least in some cases, the ERM fungal community even when a broad range of potential symbionts are available.

C. Potential Networking Abilities of Ericoid Mycorrhizal Fungi

The phylogenetic analyses described in the previous paragraphs demonstrate that **a diverse assemblage of fungi can interact with Ericaceae to form ERM**. Conversely, the **occurrence of ERM-forming fungi on plant species outside the Ericaceae** has also been frequently demonstrated. Duckett and Read (1995) reported that *R. ericae*, the best studied ERM symbiont, was also capable of forming in vitro intracellular coils in the rhizoids of **liverworts**.

Further support to this observation derived from molecular studies on leafy liverworts in the genus *Cephaloziella*. Mycelia isolated in nature from liverworts and demonstrated to form ERM on ericaceous hosts were first assigned to the genus *Hymenoscyphus* (syn. *Rhizoscyphus*) by Chambers et al. (1999). Later on, Upson et al. (2007) performed Koch's postulate for the *C. varians*—*R. ericae* association, inoculating axenically grown liverwort with an isolate of the fungus from the plant. These isolates from Antarctica could also colonise *Vaccinium macrocarpon* roots, despite the absence of ericaceous plant species from maritime and continental Antarctica.

ERM and ECM plants often co-exist in natural ecosystems such as boreal or Mediterranean forests, where ericaceous plants constitute the understorey vegetation of dominant ECM tree species (Read 1991). The traditional view that ECM and ERM fungi are taxonomically distinct

has been challenged by several papers reporting amplification of ERM sequences from ECM roots. An important discovery by Vrålstad et al. (2000) was the **strong genetic similarity among fungi of the *R. ericae* aggregate associated with ERM and ECM roots** in a boreal forest.

Following ITS sequence comparison, the ERM fungus *R. ericae* was the closest relative of fungi isolated from *Piceirhiza bicolorata*, an ECM morphotype common in post-fire sites. Phylogenetic analyses showed that fungi from ERM and ECM roots, as well as other endophytes, belong to different clades of the "*R. ericae* aggregate", first proposed by Vrålstad et al. (2002a) and later revised by Hambleton and Sigler (2005).

None of the fungal isolates from *P. bicolorata* and grouped in Clade 1 of the *R. ericae* aggregate (Fig. 14.3), corresponding to *M. variabilis*, could form ERM on ericaceous hosts (Vrålstad et al. 2002b), although a *M. variabilis* isolate from *P. bicolorata* ECM has been recently found to form ERM in *V. vitis-ideae* (Grelet et al. 2010).

Isolates assigned to the *C. finlandica*/*M. bicolor* (Clade 4 in Fig. 14.3) have been found to form ECM or ectendomycorrhiza with *Betula*, *Picea* and *Pinus* (Wilcox and Wang 1987; Vrålstad et al. 2002b). An isolate of *M. bicolor* derived from *Piceirhiza bicolorata* ECM was also demonstrated to form, at the same time, ERM on *V. myrtillus* and ECM with *Pinus sylvestris*, and to induce beneficial effects on host plant growth (Villarreal-Ruiz et al. 2004). This finding confirms previous observations by Monreal et al. (1999) that *Phialophora* (= *Cadophora*) *finlandica* could form, at least in vitro, ERM with *Gaultheria shallon*. Additional observations derive from the work of Grelet et al. (2009a), showing that *M. bicolor* obtained from *Piceirhiza bicolorata* ECM formed typical ERM structures and engaged in reciprocal transfer of carbon and nitrogen with *V. vitis-idaea*.

Bergero et al. (2000) demonstrated that several fungi (**including *Oidiodendron* spp. and sterile morphotypes**) in a Mediterranean forest were associated with both ERM and ECM plants. Fungi isolated from root tips of ECM *Quercus ilex* formed ERM with *Erica arborea* in vitro, and molecular analyses indicated that some of these *Q. ilex* fungal associates were conspecific with ERM fungi naturally occurring in *E. arborea* roots.

Potential Hyphal Connection Between ERM and Non-Ericaceous Plants Have Been Further Identified

For example, at least six ITS-RFLP types of ERM fungi, including Helotiales and *Oidiodendron*, were common

root associates of a diverse array of plant taxa within a schlerophyll forest community in south-eastern Australia (Chambers et al. 2008). Similar results were obtained by Curlevski et al. (2009) and Grelet et al. (2010), who applied the more sensitive inter-simple sequence repeat (ISSR)-PCR to investigate the occurrence of fungi with the same ISSR fingerprints in co-existing ERM and ECM plants. Shared fungal genotypes were identified between the ERM species *E. pulchella* and the ECM species *Leptospermum polygalifolium* (Curlevski et al. 2009) in a schlerophyll Australian forest, and between the ERM species *V. vitis-idaea* and the ECM species *P. sylvestris* (Grelet et al. 2010) in a Scottish boreal forest.

From the experiments described above, there is now **strong evidence that potential hyphal connections between ERM and ECM plants are a common feature in different forest ecosystems**, although the work by Grelet et al. (2010) would exclude the formation of large mycelial networks at least for *M. variabilis*, given the small genet size found for this fungus (<13 cm). However, several questions remain open on the type of interactions that ERM fungi may establish with non-ericaceous host plants, and on the functional role of these potential hyphal connections.

Some observations suggest that, rather than forming an ECM association themselves, many **ERM fungi may associate with ECM root tips in addition to the true ECM partner.**

Although one isolate of the *C. finlandica*/*M. bicolor* clade in the "*R. ericae* aggregate" has been demonstrated to form both ERM and ECM, or ectendomycorrhizal, associations with tree species under gnotobiotic conditions (Villarreal-Ruiz et al. 2004), most other isolates seem to be able to form a single mycorrhizal type, despite their common occurrence in plant roots. Bergero et al. (2000) clearly showed, in their inoculation experiments, that ERM-forming fungi isolated from ECM *Q. ilex* were often morphologically distinct from those producing the ECM. Similarly, there is no clear evidence that *M. variabilis* can actually form ECM, and it seems more likely that *M. variabilis* is an endophyte in ECM root tips formed by other fungi (Hambleton and Sigler 2005; Grelet et al. 2010). This would give support to the observation of endophytic behaviour of *M. variabilis* (Vohník et al. 2007; Ohtaka and Narisawa 2008), or the amplification of Helotiales DNA sequences from ECM formed by other fungal species (Tedersoo et al. 2009).

Occurrence of ERM Ascomycetes in Non-Ericaceous Hosts Is Not Restricted to ECM-Forming Plants

As already mentioned, Helotiales forming ERM coils in hair roots of *Woollsia pungens* (Ericaceae) under gnotobiotic conditions were isolated from the roots of 17 species from different plant families (Apiaceae, Cunoniaceae, Cyperaceae, Droseraceae, Fabaceae—Mimosoideae, Lomandraceae, Myrtaceae, Pittosporaceae, Proteaceae, Stylidiaceae) at an Australian schlerophyll forest site (Chambers et al. 2008).

In cold climates, **dark septate endophytes (DSE)** are widely distributed and frequently isolated from the roots of several plant species (see references in Jumpponen and Trappe 1998; Mandyam and Jumpponen 2005). Some members of the "*R. ericae* aggregate" are well recognised DSE: in addition to *M. variabilis*, *Cadophora finlandica* is commonly identified in the roots of mainly ECM plant species (Wilcox and Wang 1987; Mandyam and Jumpponen 2005). They have been identified also in Antarctica on *Colobanthus quitensis* and *Deschampsia antarctica* (Newsham et al. 2009). The ecological functions of DSE are not well understood, but they can provide several benefits to their host plants, including facilitation of nutrient uptake, protection from metal stress tolerance and stimulation of the mycorrhizosphere community against root diseases (Jumpponen 2001; Mandyam and Jumpponen 2005; Dos Santos Utmazian et al. 2007; Alberton et al. 2009).

A study by Abuzinadah and Read (1989) showed that *Oidiodendron* also enhances growth of *Betula pendula* on a medium containing proteins as sole nitrogen source, without producing a classical ectomycorrhizal infection. Similarly, endophytic fungi isolated from roots of the grass *Deschampsia flexuosa* and identified in the Helotiales have been shown to colonise roots and enhance nitrogen uptake by *C. vulgaris* (Ericaceae) seedlings, and vice versa (Zijlstra et al. 2005). As discussed by Curlevski et al. (2009), many questions remain on the relationships between fungi that form ERM and DSE associations, particularly with regard to the benefits conferred to plants by the DSE infection (Schulz and Boyle 2005).

The ability to infect multiple hosts raises the possibility that **ERM associations in ericoid roots and DSE associations in non-ericaceous roots might represent parts of a common mycelial network**.

Hyphal links playing important functions in nutrient exchange, including organic carbon, have been demonstrated for ECM fungi (Simard et al. 1997), and for more complex situations involving plants with different mycorrhizal status (e.g. McKendrick et al. 2000; Bidartondo et al. 2003). The role of **possible physical links between ericaceous and non-ericaceous plants via their fungal associates remains an open question**, as well as the nature of the functional relationships with these hosts. It also remains to be established whether or not the same fungus can form different types of mycorrhizal symbioses with distinct hosts in natural conditions. The classical bioassay to establish the mycorrhizal nature of a fungal isolate is the inoculation in vitro onto axenic seedlings. Although this assay remains an important test to elucidate the mycorrhizal potential of a fungal isolate, the conditions used are very different from those found in nature and the results must be interpreted with caution (Read 1996).

In addition to a possible role in nutrient exchange, the association of ERM fungi with non-ericaceous plants may be ecologically relevant in some stressed conditions. For example, genetic relatedness between ERM and ECM fungal associates was found in forests subjected to fire (Bergero et al. 2000; Vrålstad et al. 2000), and it was suggested that the ECM host, with deeper roots, could provide an efficient source for biotrophic infection of the ERM (or other) plants. A role as a reservoir of ericoid fungi has also been suggested for liverwort rhizoids by Duckett and Read (1995).

III. Exploitation of Inorganic and Organic Substrates by Ericoid Mycorrhizal Fungi

Habitats dominated by Ericaceae include most humus heathlands in the Northern hemisphere,

Mediterranean woodlands, tropical cloud forests and the dry sand plains of Australia (Read 1991; Straker 1996; Bergero et al. 2000). **Soils colonised by ERM plants** are characterised by the low availability of nutrients, due to slow litter decomposition and mineralisation processes. Here, essential nutrients such as N and P are found almost exclusively in organic forms (Read and Perez-Moreno 2003). In these ecosystems, the dominant plant species are highly dependent on the mycorrhizal symbionts for their nutrient supply. Although ERM fungi appear to have a poorly developed extraradical mycelial phase, they are able to mobilise organic nutrients rendering them accessible to the host roots (Finlay 2008).

In **mor-humus heathlands,** the **limiting nutrient is primarily nitrogen**, which is in the form of acid-hydrolysable organic compounds and insoluble humin, usually inaccessible to plants (Stribley and Read 1974). The success of Ericaceae in these stressful habitats is therefore largely related to the abilities of their ERM symbionts to improve nutrient acquisition, particularly nitrogen, from organic forms. Several studies on ERM mycelia growing in vitro (see Smith and Read 1997, 2008) have confirmed their high saprotrophic capabilities, which would facilitate nutrient mobilisation from the substrates exploited by the mycorrhizal roots and the mycelium. For example, Read and Perez-Moreno (2003) pointed out that the enzymatic capabilities of ERM fungi could extend the sources of N important to their plant hosts beyond inorganic forms to include amino acids, amino sugars, proteins, or chitin. In return for N and other nutrients, plants probably allocate up to 20 % of net primary production to their mycorrhizal symbionts (Hobbie 2006). Mobilised nutrients from organic forms can then be assimilated by plant roots, either directly or via the fungal intermediary.

A. Nitrogen and Phosphorus Uptake

Organic nitrogen (ON) accounts for up to **95 % of the soluble N pool in soils** (Abuarghub and Read 1988; Talbot and Treseder 2010). This pool

represents an important component of plant N budgets, considering that inorganic N pools in soil are insufficient to account for annual plant N uptake in many ecosystems (Kielland 1994). **Mycorrhizal plants have greater access to ON than non-mycorrhizal plants** (Schimel and Bennett 2004). The uptake of ON by ERM fungi and the subsequent transfer to the plant require multiple steps, including breakdown of polymers in soil solution, direct uptake of mono- and oligomer into mycorrhizal fungi, internal transformation of ON, and transfer across the fungus—host plant interface (Talbot and Treseder 2010). Identifying which mechanisms most strongly control each of these steps will help in understanding the extent of ON usage by mycorrhizal plants (Talbot and Treseder 2010). The **major classes of ON compounds found in soils and in soil solution include aliphatic-N, like amino-N and polysaccharide-N, and aromatic-N**, such as the compounds present in soil humus (Roberts and Jones 2008). The high soil ON content indicates that the exposure of mycorrhizal roots to ON can be equal to or greater than exposure to inorganic N in most soils (Talbot and Treseder 2010). The forms of ON that are transferred to the plant may vary among fungal and plant species, with glutamine, asparagine, and alanine being the most common ON compounds involved in this process (Chalot and Brun 1998). Systems that are dominated by ERM fungi that have the capability to produce a broad range of extracellular enzymes, such as *R. ericae* (Cairney et al. 2000; Midgley et al. 2006), may present a particularly high rate of ON uptake.

