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REVIEW

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Ericoid mycorrhiza: ecological and host specificity

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Abstract Ericoid mycorrhiza, found in plants belonging to a few families of the Ericales, is seen as the most specific of mycorrhizas, and this has generated much research into the basis of the specificity. Recently, however, non-vascular plants have been found to be able to form the same type of mycorrhiza, and the diversity of the fungal partners has expanded. This review assesses the present state of host and ecological specificity of ericoid mycorrhizas and discusses future lines of research.

Key words Mycorrhiza · Ericales · Hosts · Specificity · Ecology

Introduction

The ericoid mycorrhiza has been regarded as the most specific of mycorrhizas because of its limitation to hosts belonging to a restricted number of families of the Ericales and the participation of a small group of ascomycetous fungi as mycobionts in the association (Harley and Smith 1983). This apparent specificity has, as a conceptual framework, driven much of the research into the symbiosis. This research has explored the molecular and genetical basis of specificity and the unique biology of a mycobiont which has been selected for as a symbiotic partner under defined ecological conditions (Read 1983). However, observations over the past few years have revealed that the taxonomic diversity of both host and fungal partner is not as restricted as once thought. This review explores the implications of these observations with respect to the molecular and ecological specificity of the mycorrhiza and speculates on areas of future investigation.

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The symbionts

Although only a small number of fungi have been identified as typically ericoid mycorrhiza-forming, they are taxonomically diverse. By far the most well known and well researched is the ascomycete, *Hymenoscyphus ericae* (Read) Korf & Kernan (Helotiales) (Kernan and Finocchio 1983), which appears to be the dominant European species. Dalpe et al. (1989) isolated a slow-growing, dematiaceous hyphomycete from roots of *Vaccinium angustifolium* Ait. with cultural and mycorrhiza-forming characteristics similar to those of *H. ericae*. However, on the basis of the presence of aseptate arthroconidia they distinguished it from *H. ericae* and named it *Scytalidium vaccinii* Dalpe, Litten & Sigler sp. nov. Subsequently, Egger and Sigler (1993) used polymerase chain reaction (PCR) amplification, restriction fragment analysis and nucleotide sequencing to show that there was a very low divergence of insertions/deletions (1.2–3.5%) separating isolates of *H. ericae* and *S. vaccinii*, which indicated that the taxa are closely related and conspecific. These authors suggested that the low degree of variation in ribosomal DNA represents an anamorph-teleomorph relationship between the two species and proposed that *S. vaccinii* isolates represent North American strains derived from sexual populations in Europe. This work highlights the potential of molecular techniques to establish the relationship between *H. ericae* and other ascomycetes of similar ultrastructure or mycorrhizal morphology such as those isolated by Straker and Mitchell (1985), Reed (1987, 1989) and Hutton et al. (1994).

The use of molecular techniques also indicates that the phylogenetic relationships between different ericoid isolates may be far more complex than the morphological similarity of the anamorphs in culture suggest. Polyclonal antiserum raised against an acid phosphatase from the endophyte of the fynbos ericad *Erica hispidula* tested with antigen from various *Hymenoscyphus*–like European (INRA, Dijon; Duclos 1981) and South African (Straker and Mitchell 1985) mycorrhiza-

forming isolates showed greater immunogenic cross-reactivity with the European isolates than with the South African endophyte of *E. mauritanica* (Straker et al. 1989). Lemoine (1992) also used a highly specific polyclonal antiserum raised against acid phosphatase from *H. ericae* to distinguish between these European and South African isolates, but suggested that those from South Africa differ from *H. ericae* at the generic level. Preliminary PCR amplification and restriction fragment analysis of ribosomal DNA (D.T. Mitchell, personal communication) suggests that the same European isolates are distantly related to the single ascospore isolate of *H. ericae* used by Hughes and Mitchell (1995), and that the South African isolate from *E. mauritanica* is not closely related to the *E. hispidula* isolate. These results will have to be confirmed but appear to support some of the serological data. In a similar analysis, Perotto et al. (1995) compared South African and European isolates and concluded that the biodiversity of the different strains of ascomycetous ericoid endophytes has been underestimated, and they place the South African isolates closer to the *Oidiodendron* group than *Hymenoscyphus* (S. Perotto, personal communication). Clearly, though, rationalization of these techniques will be needed in order to obtain consistent observations.