A **significant pool of available nitrogen in heathland soils** is represented by **free amino acids** (Abuarghub and Read 1988). *R. ericae* has been shown to absorb a broad range of amino acids (Chen et al. 1999; Whittaker and Cairney 2001) and to effect transfer of aminonitrogen to its hosts, although considerable intraspecific variation can exists in this regard (Bajwa and Read 1986; Cairney et al. 2000). The utilisation of sulfur-containing amino acids by *R. ericae* is poor but, in common with other filamentous fungi, this may be enhanced under sulfur starvation (Bajwa and Read 1986). In vitro culture

studies indicate that some ERM fungi can use amino acids as their sole N source (Talbot and Treseder 2010). Amino acids vary in their frequency within proteins, with leucine, glycine and alanine being most common and tryptophan, cysteine and histidine rarest. Talbot and Treseder (2010) found that the percentage of ERM fungal species capable of using a given amino acid was significantly and positively correlated with its relative abundance in proteins.

Early work on the physiology of ERM fungi and their influence on plant nutrient uptake was on a limited number of ERM strains, mainly *R. ericae* (Leake and Read 1991). However, a single root system can harbour several ERM fungal genotypes, which may also belong to different species. It became therefore important to understand whether **this genetic diversity confers functional diversity** which could be **advantageous in nutrient-poor habitats**.

Cairney et al. (2000) studied various fungal strains isolated from hair roots of *C. vulgaris* and showed significant variations in the N utilisation pattern. While most isolates showed a preference for ammonium as a sole nitrogen source, considerable variation was observed in the abilities of isolates to utilise amino acids and proteins (e.g. BSA). In particular, large intraspecific variation was observed in the use of glutamine and BSA. Individual isolates of *R. ericae* may therefore vary considerably in their abilities to use organic nitrogen from different substrates in soil. Grelet et al. (2005) showed that the different abilities to utilise organic and mineral N sources were affected by carbon availability in a strain-specific manner. Under elevated C supply, growth differences among strains were linked to the total amount of nitrogen taken up, suggesting variation in uptake kinetics. But, under C-limiting conditions, the nitrogen-use efficiency explained strain differences, implying intraspecific variations in N metabolism. The main effect of reducing C availability was to increase the relative ability of most strains to grow on glutamine and nitrate, in comparison to ammonium. Grelet et al. (2009b) investigated whether the functional variation was maintained in symbiosis. *Vaccinium* plants were inoculated with three genetically closely related ERM fungal strains known to differ in their N use in liquid culture. ^{15}N was used to trace N uptake into shoots, and the results demonstrate that functional differences among closely related ERM fungi are maintained in symbiosis with their hosts, thus suggesting that N transfer to plant shoots in ericoid mycorrhiza is under fungal control. In the experimental conditions used by

Grelet et al. (2009b), the strain-specific effect was evident when N was supplied as ammonium or glutamine, but fungal influence on plant N uptake has also been shown for a range of amino acids (Sokolovski et al. 2002) and for nitrate (Kosola et al. 2007).

These experiments give support to the hypothesis that a **broad spectrum of symbiotic ERM fungi may provide the host plant with a wider set of acquired symbiotic functions**.

In **acidic heathland soils**, a **very low amount of free inorganic phosphate** is found, and the **main phosphorus sources are organic compounds in the form of phosphomonoesters, mainly phytates** (Cosgrove 1967; Mitchell and Gibson 2006), or phosphodiesters such as nucleic acids (Griffiths and Caldwell 1992). Phytates are usually complexed with iron and aluminium, and ERM fungi are able to access these sources (Mitchell and Read 1981). **Both extracellular and wall-bound phosphatases are produced** by a number of ERM isolates (Straker and Mitchell 1986). Phosphomonoesters are quantitatively the most important fraction of organic soil phosphorus, while phosphodiesters are found in relatively low concentrations but are potentially valuable phosphorus sources (Griffiths and Caldwell 1992; Leake and Miles 1996; Myers and Leake 1996). Leake and Miles (1996) demonstrated the ability of R. ericae to degrade DNA molecules through the production of phosphodiesterases and to directly assimilate nucleotides.

Pearson and Read (1973b) provided evidence for a movement of P-32 orthophosphate from the fungus to the plant. Two phosphate membranes transport systems were identified for a South African isolate as high affinity and low affinity systems (Straker and Mitchell 1987). Interestingly, both P uptake systems operate better under the pH conditions typically found in the acidic soils where ERM fungi live.

Many fungi can dissolve phosphate and other essential nutrients from mineral substrates by employing several mechanisms, including protonation and chelation (Gadd 2010). In terms of mineral weathering and dissolution, mycorrhizal fungi form one of the most prominent groups of soil microorganisms (Devevre et al. 1996; Jongmans et al. 1997;

Lundström et al. 2000). A number of ERM fungi has been investigated for their phosphate solubilising ability. Van Leerdam et al. (2001) showed that most isolates could solubilise the rock phosphate hydroxyapatite in the presence of ammonium, whereas Martino et al. (2003) and Gibson and Mitchell (2004) showed that O. maius and R. ericae-type isolates were capable of solubilising zinc phosphate, and some of these isolates were also able to solubilise calcium phosphate.

B. Depolymerisation of Complex Substrates

The array of hydrolytic enzymes produced by ERM fungi enables their saprotrophic growth in the absence of the host (Perotto et al. 1993, 1997), but may also mediate utilisation of organic nutrient sources by the host plant (Cairney and Burke 1998). **Ericoid fungi produce a range of extracellular enzymes that catalyse breakdown of several organic macromolecules and give plants access to breakdown products of complex polymers** that cannot be directly assimilated by either plant roots (Read and Perez-Moreno 2003). This access appears particularly important in N-limited systems, where mineralisation is insufficient to support plant N demand (Schimel and Bennett 2004).

Two classes of polymer-degrading enzymes can be recognised (Read and Perez-Moreno 2003). The first class includes several hydrolases that cleave the nutrient containing molecules themselves, whereas the second class facilitates nutrient acquisition by attacking organic molecules such as lignins, polyphenols and tannins, which may protect or precipitate essential nutrients. Lignases and polyphenol oxidases are comprised in this type of enzymes, and are expected to give a major contribution to litter decomposition as well as to plant nutrition.

ERM fungi express both types of polymer degrading enzymes. In addition to **proteases** and **chitinases**, they produce **hydrolytic enzymes**, which degrade cell wall polysaccharides such as cellulose, hemicelluloses and pectin, as well as **phenol oxidase** activities, which may facilitate host access to nitrogen and phos-

phorus within moribund plant material or from polyphenol complexes in soil (Perotto et al. 1993, 1997; Varma and Bonfante 1994; Bending and Read 1996a, b; Burke and Cairney 1997a, b; Cairney and Burke 1998; Piercey et al. 2002; Midgley et al. 2004a, 2006).

The complete use of cell wall polysaccharides, such as cellulose, is not surprising, as these enzymes are presumably required for host cell penetration. Like cellulose, the potential of ERM fungi to degrade pectin is important during penetration of the root of the host. ERM fungi have been shown to produce polygalacturonases in culture (Perotto et al. 1993, 1997; Midgley et al. 2006). *R. ericae* is also able to grow on xylans thanks to the production of at least one **xylanase** and a range of accessory enzymes (Burke and Cairney 1997a, b).

Polyphenol oxidases are known to be produced by ERM fungi (Burke and Cairney 2002). These enzymes include laccase, catechol oxidase and tyrosinase, which show a considerable overlap in substrate affinities (Burke and Cairney 2002). *R. ericae* produces laccase, along with a range of related phenol-oxidising activities (Burke and Cairney 2002). It has been suggested that **laccases** produced by ERM fungi may be engaged in a number of processes involved in the functioning of the symbioses. Suggested roles include lignin and polyphenol degradation, release of N from insoluble protein—tannin complexes and degradation of polycyclic aromatic hydrocarbon pollutants (Leake and Read 1989; Hutchison 1990; Bending and Read 1996a, b, 1997; Braun-Lüllemann et al. 1999). Cairney and Burke (1998) assessed the production of ligninolytic activities by *R. ericae*. The fact that *R. ericae* releases hydrogen peroxide (Bending and Read 1997) and hydroxyl radicals (Burke and Cairney 1998) into culture media confirms that the fungus may contribute to a form of **lignin degradation**, similar to that seen in "brown rot" fungi, in which the fragmentation of the lignin polymer is mediated by these radicals (Burke and Cairney 1998; Cairney and Burke 1998). Carbohydrate oxidase activity may further release H_2O_2, which, via production of hydroxyl radicals in the presence of Fe, may contribute to partial lignin degradation by this fungus. Burke

and Cairney (1998) examined isolates of the ERM fungus *R. ericae* for their ability to oxidize carbohydrates to their corresponding lactones and to excrete the H_2O_2 produced thereby. *R. ericae* was found to express cellobiose oxidase and glucose oxidase when grown on cellobiose and glucose respectively.

Polyphenolic compounds can reach high concentrations in heathland soils (Jalal and Read 1983). In these phenol-rich environments, the **proven ability of ERM fungi to use many monomeric phenolic compounds as carbon sources** (Leake and Read 1989) and to release the enzymes laccase and catechol oxidase (Bending and Read 1996a, b, 1997), involved in the degradation of hydrolysable polyphenols, plays a fundamental role in plant nutrition.

Bending and Read (1996b) demonstrated that, in addition to degrading tannic acid-protein precipitates by releasing polyphenol oxidase, *R. ericae* and *Oidiodendron* sp. were able to acquire nitrogen from these complexes through the expression of **extracellular acid proteases**. Such observations indicate that the ability to absorb amino acids bypassing the N mineralisation process may be widespread in organic soils. *R. ericae* is in fact known to produce extracellular proteinases that, via the hydrolysis of simple proteins, release amino acids that can be absorbed by the mycelium (Read et al. 1989; Whittaker and Cairney 2001). Interestingly, *R. ericae* also secretes an extracellular proteinase identified as a carboxyl (acid) proteinase (Leake and Read 1989) that showed optimal activity at pH 2.2, which is considerably lower than that found in acidic heathland soils (Leake and Read 1990b). However, most of the fine mycorrhizal roots of ericaceous plants are confined to the soil/litter interface, where, due to the activity of H^+-releasing processes, the pH is lower than in the bulk soil (Read et al. 1989). This interface is also the location where protein substrates accumulate. The proteinase activity of the fungus is, therefore, adapted to the specific microenvironment in which it is found. Moreover, as the pH within plant cells approaches neutral, the low pH optimum of the proteinase may also protect the plant from protein degradation (Leake and Read 1989). It has also been

proposed that excess proteinase is only released by the fungus if a suitable substrate is present in the soil (Leake and Read 1990c). The end products or amino acids can be taken up directly by the ERM fungus without further deamination and these forms of nitrogen would also be ideal for transfer from the fungus to the host plant.

ERM fungi from Ericaceae growing in the Southern hemisphere appear to be broadly similar to *R. ericae* in their abilities to utilise different carbohydrates sources, as well as amino acids and proteins as nitrogen sources (Chen et al. 1999; Whittaker and Cairney 2001; Midgley et al. 2004b).

Using **crustacean chitin as a model compound,** it was demonstrated that *R. ericae* and *Oidiodendron griseum* could cleave the polymer to its constituent subunits, which were readily assimilated (Leake and Read 1990a; Mitchell et al. 1992). A significant proportion of the N acquired by the fungus was found to be transferred to aseptically grown mycorrhizal plants of *V. macrocarpon* (Kerley and Read 1995). *R. ericae* has also been shown to supply its host plant with nitrogen directly from chitin (Kerley and Read 1997), providing strong evidence that chitinase activities are produced by the fungus during symbiosis. Bougoure and Cairney (2006) also showed that an isolate of *R. ericae* from *E. pulchella* produced both endo- and exo-acting chitinolytic activities.

Concerning the acquisition of P from polymeric organic sources, both DNA in purified form (Leake and Miles 1996) and entire nuclei (Myers and Leake 1996) have been shown to represent useable P sources for *V. macrocarpon*. P mobilisation appeared to depend upon production of phosphodiesterases by *R. ericae* (Leake and Read 1997).

A **significant consumption of C and N is required for extracellular enzyme production** (Schimel and Weintraub 2003). In fact, it has been shown that plant C supply to the fungal symbiont can control extracellular enzyme production and the acquisition of nutrients from polymeric compounds, and that ERM fungi acquire more N from high molecular weight organic nitrogen compounds when grown with the plant (Bajwa and Read 1986; Dighton

et al. 1987; Gunther et al. 1998). Under elevated glucose concentrations in the culture medium, *R. ericae* acquired a higher percentage of N from glutamine (Grelet et al. 2005), and protein mineralisation by other ERM fungal isolates also increased (Zhu et al. 1994; Eaton and Ayres 2002). Under the same glucose concentration, the expression of extracellular proteases (Nehls et al. 1999) and chitinases (Leake and Read 1990a; Bougoure and Cairney 2006) decreased in other ERM fungi. There is therefore evidence that C availability increases uptake of amino acids by ERM fungi, and that a strong C regulation of extracellular enzyme activities operates by induction/repression mechanisms (Talbot and Treseder 2010).

IV. Ericoid Mycorrhizal Fungi in Soils Enriched in Toxic Compounds

Since the first investigations by Bradley et al. (1981, 1982), **the success of Ericaceae in stressful habitats** has been ascribed to the **unique abilities of their ERM fungal partners to withstand and adapt to environmental stresses and to enhance stress tolerance in their host plants** (Cairney and Meharg 2003).

Environmental stress can shape adaptation and evolution of organisms living in changing environments, and the impact of human activities on natural environments has caused rapid and often stressful and deteriorating changes. The occurrence of adaptive processes is clearly shown by the fact that many organisms have acquired quite rapidly the ability to withstand man-made changes in the environment (Nikolaou et al. 2009).

Heavy metal toxicity represents a strong selection pressure and microbial resistance to toxic metals is widespread, with frequencies ranging from a few per cent in pristine environments to nearly 100% for bacteria growing in heavily polluted environments (Silver and Phung 2009). Adaptation of ecto- and endomycorrhizal fungi to heavy metal soil pollution of anthropic origin is also supported by several studies (e.g. Leyval et al. 1997; Meharg and Cairney 2000; Colpaert et al. 2004; Adriaensen et al. 2005; Krznaric et al. 2009).

A. Genetic and Functional Adaptation to Heavy Metals

As already described, soils naturally colonised by Ericaceae are generally acidic. **Low pH and anaerobic soil conditions facilitate mobilisation of heavy metal ions**, which are toxic above threshold concentrations (Meharg and Cairney 2000). Bradley et al. (1981, 1982) first demonstrated the **importance of ERM fungi to increase resistance** of *C. vulgaris* to heavy metals, and other authors later described metal tolerance in fungal isolates from sites with different pollution. In particular, ERM plants in association with members of the "*R. ericae* aggregate" have been shown to survive in arsenate-rich sites (Sharples et al. 2000b) and populations of arsenate resistant *R. ericae* have been isolated from *C. vulgaris* growing in As/Cu-contaminated mine soils (Sharples et al. 2001). A detailed study on more than 70 ERM fungi from polluted and non-polluted sites indicated an adaptive resistance to arsenate in the fungal populations from the mine site (Sharples et al. 2001). Strains of the ERM species *O. maius* also showed adaptation to metal pollution, as isolates from an industrial site heavily polluted with Cd, Zn and Al displayed better growth in vitro on media containing these metals, when compared with isolates from non-polluted sites (Martino et al. 2000a).

Ericoid fungal strains of *O. maius* derived from polluted and unpolluted soils also mobilised insoluble inorganic zinc compounds to different extents (Martino et al. 2003). Strains from polluted soils showed little ability to solubilise Zn from both ZnO and $Zn_3(PO_4)_2$, whereas strains from unpolluted soils showed a higher solubilisation potential. As solubilisation of insoluble metal compounds may lead to toxic metal concentrations in soils enriched with heavy metals, this mechanism may be adaptive.

In addition to being polluted by human activities, **soils may be naturally enriched in heavy metals derived from specific rock substrates**. Serpentine soils derived from ultramafic rocks are low in plant nutrients such as K and Ca, but contain high levels of potentially toxic elements such as Ni and Cr. Vallino et al. (2011) compared metal tolerance of *O. maius*

isolates derived from sites with contrasting metal pollution (industrial Cd/Zn pollution versus natural Cr/Ni pollution). Despite the small sample size, a significant difference was observed in growth of the *O. maius* isolates on media containing these metals. Strains more tolerant to Cr and Ni were those originated from the serpentine site, while strains more tolerant to Zn and Cd were isolated from the industrial sites polluted by these contaminants (Vallino et al. 2011). This would suggest, at least for some metals, a specific adaptation of *O. maius* that reflects the specific contamination in the soil of origin.

For other metals, such as copper, adaptive heavy metal tolerance is more controversial. For example, ERM fungi of *C. vulgaris* have been shown to display constitutive tolerance to copper (Bradley et al. 1982; Sharples et al. 2001). However, extracellular phosphodiesterase activity was stimulated by copper in ERM fungal isolates from mine spoil sites but not in fungi from uncontaminated sites (Gibson and Mitchell 2005). This result has been interpreted as an adaptive metal avoidance mechanism, as phosphate ions released from organic substrates by phosphatases would react with metal ions to form insoluble, and therefore less toxic, compounds (Gibson and Mitchell 2005).

Increased metal tolerance in ERM fungi can therefore arise, under selective pressure, as a consequence of new or modified phenotypic traits. As the phenotype depends on the cell genetic information, changes in gene sequences and arrangement may be expected to have arisen in those ERM fungi adapted to environmental metal stress. Mutations are one of the primary sources of genetic variation, and many environmental agents are known to induce mutations either directly or indirectly, through the production of reactive oxygen species (Hartwig 1995).

A recent study (Vallino et al. 2011) suggests that **the mutation rate positively correlated with environmental stress within the same ERM fungal species, and that mutations do not occur randomly in the genome**. Two gene regions with different functional roles in heavy metal resistance (the ribosomal ITS and the gene coding for the Cu/Zn superoxide dismutase) were compared among isolates of
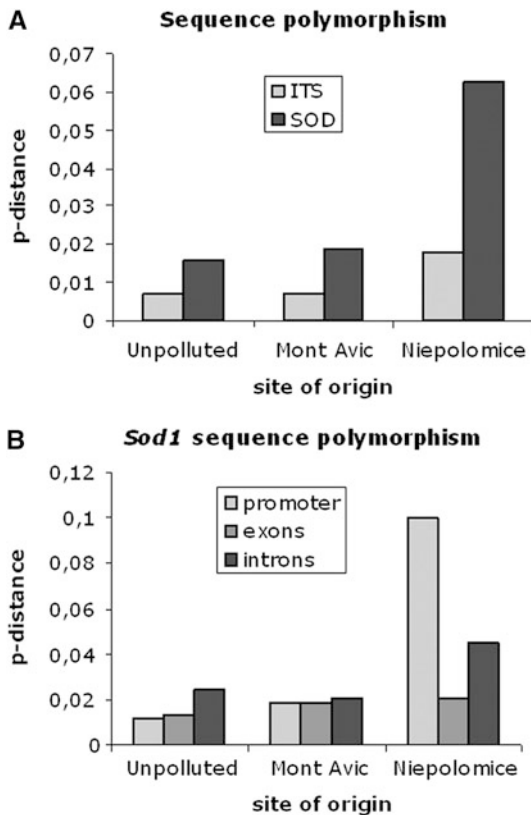
Fig. 14.4. Sequence polymorphism of the ITS and the SOD loci. (**A**) p-Distance of the ITS region and the SOD in ericoid mycorrhizal strains derived from three sites respectively non-polluted, naturally polluted (serpentine) and industrially polluted. (**B**) p-Distance of different regions of SOD locus in the same three groups of fungi (Modified from Vallino et al. (2011))

O. maius derived from non-polluted soil, and from soils with natural and industrial heavy metal pollution (Vallino et al. 2011).

The ribosomal ITS region can be considered as a "neutral" gene towards metal tolerance, whereas Cu/Zn SOD plays a major role in cell defence against toxic reactive oxygen species, which are increased by environmental stress, including heavy metals (Schützendübel and Polle 2002). The role of the *Sod1* gene (coding for the *O. maius* Cu/Zn SOD) in metal tolerance was recently demonstrated in *O. maius* by Vallino et al. (2009) and Abbà et al. (2009).

As compared with the "neutral" ITS, a higher mutation rate was found in the functional *Sod1* locus of strains from all sites (Fig. 14.4), suggesting that **genes with a functional role**

in fungal survival display a higher nucleotide polymorphism than neutral genes (Vallino et al. 2011). Moreover, a significantly higher mutation rate was found in isolates from heavily polluted industrial soils, when compared with isolates from non-polluted or naturally polluted soils (Fig. 14.4). These observations are in agreement with the finding, derived from genomic comparisons, that **fungal stress signalling pathways** are evolving rapidly and in a niche-specific fashion to **protect different species against the contrasting environmental stresses** they encounter in their diverse habitats (Nikolaou et al. 2009). Interestingly, in the *O. maius* isolates from polluted sites, most mutations were in the *Sod1* gene promoter region, rather than in the coding sequence (Vallino et al. 2011), suggesting that mutagenesis induced by environmental stress may also target specific gene regions. A similar situation was observed in the promoter region of metallothionein genes in a cadmium tolerant population of the collembola *Orchesella cincta* (Janssens et al. 2007).

B. Cellular and Molecular Mechanisms of Heavy Metal Tolerance

Metals are directly or indirectly involved in all aspects of microbial growth, metabolism and differentiation (Gadd 1993, 2010). Several metals play essential functions in the organisms, and insufficient levels of essential metals can result in stress responses just as severe as those resulting from excess metals. Therefore, cells must have developed mechanisms to maintain metal homeostasis (Tomsett 1993). By contrast, some heavy metals such as Cs, Al, Cd, Hg and Pb have no known functions in most organisms and are therefore toxic at all concentrations. Molecular recognition allows organisms to differentiate between essential and non-essential ions and, if necessary, to partition them in different ways.

The cellular and molecular mechanisms potentially involved in **metal tolerance in fungi**, like in other organisms, can be classified into three groups: (i) *avoidance* mechanisms that restrict entry of metal ions into the cytoplasm, and relies on decreased uptake or increased efflux of metal ions, or by their

immobilisation outside the hypha by biosorption to cell walls, pigments and extracellular polysaccharides, (ii) *sequestration* mechanisms that reduce the concentration of free metal ions in the cytosol and include metal-binding peptides and proteins which regulate metal ion homeostasis, as well as intracellular compartmentation and (iii) *antioxidative* mechanisms that allow the fungus to directly or indirectly counteract accumulation of ROS and oxidative stress. Some of these mechanisms are constitutively present, whereas others are only activated when metals exceed a threshold value (Colpaert et al. 2011). Most information on the mechanisms of heavy metal tolerance in mycorrhizal fungi derive from work on ECM fungi (e.g. see Courbot et al. 2004; Bellion et al. 2006; Colpaert et al. 2011), although the most recent work on ERM fungi, described later in this chapter, opens up the possibility to use some ERM isolates as model systems to investigate heavy metal tolerance.

Fungal mycelia often have a high sorption capacity for metals, so binding of metals to the hyphal surface may represent a substantial fraction of the metal accumulated by mycelia (Gadd 1993, 2010; Leyval et al. 1997). Biosorption to cell walls, pigments and extracellular polysaccharides have been reported for ERM fungi (Bradley et al. 1981; Denny and Ridge 1995; Martino et al. 2000a).

Fungal weathering can release essential metals and nutrients from insoluble minerals, but it can also increase final concentrations of toxic metals in the soil. **Biotransformation of solubilised metals into insoluble organic forms** is a **common phenomenon in both ECM and ERM fungi** (Gadd 2010). Biotransformation of Zn was observed in *O. maius* isolates from soils heavily polluted with heavy metals which were found to cause precipitation of organic zinc (Martino et al. 2003). Similarly, *R. ericae* exposed to uranium demonstrated a high tolerance to uranium oxides and was found to form extracellular crystalline precipitates of uranium-containing minerals on the fungal mycelia (Fomina et al. 2007), likely with the involvement of oxalic acid.

Fungi can respond to the presence of metals with the **release of specific proteins in the surrounding medium**. It was found for example that the presence of zinc in the culture medium sharply increased in *O. maius* the secretion and activity of extracellular enzymes that hydrolyse the pectin component of plant cell walls (Martino et al. 2000b). The significance of this increased production is unclear, but oligalacturonans may function as better metal chelators than larger polymers, thus protecting the fungus during saprotrophic growth. Zinc ions also induced in *O. maius* a general change in the array of secreted proteins, with a shift towards the production of more basic, low molecular weight polypeptides (Martino et al. 2002).

Intracellular metal concentrations in fungi may be regulated by transport, including efflux mechanisms and internal compartmentation. **Metal transporters** are major players in keeping the cytosolic concentrations compatible with metabolic activities, and they may either extrude metal ions out of the cell as plasma-membrane transporters, or sequestrate metals in intracellular compartments, usually the vacuole (Fig. 14.5). Most of our knowledge on metal transporters in fungi comes from studies on the yeast *Saccharomyces cerevisiae* (Rutherford and Bird 2004), but both mechanisms have been identified in ECM fungi (e.g. Blaudez et al. 2000; Blaudez and Chalot 2011; Bolchi et al. 2011; Colpaert et al. 2011).

In ERM fungi, **metal transporters involved in metal efflux** were identified by Sharples et al. (2000b, 2001) in arsenate-resistant isolates of *R. ericae*. These isolates accumulated arsenate, which is a phosphate analogue and is transported by the phosphate uptake system. However, compared to isolates from unpolluted sites, *R. ericae* isolates from As/Cu mines had the capacity to reduce arsenate to arsenite, which was rapidly expelled from their mycelium via an increased efflux system (Sharples et al. 2000b, 2001).

Fungi may also decrease cytosolic free metal through the synthesis of a variety of **metal-binding peptides and proteins**. Among them, essential components of Cd detoxification pathways
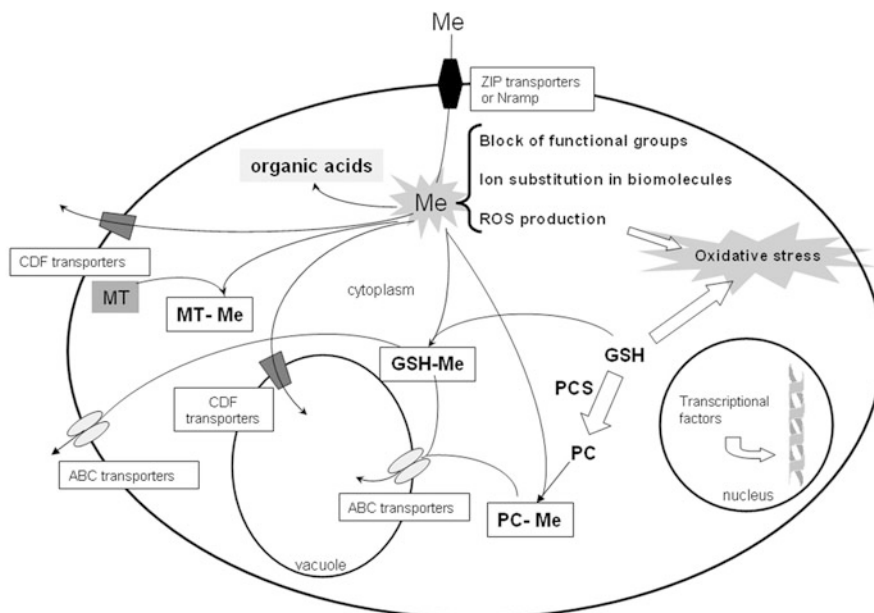
Fig. 14.5. Mechanisms involved in the intracellular detoxification or organellar compartmentation of heavy metals. A variety of mechanisms may be involved in transport phenomena contributing to decreased uptake and/or increased efflux. A variety of specific or non-specific mechanisms may also lead to intracellular chelation and intracellular precipitation, and to ROS detoxification

in various organisms are thiol compounds, including reduced glutathione, phytochelatins, and metallothioneins. **Metallothioneins** have been identified in most types of mycorrhizal fungi: they have been studied in both ECM fungi (Courbot et al. 2004; Bellion et al. 2006; Ramesh et al. 2009; Bolchi et al. 2011) and arbuscular mycorrhizal fungi (Lanfranco et al. 2002; Gonzlez-Guerrero et al. 2007), where they were mostly found to be regulated. A cDNA coding for a Cu-metallothionein was found in the ERM fungus *O. maius* by Vallino et al. (2005), but gene expression was not affected by Zn treatment.

Thiol compounds such as glutathione and phytochelatins are major players in the anti-oxidative response in plants and in some fungi (Courbot et al. 2004; Yadav 2010). There is currently only very scanty information on the thiol-dependent antioxidant systems in ERM fungi, with the identification of a thioredoxin gene in the *O. maius* cDNA library (Vallino et al. 2005). By contrast, the complete thiol-dependent antioxidant systems of the ECM fungus *Laccaria bicolor* (Morel et al. 2008) and of *Tuber melanosporum* (Bolchi et al. 2011) have been recently characterised at the genome scale.

In addition to thiols compounds, fungi can protect themselves from the oxidative stress caused by heavy metals through the synthesis **of enzymatic antioxidants** such as **catalase, peroxidase and superoxide dismutase** (Jacob et al. 2001; Guelfi et al. 2003; Todorova et al. 2008). In *O. maius* Zn, two extracellular proteins showed high homology with superoxide dismutases (SOD), that play a protective role against free O^{2-} radical toxicity, catalysing their conversion in hydrogen peroxide and oxygen (Fridovich 1995). Their induction by heavy metals has been described by several authors in plants, animals and microorganisms (Chongpraditnun et al. 1992; Yoo et al. 1999; Vido et al. 2001). Treatment with high concentrations of zinc ions resulted in an increased amount and activity of both intracellular and extracellular SOD enzymes in an *O. maius* isolate from a polluted site (Martino et al. 2002; Vallino et al. 2009). The Cu/Zn SOD seems to be induced in *O. maius* specifically by zinc, as suggested by a comparative high-throughput proteomic investigation to elucidate common and specific responses of *O.*

maius Zn to zinc and cadmium (Chiapello et al., unpublished data). The increased production of an extracellular Cu/Zn SOD, by binding metals and by performing its specific enzyme activity, could help both the fungus and the host plant to cope with the ROS formed in the extracellular medium, caused by metal pollution. Further demonstration of the important role of the Cu/Zn SOD enzyme in *O. maius* metal tolerance derived from heterologous expression of the corresponding gene in yeast (Vallino et al. 2009) and from the gene knock-out via homologous recombination and gene disruption (Abbà et al. 2009). Compared with the wild-type strain, the *O. maius* SOD1-null mutants showed a significant increase in zinc and cadmium sensitivity.

C. Omics Approaches to the Study of Heavy Metal Tolerance

Investigations on specific components (e.g. transporters, SODs, chelating metabolites) described in the previous paragraphs demonstrate that they may play significant roles in heavy metal tolerance in ERM fungi. However, this targeted approach often relies on existing knowledge acquired in other biological systems, and may miss specific mechanisms operating in the organisms under investigation, or general mechanisms so far unidentified. Untargeted approaches are therefore an important source of novel information, especially if they are supported by functional assays (Ruytinx et al. 2011).

The first attempt to investigate ERM fungal genes involved in zinc metal tolerance was through the sequencing of a small EST collection (Vallino et al. 2005). Variation in **gene expression** after treatment with high concentrations of Zn was monitored on 130 unigenes by reverse Northern blot hybridisation (Fig. 14.6): 16 unigenes were shown to be either up or down regulated. Among the differentially regulated genes, Vallino et al. (2005) could not find any previously reported heavy metal responsive or stress-related genes.

Most of the genes identified in the EST library and included in the cell defence category (e.g. Cu-metallothionein, Cu/Zn SOD, ascorbate peroxidase, thioredoxin, heat shock proteins) were not affected by the Zn treatment. This may be because the tolerant strain *O. maius* Zn (whose mRNA was used to construct the library and to hybridise the reverse Northern blots) does not perceive the metal concentration in the growth medium (10 mM Zn) as a serious stress condition, or because these genes may be involved at an earlier stage.

An untargeted approach using **genomic microarray** has been recently applied to *Cadophora finlandica* (Gorfer et al. 2009). *C. finlandica*, as described in Sect. II.C, can potentially associate with both ERM and ECM hosts (Vrålstad et al. 2002b). It is frequently found in heavy metal polluted habitats, and a possible functional role in heavy metal resistance by the host plants has been suggested (Vrålstad et al. 2002a). The genomic microarray analysis was conducted on a metal tolerant isolate of *C. finlandica* derived from an ECM *Salix caprea* root tip growing on a heavily contaminated soil (Dos Santos Utmazian et al. 2007) and compared the expression profiles on media supplemented with different metals (Gorfer et al. 2009). Many of the newly identified heavy metal regulated genes in *C. finlandica* encoded proteins of unknown functions or proteins with no established roles in heavy metal detoxification, but a large set of regulated genes were predicted to encode extracellular or plasma membrane proteins (e.g. transporters or secreted proteins). The majority of heavy metal defence activities in *C. finlandica* is therefore thought to take place outside the cell (Gorfer et al. 2009). It remains to be established if the *C. finlandica* isolate investigated in this study is truly mycorrhizal on ericaceous hosts.

Construction of cDNA libraries from ERM fungi growing on metal-containing medium, coupled with a functional assay in metal sensitive yeast mutants has allowed the identification of novel genes involved in metal tolerance in *O. maius*. In particular, a cDNA library obtained from *O. maius* growing on Cd was transformed in the *S. cerevisiae* cadmium-sensitive mutant y*ap1*. Most yeast transformants able to grow on cadmium were found
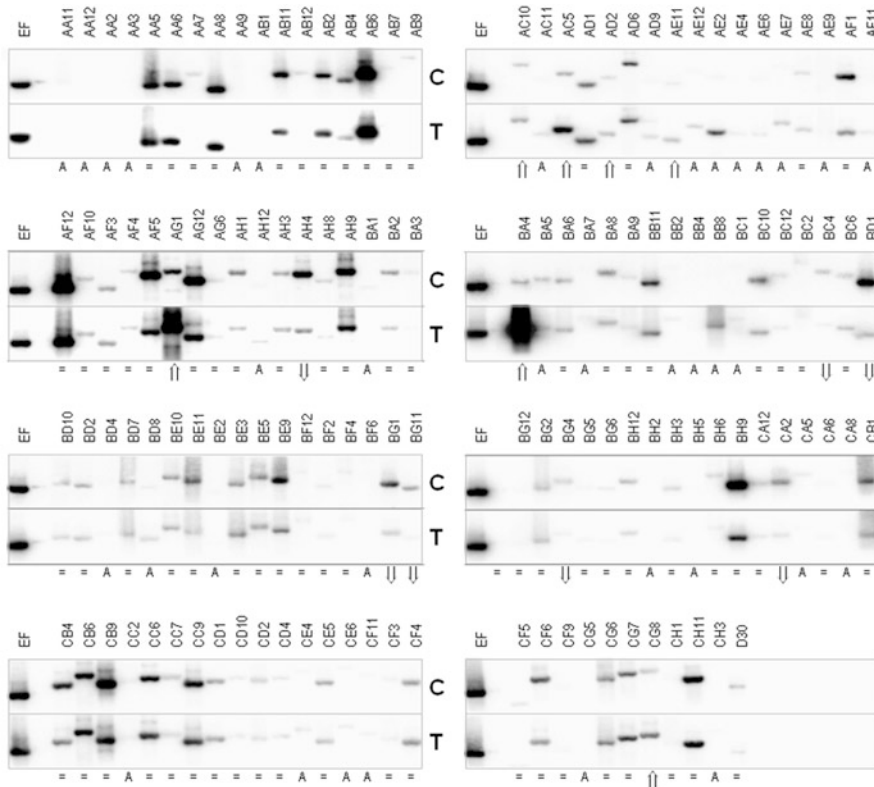
Fig. 14.6. Reverse Northern blot hybridisation of part of the *O. maius* Zn EST unigenes identified by Vallino et al. (2005), with two probes obtained from mycelia grown for 20 days in the absence of Zn (*C*) or with addition of 10 mM Zn (*T*). *EF* Elongation factor 1α (housekeeping gene used for data normalisation), *A* absent signal (ratio signal/background <3), ⇑ up-regulated genes (ratio treated/control >3.0), ⇓ down-regulated genes (ratio treated/control <0.3), = not regulated genes (ratio treated/control between 0.3 and 3.0) (Modified from Vallino et al. (2005))

to harbour an insert coding for the same PLAC8 domain-containing protein, named OmFCR (*O. maius* fungal cadmium resistance) because of the sequence similarity with a plant protein involved in cadmium resistance (Abbà et al. 2011). The PLAC8 domain is widespread and evolutionary conserved in several proteins found in all eukaryotic kingdoms, but has no assigned biological role.

When tested on a number of different metals and stressful conditions, the OmFCR protein was found to confer specific resistance to cadmium. Direct measurement of cadmium in exposed yeast cells indicate no changes in this metal content caused by the presence of OmFCR (Abbà et al. 2011). This result would exclude that OmFCR is either a membrane efflux pump or a heavy metal chelator, as the expression of an efflux pump would decrease the cellular Cd content in the cell, while an intracellular chelator would increase it. The use of the yeast two-hybrid assay, followed by a number of experiments with specific yeast mutants illustrated by Abbà et al. (2011), indicates that OmFCR confers cadmium resistance to yeast cells through its interaction with Mlh3p, a subunit of the mismatch repair (MMR) system. The MMR pathway repairs base-base mismatches and insertion/deletion loops that arise from DNA duplication, as well as mismatches in heteroduplexes that are formed during recombination (Fishel and Kolodner 1995).

Cadmium is known to **bind to the MMR system** and to **reduce its capacity to recognise small misalignments and base—base mismatches** by disrupting its structure and function (Jin et al. 2003). Unlike many other genotoxic metal ions, cadmium, in fact, does not inflict direct damage on DNA, proteins
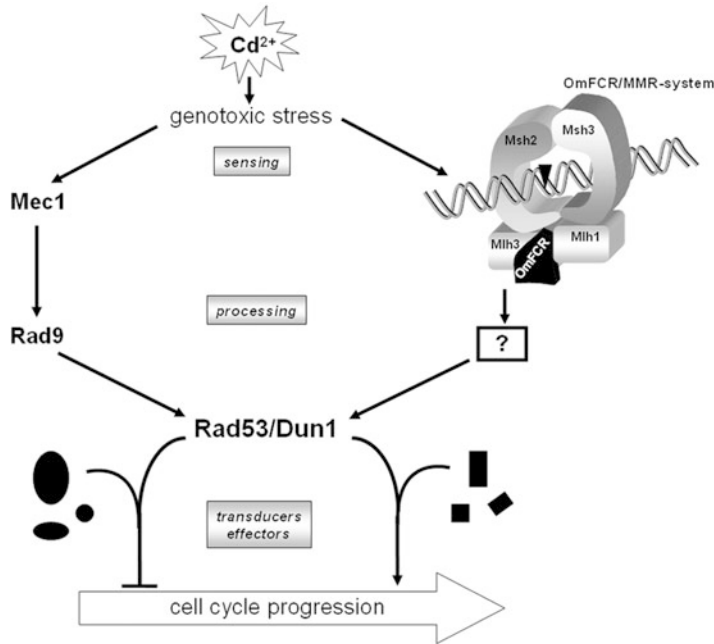
Fig. 14.7. Working model for OmFCR. The genotoxic stress caused by cadmium might recruit the MMR system, which, in its turn, might promote the firing of OmFCR through protein—protein interactions with Mlh3p. The signalling pathway promoted by OmFCR appears to merge with the final part of the Mec1p-dependent phosphorylation cascade, at the Rad53p/ Dun1p level. In pFL61-transformed cells (*left*) Dun1p is likely to recruit effector proteins that cause cell cycle arrest, while the presence of OmFCR (*right*) might enlist alternative effector proteins that ultimately allow the progression of cell division (Modified from Abbà et al. (2011))

and lipids through the generation of reactive oxygen species (ROS), but it targets proteins that are directly or indirectly involved in DNA repair and in antioxidant defence, altering their functions and ultimately causing toxic, mutagenic and carcinogenic effects. Although a first hypothesis could be a direct role of OmFCR in the repair of DNA damage caused by cadmium, the experiments described by Abbà et al. (2011) suggest that OmFCR may rather take part to the fairly unexplored role of the MMR system in connecting the recognition of DNA lesions with downstream signalling cascades that ultimately lead to cell cycle checkpoints (Fig. 14.7).

V. Ericoid Mycorrhizal Fungi as Model Systems

Although mycorrhizae are critical elements of terrestrial ecosystems, we have just begun to understand the molecular interactions between mycorrhizal fungi and their host plants. The release of the complete *Laccaria bicolor* and *Tuber melanosporum* genomes (Martin et al. 2008, 2010) and the on-going genome sequencing of additional mycorrhizal fungi by the DOE Joint Genome Institute (http://www.jgi. doe.gov/) are going to provide unprecedented opportunities for studying the biology and the evolution underlying the symbiotic lifestyle. These new fungal genome sequencing programs proposed by Francis Martin within the international project "*Exploring the Genome Diversity of Mycorrhizal Fungi to Understand the Evolution and Functioning of Symbiosis in Woody Shrubs and Trees*" and the following comparison with various pathogenic and saprobic fungal genomes are likely to shed light on the complexity of plant—fungus associations and lead to the identification of the key gene set associated with mycorrhizal symbiosis.

Investigations on specific genes mediating symbiotic events in mycorrhiza formation have hitherto been based exclusively on the study of non-mycorrhizal (Myc-) plant mutants interacting with AM fungi (Parniske 2004). An equivalent development of molecular and genetic approaches is now required at the fungal side to obtain a complete picture of the symbiosis (Helber and Requena 2008). **Gene function assignment will represent a critical step in the identification and the study of genes essential for symbiosis,** but this procedure depends on the development of high-throughput methodologies to test gene function in vivo or in vitro. One of such approaches is the genetic transformation, i.e. the incorporation of exogenous DNA that causes the change or disruption of a gene followed by phenotype alterations.

The first evidence for **genetic transformation** of a mycorrhizal fungus was the successful transformation of the basidiomycete *L. laccata* using a protoplast-PEG-based transformation (Barrett et al. 1990).

Since then, other mycorrhizal species were stably transformed: several ectomycorrhizal (ECM) fungi, such as *Hebeloma cylindrosporum* (Marmeisse et al. 1992; Pardo et al. 2002; Combier et al. 2003), *Paxillus involutus* (Bills et al. 1995; Pardo et al. 2002), *Laccaria bicolor* (Bills et al. 1999; Kemppainen et al. 2005), *Suillus bovinus* (Hanif et al. 2002; Pardo et al. 2002) and *Pisolithus tinctorius* (Rodriguez-Tovar et al. 2005), and one ERM fungus, the ascomycete *Oidiodendron maius* strain Zn (Martino et al. 2007). However, arbuscular mycorrhizal fungi and other ECM fungi, such as *Tuber borchii*, seem to be recalcitrant to stable transformation (Forbes et al. 1998; Grimaldi et al. 2005; Helber and Requena 2008). All these genetic transformations led to the random integration of a selectable marker into the host genome. Random mutagenesis is a powerful approach to the creation of large collections of random mutants which can be then screened for a phenotype of interest. Combier and colleagues, for example, selected ten Myc-transformants blocked at the early stages of ectomycorrhiza formation from a *H. cylindrosporum* collection of random mutants, although the identification and the functional assignment of the mutated genes were not undertaken (Combier et al. 2004).

The most direct ways to explore gene function are generally the complete and stable inactivation of the target gene via homologous recombination at the wild-type locus (gene knock-out) or the down-regulation of its expression by the introduction of an antisense RNA molecule (gene knock-down). As demonstrated by Kemppainen et al. (2009), gene knock-down may represent a reliable alternative to disruption experiments for functional analysis of genes, especially in fungi with multinuclear hyphae, such as *L. bicolor*, or in fungi with a low frequency of homologous recombination. Yet, gene silencing does not ensure the complete suppression of the target gene expression as opposed to null mutants.

Although **gene disruption in mycorrhizal fungi** is limited by the high frequency of ectopic integrations of the transforming DNA molecule, the first targeted gene inactivation in a mycorrhizal fungus has proved to be a successful approach to study genes involved in the mycorrhization process (Abbà et al. 2009). The stable inactivation of the Cu/Zn superoxide dismutase (SOD1) gene by *Agrobacterium*-mediated transformation (AMT) has demonstrated, in fact, that in addition to a general role as an anti-oxidant enzyme, **SOD1 is involved in the *O. maius* morphogenetic responses to the symbiotic partner.**

SODs play a role in both pathogenic and symbiotic interactions between fungi and other organisms (Scott and Eaton 2008). An up-regulation of fungal ROS scavenger machinery during the early stages of the interaction has been demonstrated in pathogenic fungi (Hwang et al. 2002; Cox et al. 2003; Brown et al. 2008), as well as in arbuscular (Lanfranco et al. 2005) and ectomycorrhizal (Baptista et al. 2007) fungi. Cu/Zn SOD is the first fungal protein with a demonstrated role in the establishment of the ERM symbiosis, and the disruption of the corresponding gene is proposed to cause an imbalance in the redox homeostasis during host colonisation and an alteration in the delicate dialogue between *O. maius* and its host plant (Abbà et al. 2009).

Besides the possibility of gene deletion by homologous recombination, *O. maius* can be easily transformed by **random insertional mutagenesis.** A collection of more than 2000 random mutants is already available to be screened for the phenotype of interest and transformation with plasmid vectors for GFP tagging has been successfully used for protein localisation (Abbà, personal communication).

O. maius can be easily grown in vitro, where it reproduces asexually by forming conidia with just a single haploid nucleus. Moreover, the haploid monokaryotic status implies that the

modifications of the wild-type phenotype, if detectable, can be observed even when mutations are recessive. This characteristic makes this fungus a good candidate to study mutants, because uninucleated spores can germinate and produce a homokaryotic mycelium with all the nuclei carrying the mutation.

Fungal genes potentially implicated in the mycorrhization process have been so far studied in the non-mycorrhizal filamentous fungus *Magnaporthe oryzae* (Heupel et al. 2010; Kloppholz et al. 2011). Although there are several types of mycorrhiza in terms of ecological significance and host—fungus association, a mycorrhizal fungus like *O. maius* might represent a more suitable heterologous system for studying fungal genes involved in the symbiosis. The feasibility of stable transformations and targeted gene disruptions in *O. maius* might open new possibilities to study the biological role of symbiosis-regulated genes, especially of those encoding effector-like secreted proteins, which have been hypothesised to interact with host plant proteins or alter their expression during the symbiosis interaction (Martin et al. 2008).

VI. Conclusions

A classical view is that distinct mycorrhizal types exist in nature, each with a well described morphology of the plant—fungus association (Smith and Read 1997) and a distinct range of symbiotic fungi. Although the classification into mycorrhizal types has been important to describe the diversity of symbiotic plant—fungus associations, it has lead to the view of a sharp separation among endo- and ectomycorrhizae. However, starting from the observation of common fungal taxa in ECM and ERM plants in nature (Bergero et al. 2000; Vrålstad et al. 2000, 2002a; Grelet et al. 2010), it was shown that the same fungus isolated from ECM roots could form ERM on ericaceous hosts and improve plant growth and/or nutrition (Villarreal-Ruiz et al. 2004; Grelet et al. 2009a). Although the range of potential interactions of ERM fungi is a delicate issue still

open to debate, the observations on *M. bicolor* (Villarreal-Ruiz et al. 2004) indicate, at least for some ERM fungi, a phenotypic plasticity in their interactions with plants of different mycorrhizal status and contribute to weaken the boundaries between the classical mycorrhizal types. Similar conclusions also derive from studies on the taxonomic diversity of symbiotic fungi in other mycorrhizal symbioses. For example, well known ECM fungi have been found to associate with mycoheterotrophic and mixotrophic forest orchids, where they form typical endomycorrhizal coils. This phenotypic plasticity of ECM fungi in orchids has been demonstrated for both ascomycetes (e.g. Selosse et al. 2004) and basidiomycetes (e.g. Selosse et al. 2002; Girlanda et al. 2006).

There is increasing evidence that fungi taxonomically related to ERM fungi are common associates of plants with different mycorrhizal status (e.g. Chambers et al. 2008; Ohtaka and Narisawa 2008; Tedersoo et al. 2009), with a continuum from loose non-mycorrhizal to mycorrhizal associations between ericoid fungi and host plants, possibly related to environmental conditions and to the host plant species. It becomes now important to investigate whether these potential hyphal connections, irrespective of the morphology of fungus—plant interactions, do contribute to a functional mycelial network that may allow nutrient transfer in a mixed plant community, similar (or perhaps even connected) to the wood-wide web first demonstrated for ECM plants by Simard et al. (1997).

All ERM fungi appear to be well equipped with an arsenal of hydrolytic enzymes that are instrumental in the litter decomposition in heathlands as well as in woodlands.

Given the ECM potential of *M. bicolor* isolates, it is quite interesting that the genomic sequencing of the ECM fungus *Laccaria bicolor* indicates a strong reduction in the number of genes involved in the production of hydrolytic enzymes, as compared to saprotrophic fungi (Martin and Selosse 2008). However, it should be noted that some ECM forming ascomycetes, such as *Sphaerosporella brunnea* and *Geopyxis carbonaria*, that colonise seedlings particularly after fire, effectively degrade cellulose, produce phenol oxidase and degrade lignin (see Egger 2006). A genomic investigation of

wider range of ERM and ECM fungi will be important to provide a more comprehensive understanding of the saprotrophic potential of these ecto- and endomycorrhizal fungi.

Biochemical, physiological and molecular studies have allowed us to gain better insights on the mechanisms of stress tolerance of ERM fungi, especially towards heavy metals (see Sect. IV). The identification of these mechanisms provides us with some clues to better understand the success observed for members of the "R. ericae aggregate" and O. maius in colonising heavy metal polluted soils. More mechanisms likely enable ERM fungi to protect themselves and their host plant from toxic compounds, and will hopefully emerge from genome wide approaches as well as from comparisons between metal tolerant and metal sensitive ERM strains (Murat et al. 2011).

References

Abbà S, Khouja HR, Martino E, Archer DB, Perotto S (2009) SOD1-Targeted gene disruption in the ericoid mycorrhizal fungus *Oidiodendron maius* reduces conidiation and the capacity for mycorrhization. Mol Plant Microbe Interact 22:1412–1421

Abbà S, Vallino M, Daghino S, Di Vietro L, Borriello R, Perotto S (2011) A PLAC8-containing protein from an endomycorrhizal fungus confers cadmium resistance to yeast cells by interacting with Mlh3p. Nucleic Acids Res 39:7548–7563

Abuarghub SM, Read DJ (1988) The biology of mycorrhizal in the Ericaceae. XI. The distribution of nitrogen in soil of a typical upland callunetum with special reference to the free amino-acids. New Phytol 108:425–431

Abuzinadah RA, Read DJ (1989) The role of proteins in the nitrogen nutrition of ectomycorrhizal plants. V. Nitrogen transfer in birch (*Betula pendula*) grown in association with mycorrhizal and non-mycorrhizal fungi. New Phytol 112:61–68

Adriaensen K, Vrålstad T, Noben JP, Vangronsveld J, Colpaert JV (2005) Copper adapted *Suillus luteus*, a symbiotic solution for pines colonising Cu mine spoil. Appl Environ Microbiol 11:7279–7284

Alberton O, Kuyper TW, Summerbell RC (2009) Dark septate root endophytic fungi increase growth of Scots pine seedlings under elevated CO_2 through enhanced nitrogen use efficiency. Plant Soil 328:459–470

Allen TR, Millar T, Berch SM, Berbee ML (2003) Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. New Phytol 160:255–272

Bajwa R, Read DJ (1986) Utilization of mineral and amino N sources by the ericoid mycorrhizal endophyte *Hymenoscyphus ericae* and by mycorrhizal and non-mycorrhizal seedlings of *Vaccinium*. Trans Br Mycol Soc 87:269–277

Baptista P, Martins A, Pais MS, Tavares RM, Lino-Neto T (2007) Involvement of reactive oxygen species during early stages of ectomycorrhiza establishment between *Castanea sativa* and *Pisolithus tinctorius*. Mycorrhiza 17:185–193

Barrett V, Dixon RK, Lemke PA (1990) Genetic transformation of a mycorrhizal fungus. Appl Microbiol Biotechnol 33:313–316

Bellion M, Courbot M, Jacob C, Blaudez D, Chalot M (2006) Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi. FEMS Microbiol Lett 254:173–181

Bending GD, Read DJ (1996a) Effects of the soluble polyphenol tannic acid on the activities of ericoid and ectomycorrhizal fungi. Soil Biol Biochem 28:1595–1602

Bending GD, Read DJ (1996b) Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. Soil Biol Biochem 28:1603–1612

Bending GD, Read DJ (1997) Lignin and soluble-phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. Mycol Res 101:1348–1354

Berch SM, Allen TR, Berbee ML (2002) Molecular detection, community structure and phylogeny of ericoid mycorrhizal fungi. Plant Soil 244:55–66

Bergero R, Perotto S, Girlanda M, Vidano G, Luppi AM (2000) Ericoid mycorrhizal fungi are common root associates of a Mediterranean ectomycorrhizal plant (*Quercus ilex*). Mol Ecol 9:1639–1649

Bidartondo MI, Bruns TD, Weiss M, Sergio C, Read DJ (2003) Specialized cheating of the ectomycorrhizal symbiosis by an epiparasitic liverwort. Proc Biol Sci R Soc 270:835–842

Bills SN, Richter DL, Podila GK (1995) Genetic transformation of the ectomycorrhizal fungus *Paxillus involutus* by particle bombardment. Mycol Res 99:557–561

Bills SN, Podila GK, Hiremath S (1999) Genetic engineering of an ectomycorrhizal fungus *Laccaria bicolor* for use as a biological control agent. Mycologia 91:237–242

Blaudez D, Chalot M (2011) Characterization of the ER-located zinc transporter ZnT1 and identification of a vesicular zinc storage compartment in *Hebeloma cylindrosporum*. Fungal Genet Biol 48:496–503

Blaudez D, Botton B, Chalot M (2000) Cadmium uptake and subcellular compartmentation in the ectomycorrhizal fungus *Paxillus involutus*. Microbiology 146:1109–1117

Bolchi A, Ruotolo R, Marchini G, Vurro E, Sanità di Toppi L, Kohler A, Tisserant E, Martin F, Ottonello S (2011) Genome-wide inventory of metal homeostasis-related gene products including a functional phytochelatin synthase in the hypogeous mycorrhizal fungus *Tuber melanosporum*. Fungal Genet Biol 48:573–584

Bonfante-Fasolo P (1980) Occurrence of a basidiomycetes in living cells of mycorrhizal hair roots of *Calluna vulgaris*. Trans Br Mycol Soc 75:320–325

Bonfante-Fasolo P, Gianinazzi-Pearson V (1979) Ultrastructural aspects of endomycorrhiza in the Ericaceae. I. Naturally infected hair roots of *Calluna vulgaris* L. Hull. New Phytol 83:739–744

Bougoure DS, Cairney JWG (2005a) Assemblages of ericoid mycorrhizal and other root-associated fungi from *Epacris pulchella* (Ericaceae) as determined by culturing and direct DNA extraction from roots. Environ Microbiol 7:819–827

Bougoure DS, Cairney JWG (2005b) Fungi associated with hair roots of *Rhododendron lochiae* (Ericaceae) in an Australian tropical cloud forest revealed by culturing and culture-independent molecular methods. Environ Microbiol 7:1743–1754

Bougoure DS, Cairney JWG (2006) Chitinolytic activities of ericoid mycorrhizal and other root-associated fungi from *Epacris pulchella* (Ericaceae). Mycol Res 110:328–334

Bougoure DS, Parkin PI, Cairney JWG, Alexander IJ, Anderson IC (2007) Diversity of fungi in hair roots of Ericaceae varies along a vegetation gradient. Mol Ecol 16:4624–4636

Bradley R, Burt AJ, Read DJ (1981) Mycorrhizal infection and resistance to heavy metal toxicity in *Calluna vulgaris*. Nature 292:335–337

Bradley R, Burt AJ, Read DJ (1982) The biology of mycorrhiza in the Ericaceae VIII. The role of the mycorrhizal infection in heavy metal resistance. New Phytol 91:197–209

Braun-Lüllemann A, Hüttermann A, Majcherczyk A (1999) Screening of ectomycorrhizal fungi for degradation of polycyclic aromatic hydrocarbons. Appl Microbiol Biotechnol 53:127–132

Brown SH, Yarden O, Gollop N, Chen S, Zveibil A, Belausov E, Freeman S (2008) Differential protein expression in *Colletotrichum acutatum*: changes associated with reactive oxygen species and nitrogen starvation implicated in pathogenicity on strawberry. Mol Plant Pathol 9:171–190

Burke RM, Cairney JWG (1997a) Carbohydrolase production by the ericoid mycorrhizal fungus *Hymenoscyphus ericae* under solid state fermentation conditions. Mycol Res 101:1135–1139

Burke RM, Cairney JWG (1997b) Purification and characterisation of a β-1,4-endoxylanase from the ericoid mycorrhizal fungus *Hymenoscyphus ericae*. New Phytol 135:345–352

Burke RM, Cairney JWG (1998) Carbohydrate oxidases in ericoid and ectomycorrhizal fungi: a possible source of Fenton radicals during the degradation of lignocellulose. New Phytol 139:637–645

Burke RM, Cairney JWG (2002) Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi. Mycorrhiza 12:105–116

Cairney JWG, Burke RM (1998) Extracellular enzyme activities of the ericoid mycorrhizal endophyte *Hymenoscyphus ericae* (Read) Korf & Kernan: their likely roles in decomposition of dead plant tissue in soil. Plant Soil 205:181–192

Cairney JWG, Meharg AA (2003) Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. Eur J Soil Sci 54:735–740

Cairney JWG, Sawyer NA, Sharples JM, Meharg AA (2000) Intraspecific variation in nitrogen source utilisation by isolates of the ericoid mycorrhizal fungus *Hymenoscyphus Ericae* (Read) Korf and Kernan. Soil Biol Biotechnol 32:1319–1322

Chalot M, Brun A (1998) Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. FEMS Microbiol Rev 22:21–44

Chambers SM, Williams PG, Seppelt RD, Cairney JWG (1999) Molecular identification of *Hymenoscyphus* sp. From rhizoids of the leafy liverwort *Cephaloziella exiliflora* (Tayl.) Steph. In Australia and Antarctica. Mycol Res 103:286–288

Chambers SM, Liu G, Cairney JWG (2000) ITS rDNA sequence comparison of ericoid mycorrhizal endophytes from *Woollsia pungens*. Mycol Res 104:168–174

Chambers SM, Curlevski NJ, Cairney JWG (2008) Ericoid mycorrhizal fungi are common root inhabitants of non-Ericaceae plants in a south-eastern Australian sclerophyll forest. FEMS Microbiol Ecol 65:263–270

Chen A, Chambers SM, Cairney JWG (1999) Utilisation of organic nitrogen and phosphorus sources by mycorrhizal endophytes of *Woollsia pungens* (Cav.) F. Muell. (Epacridaceae). Mycorrhiza 8:181–187

Chongpraditnun P, Mori S, Chino M (1992) Excess copper induces a cytosolic Cu, Zn-superoxide dismutase in soybean root. Plant Cell Physiol 33:239–244

Colpaert JV, Muller LAH, Lambaerts M, Adriaensen K, Vangronsveld J (2004) Evolutionary adaptation to Zn toxicity in populations of suilloid fungi. New Phytol 162:549–559

Colpaert JV, Wevers JHL, Krznaric E, Adriaensen K (2011) How metal-tolerant ecotypes of ectomycorrhizal fungi protect plants from heavy metal pollution. Ann For Sci 68:17–24

Combier JP, Melayah D, Raffier C, Gay G, Marmeisse R (2003) *Agrobacterium tumefaciens*-mediated transformation as a tool for insertional mutagenesis in the symbiotic ectomycorrhizal fungus *Hebeloma cylindrosporum*. FEMS Microbiol Lett 220:141–148

Combier JP, Melayah D, Raffier C, Pepin R, Marmeisse R, Gay G (2004) Nonmycorrhizal (Myc-) mutants of *Hebeloma cylindrosporum* obtained through insertional mutagenesis. Mol Plant Microbe Interact 17:1029–1038

Cosgrove DJ (1967) Metabolism of organic phosphates in soil. In: McLaren AD, Peterson GH (eds) Soil biochemistry. Dekker, New York, pp 216–228

Courbot M, Diez L, Ruotolo R, Chalot M, Leroy P (2004) Cadmium-responsive thiols in the ectomycorrhizal fungus *Paxillus involutus*. Appl Environ Microbiol 70:7413–7417

Couture M, Fortin JA, Dalpé Y (1983) *Oidiodendron griseum* Robak: an endophyte of ericoid mycorrhiza in *Vaccinium* spp. New Phytol 95:375–380

Cox GM, Harrison TS, McDade HC, Taborda CP, Heinrich G, Casadevall A, Perfect JR (2003) Superoxide dismutase influences the virulence of *Cryptococcus neoformans* by affecting growth within macrophages. Infect Immun 71:173–180

Curlevski NJA, Chambers SM, Anderson IC, Cairney JWG (2009) Identical genotypes of an ericoid mycorrhiza-forming fungus occur in roots of *Epacris pulchella* (Ericaceae) and *Leptospermum polygalifolium* (Myrtaceae) in an Australian sclerophyll forest. FEMS Microbiol Ecol 67:411–420

Dalpé Y (1986) Axenic synthesis of ericoid mycorrhiza in *Vaccinium angustifolium* Ait. by *Oidiodendron* species. New Phytol 103:391–396

Dalpé Y (1989) Ericoid mycorrhizal fungi in the Myxotrichaceae and Gymnoascaceae. New Phytol 113:523–527

Dalpé Y, Litten W, Sigler L (1989) *Scytalidium vaccinii* sp. nov., an ericoid endophyte of *Vaccinium angustifolium* roots. Mycotaxon 35:371–377

Denny HJ, Ridge I (1995) Fungal slime and its role in the mycorrhiza amelioration of zinc toxicity to higher plants. New Phytol 130:251–257

Devevre O, Garbaye J, Botton B (1996) Release of complexing organic acids by rhizosphere fungi as a factor in Norway Spruce yellowing in acidic soils. Mycol Res 100:1367–1374

Dighton J, Thomas ED, Latter PM (1987) Interactions between tree roots, mycorrhizas, a saprotrophic fungus and the decomposition of organic substrates in a microcosm. Biol Fertil Soils 4:145–150

Dos Santos Utmazian MN, Schweiger P, Sommer P, Gorfer M, Strauss J (2007) Influence of *Cadophora finlandica* and other microbial treatments on cadmium and zinc uptake in willows grown on polluted soil. Plant Soil Environ 53:158–166

Duckett JG, Read DJ (1995) Ericoid mycorrhizas and rhizoid-ascomycete associations in liverworts share the same mycobiont: isolation of the partners and resynthesis of the associations in vitro. New Phytol 129:439–447

Duddridge J, Read DJ (1982) An ultrastructural analysis of the development of mycorrhizas in *Rhododendron pontium*. Can J Bot 60:2345–2356

Eaton GK, Ayres MP (2002) Plasticity and constraint in growth and protein mineralization of ectomycorrhizal fungi under simulated nitrogen deposition. Mycologia 94:921–932

Egger KN (2006) The surprising diversity of ascomycetous mycorrhizas. New Phytol 170:421–423

Egger KN, Sigler L (1993) Relatedness of the ericoid endophytes *Scytalidium vaccinii* and *Hymenoscyphus ericae* inferred from analysis of ribosomal DNA. Mycologia 85:219–230

Englander L, Hull RJ (1980) Reciprocal transfer of nutrients between ericaceous plants and a *Clavaria* sp. New Phytol 84:661–667

Finlay RD (2008) Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. J Exp Bot 59:1115–1126

Fishel R, Kolodner RD (1995) Identification of mismatch repair genes and their role in the development of cancer. Curr Opin Genet Dev 5:382–395

Fomina M, Charnock JM, Hillier S, Alvarez R, Gadd GM (2007) Fungal transformations of uranium oxides. Environ Microbiol 9:1696–1710

Forbes PJ, Millian S, Hooker JE, Harrier LA (1998) Transformation of the arbuscolar mycorrhiza *Gigaspora rosea* by particle bombardment. Mycol Res 102:497–501

Fridovich I (1995) Superoxide radicals and superoxide dismutase. Annu Rev Biochem 5:321–324

Gadd GM (1993) Interactions of fungi with toxic metals. New Phytol 124:25–60

Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156:609–643

Genney DR, Alexander IJ, Hartley SE (2000) Exclusion of grass roots from soil organic layers by *Calluna*: the role of ericoid mycorrhizas. J Exp Bot 51:1117–1125

Gibson BR, Mitchell DT (2004) Nutritional influences on the solubilization of metal phosphate by ericoid mycorrhizal fungi. Mycol Res 108:947–954

Gibson BR, Mitchell DT (2005) Phosphatases of ericoid mycorrhizal fungi: kinetic properties and the effect of copper on activity. Mycol Res 109:478–486

Girlanda M, Selosse MA, Cafasso D, Brilli F, Delfine S, Fabbian R, Ghignone S, Pinelli P, Segreto R, Loreto F, Cozzolino S, Perotto S (2006) Inefficient photosynthesis in the Mediterranean orchid *Limodorum abortivum* is mirrored by specific association to ectomycorrhizal Russulaceae. Mol Ecol 15:491–504

Gonzlez-Guerrero M, Cano C, Azcón-Aguilar C, Ferrol N (2007) GintMT1 encodes a functional metallothionein in *Glomus intraradices* that responds to oxidative stress. Mycorrhiza 17:327–335

Gorfer M, Persak H, Berger H, Brynda S, Bandian D, Strauss J (2009) Identification of heavy metal regulated genes from the root associated ascomycete *Cadophora finlandica* using a genomic microarray. Mycol Res 113:1377–1388

Grelet GA, Meharg AA, Alexander IJ (2005) Carbon availability affects nitrogen source utilisation by *Hymenoscyphus ericae*. Mycol Res 109:469–477

Grelet GA, Johnson D, Paterson E, Anderson IC, Alexander IJ (2009a) Reciprocal carbon and nitrogen transfer between an ericaceous dwarf shrub and fungi isolated from *Piceirhiza bicolorata* ectomycorrhizas. New Phytol 182:359–366

Grelet GA, Meharg AA, Duff EI, Anderson IC, Alexander IJ (2009b) Small genetic differences between ericoid mycorrhizal fungi affect nitrogen uptake by *Vaccinium*. New Phytol 181:708–718

Grelet GA, Johnson D, Vrålstad T, Alexander IJ, Anderson IC (2010) New insights into the mycorrhizal *Rhizoscyphus ericae* aggregate: spatial structure and co-colonization of ectomycorrhizal and ericoid roots. New Phytol 188:210–222

Griffiths RP, Caldwell BA (1992) Mycorrhizal mat communities in forest soils. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB International, Wallingford, pp 98–105

Grimaldi B, de Raaf MA, Filetici P, Ottonello S, Ballario P (2005) *Agrobacterium*-mediated gene transfer and enhanced green fluorescent protein visualization in the mycorrhizal ascomycete *Tuber borchii*: a first step towards truffle genetics. Curr Genet 48:69–74

Guelfi A, Azevedo RA, Lea PJ, Molina SMG (2003) Growth inhibition of the filamentous fungus *Aspergillus nidulans* by cadmium: an antioxidant enzyme approach. J Gen Appl Microbiol 49:63–73

Gunther H, Perner B, Gramss G (1998) Activities of phenol oxidizing enzymes of ectomycorrhizal fungi in axenic culture and in symbiosis with Scots pine (*Pinus sylvestris* L.). J Basic Microbiol 38:197–206

Hambleton S, Currah RS (1997) Fungal endophytes from the roots of alpine and boreal Ericaceae. Can J Bot 75:1570–1581

Hambleton S, Sigler L (2005) Meliniomyces, a new anamorph genus for root-associated fungi with phylogenetic affinities to *Rhizoscyphus ericae* (*Hymenoscyphus ericae*), Leotiomycetes. Stud Mycol 53:1–27

Hambleton S, Egger KN, Currah RS (1998) The genus *Oidiodendron*: species delimitation and phylogenetic relationships based on nuclear ribosomal DNA analysis. Mycologia 90:854–868

Hanif M, Pardo A, Gorfer M, Raudaskoski M (2002) T-DNA transfer and integration in the ectomycorrhizal fungus *Suillus bovinus* using hygromycin B as a selectable marker. Curr Genet 41:183–188

Hartwig A (1995) Current aspects in metal genotoxicity. Biometals 8:3–11

Helber N, Requena N (2008) Expression of the fluorescence markers DsRed and GFP fused to a nuclear localization signal in the arbuscolar mycorrhizal fungus *Glomus intraradices*. New Phytol 177:537–548

Heupel S, Roser B, Kuhn H, Lebrun MH, Villalba F, Requena N (2010) Erl1, a novel Era-Like GTPase from *Magnaporthe oryzae*, is required for full root virulence and is conserved in the mutualistic symbiont *Glomus intraradices*. Mol Plant Microbe Interact 23:67–81

Hobbie EA (2006) Carbon allocation to ectomycorrhizal fungi correlates with belowground allocation in culture studies. Ecology 87:563–569

Hutchison LJ (1990) Studies on the systematics of ectomycorrhizal fungi in axenic culture. III. Patterns of polyphenol oxidase activity. Mycologia 82:424–435

Hutton BJ, Dixon KW, Sivasithamparam K (1994) Ericoid endophytes of Western Australian heaths (Epacridaceae). New Phytol 127:557–566

Hwang CS, Rhie GE, Oh JH, Huh WK, Yim HS, Kang SO (2002) Copper- and zinc-containing superoxide dismutase (Cu/ZnSOD) is required for the protection of *Candida albicans* against oxidative stresses and the expression of its full virulence. Microbiology 148:3705–3713

Jacob C, Courbot M, Brun A, Steinman HM, Jacquot J-P, Botton B, Chalot M (2001) Molecular cloning, characterization and regulation by cadmium of a superoxide dismutase from the ectomycorrhizal fungus *Paxillus involutus*. Eur J Biochem 268:3223–3232

Jalal MAF, Read DJ (1983) The organic acid composition of *Calluna* heathland soil with special reference to phyto- and fungi-toxicity. I. Isolation and identification of organic acids. Plant Soil 70:257–272

Janssens TKS, Mariën J, Cenijn P, Legler J, van Straalen NM, Dick RD (2007) Recombinational microevolution of functionally different metallothionein promoter alleles from *Orchesella cincta*. BMC Evol Biol 7:88–107

Jin YH, Clark AB, Slebos RJC, Al-Refai H, Taylor JA, Kunkel TA, Resnick MA, Gordenin DA (2003) Cadmium is a mutagen that acts by inhibiting mismatch repair. Nat Genet 34:326–329

Jongmans AG, van Breemen N, Lundström US, van Hees PAW, Finlay RD, Srinivasan M, Unestam T, Giesler R, Melkerud P-A, Olsson M (1997) Rock-eating fungi. Nature 389:682–683

Jumpponen A (2001) Dark septate endophytes – are they mycorrhizal? Mycorrhiza 11:207–211

Jumpponen A, Trappe JM (1998) Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol 140:295–310

Kemppainen M, Circosta A, Tagu D, Martin F, Pardo A (2005) *Agrobacterium*-mediated transformation of the ectomycorrhizal symbiont *Laccaria bicolor* S238N. Mycorrhiza 16:19–22

Kemppainen M, Duplessis S, Martin F, Pardo AG (2009) RNA silencing in the model mycorrhizal fungus *Laccaria bicolor*: gene knock-down of nitrate reductase results in inhibition of symbiosis with *Populus*. Environ Microbiol 11:1878–1896

Kerley SJ, Read DJ (1995) The biology of mycorrhiza in the Ericaceae. XVIII. Chitin degradation by *Hymenoscyphus ericae* and transfer of chitin-nitrogen to the host plant. New Phytol 131:369–375

Kerley SJ, Read DJ (1997) The biology of mycorrhiza in the Ericaceae. XIX. Fungal mycelium as a nitrogen source for the ericoid mycorrhizal fungus *Hymenoscyphus ericae* and its host plants. New Phytol 136:691–701

Kernan MJ, Finocchio F (1983) A new discomycetes associated with the roots of *Monotropa uniflora* (Ericaceae). Mycologia 75:916–920

Kielland K (1994) Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. Ecology 75:2373–2383

Kjøller R, Olsrud M, Michelsen A (2010) Co-existing ericaceous plant species in a subarctic mire community share fungal root endophytes. Fungal Ecol 3:205–214

Kloppholz S, Kuhn H, Requena N (2011) A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. Curr Biol 21:1204–1209

Kosola KR, Workmaster BAA, Spada PA (2007) Inoculation of cranberry (*Vaccinium macrocarpon*) with the ericoid mycorrhizal fungus *Rhizoscyphus ericae* increases nitrate influx. New Phytol 176:184–196

Kron KA, Judd WS, Stevens PF, Crayn DM, Anderberg AA, Gadek PA, Quinn CJ, Luteyn JL (2002) Phylogenetic classification of Ericaceae: molecular and morphological evidence. Bot Rev 68:335–423

Krznaric E, Wevers JHL, Cloquet C, Vangronsveld J, Vanhaecke F, Colpaert JV (2009) Zn pollution counteracts Cd toxicity in metal-tolerant ectomycorrhizal fungi and their host plant, *Pinus sylvestris*. Environ Microbiol 12:2133–2141

Lacourt I, Girlanda M, Perotto S, Del Pero M, Zuccon D, Luppi AM (2002) Nuclear ribosomal sequence analysis of *Oidiodendron*: towards a redefinition of ecologically relevant species. New Phytol 149:565–576

Lanfranco L, Bolchi A, Ros EC, Ottonello S, Bonfante P (2002) Differential expression of a metallothionein gene during the presymbiotic versus the symbiotic phase of an arbuscular mycorrhizal fungus. Plant Physiol 130:58–67

Lanfranco L, Novero M, Bonfante P (2005) The mycorrhizal fungus *Gigaspora margarita* possesses a CuZn superoxide dismutase that is up-regulated during symbiosis with legume hosts. Plant Physiol 137:1319–1330

Leake JR, Miles W (1996) Phosphodiesters as mycorrhizal P sources. I. Phosphodiesterase production and the utilization of DNA as a phosphorus source by the ericoid mycorrhizal fungus *Hymenoscyphus ericae*. New Phytol 132:435–443

Leake JR, Read DJ (1989) The effect of phenolic compounds on nitrogen mobilisation by ericoid

mycorrhizal systems. Agric Ecosyst Environ 29:225–236

Leake JR, Read DJ (1990a) Chitin as a nitrogen source for mycorrhizal fungi. Mycol Res 94:993–995

Leake JR, Read DJ (1990b) Proteinase activity in mycorrhizal fungi. I. The effect of extracellular pH on the production and activity of proteinase by ericoid endophytes from soils of contrasted pH. New Phytol 115:243–250

Leake JR, Read DJ (1990c) Proteinase activity in mycorrhizal fungi. II. The effects of mineral and organic nitrogen sources on induction of extracellular proteinase in *Hymenoscyphus ericae* (Read) Korf and Kernan. New Phytol 116:123–128

Leake JR, Read DJ (1991) Experiments with ericoid mycorrhiza. In: Norris JR, Read DJ, Varma AK (eds) Methods in microbiology, vol 23. Elsevier, Oxford, pp 435–459

Leake JR, Read DJ (1997) Mycorrhizal fungi in terrestrial habitats. In: Wicklow D, Söderström B (eds) The mycota IV: environmental and microbial relationships. Springer, Berlin Heidelberg New York, pp 281–301

Leyval C, Turnau K, Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7:139–153

Liu G, Chambers SM, Cairney JWG (1998) Molecular diversity of ericoid mycorrhizal endophytes isolated from *Woollsia pungens*. New Phytol 140:145–153

Lundström US, Van Breemen N, Bain D (2000) The podzolization process. A review. Geoderma 94:91–107

Mandyam K, Jumpponen A (2005) Seeking the elusive function of the root-colonising dark septate endophytic fungi. Stud Mycol 53:173–189

Marmeisse R, Gay G, Debaud JC, Casselton LA (1992) Genetic transformation of the symbiotic basidiomycete fungus *Hebeloma cylindrosporum*. Curr Genet 22:41–45

Martin F, Selosse M-A (2008) The *Laccaria* genome: a symbiont blueprint decoded. New Phytol 180:296–310

Martin F, Aerts A, Ahren D, Brun A, Danchin EGJ, Duchaussoy F, Gibon J, Kohler A, Lindquist E, Pereda V et al (2008) The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. Nature 452:88–92

Martin F, Kohler A, Murat C, Balestrini R, Coutinho PM, Jaillon O, Montanini B, Morin E, Noel B, Percudani R et al (2010) Perigord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. Nature 464:1033–1038

Martino E, Turnau K, Girlanda M, Bonfante P, Perotto S (2000a) Ericoid mycorrhizal fungi from heavy metal polluted soils: their identification and growth in the presence of zinc ions. Mycol Res 104:338–344

Martino E, Coisson JD, Lacourt I, Favaron F, Bonfante P, Perotto S (2000b) Influence of heavy metals on production and activity of pectinolytic enzymes in ericoid mycorrhizal fungi. Mycol Res 104:825–833

Martino E, Franco B, Piccoli G, Stocchi V, Perotto S (2002) Influence of zinc ions on protein secretion in a heavy metal tolerant strain of the ericoid mycorrhizal fungus Oidiodendron maius. Mol Cell Biochem 231:179–185

Martino E, Perotto S, Parsons R, Gadd GM (2003) Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. Soil Biol Biotechnol 35:133–141

Martino E, Murat C, Vallino M, Bena A, Perotto S, Spanu P (2007) Imaging mycorrhizal fungal transformants that express EGFP during ericoid endosymbiosis. Curr Genet 52:65–75

Massicotte HB, Melville LH, Peterson RL (2005) Structural characteristics of root-fungal interactions for five ericaceous species in Eastern Canada. Can J Bot 83:1057–1064

McKendrick SL, Leake JR, Read DJ (2000) Symbiotic germination and development of myco-heterotrophic plants in nature: transfer of carbon from ectomycorrhizal Salix repens and Betula pendula to the orchid Corallorhiza trifida through shared hyphal connections. New Phytol 145:539–548

McLean CB, Cunnington JH, Lawrie AC (1999) Molecular diversity within and between ericoid endophytes from the Ericaceae and Epacridaceae. New Phytol 144:351–358

Meharg AA, Cairney JWG (2000) Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. Adv Ecol Res 30:69–112

Midgley DJ, Chambers SM, Cairney JWG (2004a) Inorganic and organic substrates as sources of nitrogen and phosphorus for multiple genotypes of two ericoid mycorrhizal fungal taxa from Woollsia pungens Cav. (Muell.) and Leucopogon parviflorus (Andr.) Lindl. (Ericaceae). Austral J Bot 52:63–71

Midgley DJ, Chambers SM, Cairney JWG (2004b) Utilisation of carbon substrates by multiple genotypes of ericoid mycorrhizal fungal endophytes from eastern Australian Ericaceae. Mycorrhiza 14:245–251

Midgley DJ, Chambers SM, Cairney JWG (2004c) Distribution of ericoid mycorrhizal endophytes and root-associated fungi in neighbouring Ericaceae plants in the field. Plant Soil 259:137–151

Midgley DJ, Jordan LA, Saleeba JA, McGee PA (2006) Utilisation of carbon substrates by orchid and ericoid mycorrhizal fungi from Australian dry sclerophyll forests. Mycorrhiza 16:175–182

Mitchell DT, Gibson BR (2006) Ericoid mycorrhizal association: ability to adapt to a broad range of habitats. Mycologist 20:2–9

Mitchell DT, Read DJ (1981) Utilization of inorganic and organic phosphates by the mycorrhizal endophytes of Vaccinium macrocarpon and Rhododendron ponticum. Trans Br Mycol Soc 76:255–260

Mitchell DT, Sweeney M, Kennedy A (1992) Chitin degradation by Hymenoscyphus ericae and the influence of H. ericae on the growth of ectomycorrhizal fungi. In: Read DJ, Lewis DH, Fitter AH, Alexander IJ (eds) Mycorrhizas in ecosystems. CAB International, Wallingford, pp 246–251

Monreal M, Berch SM, Berbee M (1999) Molecular diversity of ericoid mycorrhizal fungi. Can J Bot 77:1580–1594

Morel M, Kohler A, Martin F, Gelhaye E, Rouhier N (2008) Comparison of the thiol-dependent antioxidant systems in the ectomycorrhizal Laccaria bicolor and the saprotrophic Phanerochaete chrysosporium. New Phytol 180:391–407

Murat C, Zampieri E, Vallino M, Daghino S, Perotto S, Bonfante P (2011) Genomic suppression subtractive hybridization as a tool to identify differences in mycorrhizal fungal genomes. FEMS Microbiol Lett 318:115–120

Myers MD, Leake JR (1996) Phosphodiesters as mycorrhizal P sources. II. Ericoid mycorrhiza and the utilization of nuclei as a phosphorus source by Vaccinium macrocarpon. New Phytol 132:445–451

Nehls U, Kleber R, Wiese J, Hampp R (1999) Isolation and characterization of a general amino acid permease from the ectomycorrhizal fungus Amanita muscaria. New Phytol 144:343–349

Newsham KK, Upson R, Read DJ (2009) Mycorrhizas and dark septate root endophytes in polar regions. Fungal Ecol 2:10–20

Nikolaou E, Stumpf M, Quinn J, Stansfield I, Brown AJP (2009) Phylogenetic diversity of stress signalling pathways in fungi. BMC Evol Biol 9:44–53

Ohtaka N, Narisawa K (2008) Molecular characterization and endophytic nature of the root-associated fungus Meliniomyces variabilis (LtVB3). J Gen Plant Pathol 74:24–31

Pardo AG, Hanif M, Raudaskoski M, Gorfer M (2002) Genetic transformation of ectomycorrhizal fungi mediated by Agrobacterium tumefaciens. Mycol Res 106:132–137

Parniske M (2004) Molecular genetics of the arbuscular mycorrhizal symbiosis. Curr Opin Plant Biol 7:414–421

Pearson V, Read DJ (1973a) The biology of mycorrhizae in the Ericaceae. I. The isolation of the endophyte and synthesis of mycorrhizas in aseptic culture. New Phytol 72:371–379

Pearson V, Read DJ (1973b) Biology of mycorrhiza in Ericaceae. II. Transport of carbon and phosphorus by endophyte and mycorrhiza. New Phytol 72:1325–1331

Perotto R, Bettini V, Bonfante P (1993) Evidence of two polygalacturonases produced by a mycorrhizal ericoid fungus during saprotrophic growth. FEMS Microbiol Lett 114:85–92

Perotto S, Actis Perino E, Perugini J, Bonfante P (1996) Molecular diversity of fungi from ericoid mycorrhizal roots. Mol Ecol 5:123–131

Perotto S, Coisson JD, Perugini I, Cometti V, Bonfante P (1997) Production of pectin-degrading enzymes by ericoid mycorrhizal fungi. New Phytol 135:151–162

Peterson RL, Massicotte HB (2004) Exploring structural definitions of mycorrhizas, with emphasis on nutrient-exchange interfaces. Can J Bot 82:1074–1088

Peterson TAW, Mueller C, Englander L (1980) Anatomy and ultrastructure of a Rhododendron root–fungus association. Can J Bot 58:2421–2433

Piercey MM, Thormann MN, Currah RS (2002) Saprotrophic characteristics of three fungal taxa from ericalean roots and their association with the roots of Rhododendron groenlandicum and Picea mariana in culture. Mycorrhiza 12:175–180

Ramesh G, Podila GK, Gay G, Marmeisse R, Reddy MS (2009) Different patterns of regulation for the copper and cadmium metallothioneins of the ectomycorrhizal fungus Hebeloma cylindrosporum. Appl Environ Microbiol 75:2266–2274

Rasmussen HN, Rasmussen FN (2009) Orchid mycorrhiza: implications of a mycophagous life style. Oikos 118:334–345

Read DJ (1974) Pezizella ericae sp. nov., the perfect state of a typical mycorrhizal endophyte of the Ericaceae. Trans Br Mycol Soc 63:381–383

Read DJ (1991) Mycorrhizas in ecosystems. Experientia 47:376–391

Read DJ (1996) The structure and function of the ericoid mycorrhizal root. Ann Bot 77:365–374

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems – a journey towards relevance? New Phytol 157:475–492

Read DJ, Leake JR, Langdale AR (1989) The nitrogen nutrition of mycorrhizal fungi and their host plants. In: Boddy L, Marchant R, Read DJ (eds) Nitrogen, phosphorus and sulphur utilization by fungi. Cambridge University Press, Cambridge, pp 181–204

Roberts P, Jones D (2008) Critical evaluation of methods for determining total protein in soil solution. Soil Biol Biochem 40:1485–1495

Rodriguez-Tovar AV, Ruiz-Medrano R, Herrera-Martinez A, Barrera-Figueroa BE, Hidalgo-Lara ME, Reyes-Marquez BE, Cabrera-Ponce JL, Valdes M, Xoconostle-Cazares B (2005) Stable genetic transformation of the ectomycorrhizal fungus Pisolithus tinctorius. J Microbiol Methods 63:45–54

Rutherford JC, Bird AJ (2004) Metal-responsive transcription factors that regulate iron, zinc, and copper homeostasis in eukaryotic cells. Eukaryot Cell 3:1–13

Ruytinx J, Craciun AR, Verstraelen K, Vangronsveld J, Colpaert JV, Verbruggen N (2011) Transcriptome analysis by cDNA-AFLP of Suillus luteus Cd-tolerant and Cd-sensitive isolates. Mycorrhiza 21:145–154

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. Ecology 85:591–602

Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–563

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109:661–686

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J Exp Bot 53:1351–1365

Scott B, Eaton CJ (2008) Role of reactive oxygen species in fungal cellular differentiations. Curr Opin Microbiol 11:488–493

Selosse MA, Weiß M, Jany JL, Tillier A (2002) Communities and populations of sebacinoid basidiomycetes associated with the achlorophyllous orchid Neottia nidus-avis (L.) L.C.M. Rich. and neighbouring tree ectomycorrhizae. Mol Ecol 11:1831–1844

Selosse MA, Faccio A, Scappaticci G, Bonfante P (2004) Chlorophyllous and achlorophyllous specimens of Epipactis microphylla (Neottieae, Orchidaceae) are associated with ectomycorrhizal septomycetes, including truffles. Microbiol Ecol 47:416–426

Selosse MA, Setaro S, Glatard F, Richard F, Urcelay C, Weiss M (2007) Sebacinales are common mycorrhizal associates of Ericaceae. New Phytol 174:864–878

Setaro S, Weiss M, Oberwinkler F, Kottke I (2006) Sebacinales form ectendomycorrhizas with Cavendishia nobilis, a member of the Andean clade of Ericaceae, in the mountain rain forest of southern Ecuador. New Phytol 169:355–365

Sharples JM, Chambers SM, Meharg AA, Cairney JWG (2000a) Genetic diversity of root-associated fungal endophytes from Calluna vulgaris at contrasting field sites. New Phytol 148:153–162

Sharples JM, Meharg AA, Chambers SM, Cairney JWG (2000b) Mechanism of arsenate resistance in the ericoid mycorrhizal fungus Hymenoscyphus ericae. Plant Physiol 124:1327–1334

Sharples JM, Meharg AA, Chambers SM, Cairney JWG (2001) Arsenate resistance in the ericoid mycorrhizal fungus *Hymenoscyphus ericae*. New Phytol 151:265–270

Silver S, Phung LT (2009) Heavy metals, bacterial resistance. In: Schaechter M (ed) Encyclopedia of microbiology. Elsevier, Oxford, pp 220–227

Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R (1997) Net transfer of carbon between ectomycorrhizal tree species in the field. Nature 388:579–582

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic, London

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, London

Sokolovski SG, Meharg AA, Maathuis FJM (2002) *Calluna vulgaris* root cells show increased capacity for amino acid uptake when colonized with the mycorrhizal fungus *Hymenoscyphus ericae*. New Phytol 155:525–530

Straker CJ (1996) Ericoid mycorrhiza: ecological and host specificity. Mycorrhiza 6:215–225

Straker CJ, Mitchell DT (1986) The activity and characterization of acid phosphatase in endomycorrhizal fungi of the Ericaceae. New Phytol 104:243–256

Straker CJ, Mitchell DT (1987) Kinetic characterization of dual phosphate uptake system in the endomycorrhizal fungus of *Erica hispidula* L. New Phytol 106:129–137

Stribley DP, Read DJ (1974) Biology of mycorrhiza in Ericaceae. 4. Effect of mycorrhizal infection on uptake of N-15 from labeled soil by *Vaccinium macrocarpon* Ait. New Phytol 73:1149–1155

Talbot JM, Treseder KK (2010) Controls over mycorrhizal uptake of organic nitrogen. Pedobiologia 53:169–179

Tedersoo L, Pärtel K, Jairus T, Gates G, Põldmaa K, Tamm H (2009) Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the Helotiales. Environ Microbiol 11:3166–3178

Todorova D, Nedeva D, Abrashev R, Tsekova K (2008) Cd (II) stress response during the growth of *Aspergillus niger* B 77. J Appl Microbiol 104:178–184

Tomsett BA (1993) Genetic and molecular biology of metal tolerance in fungi. In: Jennings DH (ed) Stress tolerance of fungi. Dekker, New York, pp 69–95

Upson R, Read DJ, Newsham KK (2007) Widespread association between the ericoid mycorrhizal fungus *Rhizoscyphus ericae* and a leafy liverwort in the maritime and sub-Antarctic. New Phytol 176:460–471

Vallino M, Drogo V, Abbà S, Perotto S (2005) Gene expression of the ericoid mycorrhizal fungus *Oidiodendron maius* in the presence of high zinc concentrations. Mycorrhiza 15:333–344

Vallino M, Martino E, Boella F, Murat C, Chiapello M, Perotto S (2009) Cu, Zn superoxide dismutase and zinc stress in the metal-tolerant ericoid mycorrhizal fungus *Oidiodendron maius* Zn. FEMS Microbiol Lett 293:48–57

Vallino M, Zampieri E, Murat C, Girlanda M, Picarella S, Pitet M, Portis E, Martino E, Perotto S (2011) Specific regions in the Sod1 locus of the ericoid mycorrhizal fungus *Oidiodendron maius* from metal enriched soils show different sequence polymorphism. FEMS Microbiol Ecol 75:321–331

Van Leerdam DM, Williams PA, Cairney JWG (2001) Phosphate solubilising abilities of ericoid mycorrhizal endophytes of *Woollsia pungens* (Epacridaceae). Austral J Bot 49:75–80

Van der Wal A, De Boer W, Klein Gunnewiek PJA, van Veen JA (2009) Possible mechanism for Spontaneous establishment of Calluna vulgaris in a recently abandoned agricultural field. Restor Ecol 17:308–313

Varma A, Bonfante P (1994) Utilisation of cell-wall related carbohydrates by ericoid mycorrhizal endophytes. Symbiosis 16:301–313

Vido K, Spector D, Lagniel G, Lopez S, Toledano MB, Labarre J (2001) A proteome analysis of the cadmium response in *Saccharomyces cerevisiae*. J Biol Chem 276:8469–8474

Villarreal-Ruiz L, Anderson IC, Alexander IJ (2004) Interaction between an isolate from the *Hymenoscyphus ericae* aggregate and roots of *Pinus* and *Vaccinium*. New Phytol 164:183–192

Vohník M, Fendrych M, Albrechtov J, Vostka M (2007) Intracellular colonization of Rhododendron and Vaccinium roots by *Cenococcum geophilum*, *Geomyces pannorum* and *Meliniomyces variabilis*. Folia Microbiol 52:407–414

Vrålstad T (2004) Are ericoid and ectomycorrhizal fungi partof a common guild? New Phytol 164:7–10

Vrålstad T, Fossheim T, Schumacher T (2000) *Piceirhiza bicolorata*- the ectomycorrhizal expression of the *Hymenoscyphus ericae* aggregate? New Phytol 145:549–563

Vrålstad T, Myhre E, Schumacher T (2002a) Molecular diversity and phylogenetic affinities of symbiotic root-associated ascomycetes of the Helotiales in burnt and metal polluted habitats. New Phytol 155:131–148

Vrålstad T, Schumacher T, Taylor AFS (2002b) Mycorrhizal synthesis between fungal strains of the *Hymenoscyphus ericae* aggregate and potential ectomycorrhizal and ericoid hosts. New Phytol 153:143–152

Weiss M (2004) Sebacinales: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. Mycol Res 108:1003–1010

Whittaker SP, Cairney JWG (2001) Influence of amino acids on biomass production by ericoid mycorrhizal

endophytes from *Woollsia pungens* (Epacridaceae). Mycol Res 105:105–111

Wilcox HE, Wang CJK (1987) Ectomycorrhizal and ectoendomycorrhizal associations of *Phialophora finlandia* with *Pinus resinosa*, *Picea rubens* and *Betula alleghaniensis*. Can J For Res 17:976–990

Xiao G, Berch SM (1996) Diversity and abundance of ericoid mycorrhizal fungi of *Gaultheria shallon* on forest clearcuts. Can J Bot 74:337–346

Yadav SK (2010) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S Afr J Bot 76:167–171

Yoo HY, Chang MS, Rho HM (1999) Heavy metal-mediated activation of the rat Cu/Zn superoxide dismutase gene via a metal-responsive element. Mol Gen Genet 262:310–313

Zhang YH, Zhuang WY (2004) Phylogenetic relationships of some members in the genus *Hymenoscyphus* (Ascomycetes, Helotiales). Nova Hedwigia 78:475–484

Zhu H, Dancik BP, Higginbotham KO (1994) Regulation of extracellular proteinase production in an ectomycorrhizal fungus *Hebeloma crustuliniforme*. Mycologia 86:227–234

Zijlstra JD, van Hof P, Baar J, Verkley GJM, Summerbell RC, Paradi I, Braakhekke WG, Berendse F (2005) Diversity of symbiotic root endophytes of the Helotiales in ericaceous plants and the grass *Deschampsia flexuosa*. Stud Mycol 53:147–162