Fungi of the hyphomycete genus, *Oidiodendron*, have been consistently identified as mycorrhizal and include *O. griseum* Robak (Couture et al. 1983; Dalpe 1986; Xiao and Berch 1992), *O. maius* Barron (Douglas et al. 1989), *O. rhodogenum* Robak and *O. cerealis* (Thum.) Barron (Dalpe 1986), *O. periconiodes* Morall (Currah et al. 1993) and *O. chlamydosporicum* Morrall, *O. citrinum* Barron, *O. flavum* Szilvinyi and *O. scytaloides* W. Gams & Soderstrom (Dalpe 1991). Although not isolated from ericaceous hosts but from soil, species of the Myxotrichaceae have been shown able to form typical ericoid mycorrhizas (Dalpe 1989): these fungi are *Myxotrichum setosum* (Eidam) Orr & Kuehn and *Gymnascella dankaliensis* (Castellani) Currah, both with *Oidiodendron* anamorphs, and *Pseudogymnoascus roseus* Raillo with a *Gymnoascus* anamorph.

In a study on the identification of mycorrhizas of alpine plant communities, Stoyke and Currah (1991) isolated a dark, simply septate hyphal form from ericaceous and rosaceous hosts. The fungus produced intracortical sclerotia of compact, pigmented and irregularly shaped hyphae in resynthesized mycorrhizas in *Menziesia ferruginea* (Ericaceae) but no intracellular hyphal coils. Because of these characteristics, it is doubtful whether this isolate, identified as *Phialocephala fortinii* Wang & Wilcox, can be considered an ericoid mycorrhizal fungus sensu stricto.

The first indirect evidence that a basidiomycete could be an ericoid endophyte was provided by Seviour et al. (1973), who observed the continual association of basidiocarps of *Clavaria* sp. with rhododendrons and ericas being used in hybridization trials. These authors suggested, from the use of polyclonal antiserum raised against basidiocarp soluble proteins and subsequent immunofluorescence of fungal peletons in mycorrhizal roots of the plants, that *Clavaria* is able to form ericoid mycorrhizas. Englander and Hull (1980) provided further circumstantial evidence for this relationship with the use of ^{14}C and ^{32}P radioisotopes, whose distribution following feeding to rhododendron plants and associated basidiocarps of *Clavaria argillacea* Fr. suggested a bidirectional movement of nutrients between them. Petersen and Litten (1989) identified a new species of *Clavaria, C. oronoensis* Petersen & Litten, which was found growing in pots of *V. angustifolium* and suggested a mycorrhizal relationship. Confirmation of *Clavaria* sp. as an ericoid endophyte came from Mueller et al. (1986), who localized, immunocytochemically, both *H. ericae* and a *Clavaria* species in roots of *Rhododendron* using polyclonal antisera raised against mycelium and fruit bodies of the respective fungi. Basidiomycetes have also been observed by transmission electron microscopy in roots of *Rhododendron* (Peterson et al. 1980) and *Calluna vulgaris* L. (Bonfante-Fasolo 1980). In both cases the authors were able to distinguish the basidiomycete from an ascomycete, forming the same type of association in adjacent cells, by the presence of dolipore septa. Bonfante-Fasolo (1980) suggested that the characteristics of the septa and cell wall structure in *Calluna* were reminiscent of those of *Tulasnella fuscoviolaceae*, an orchid endophyte. The weight of evidence from these studies suggests that basidiomycetes are able to form mycorrhizas of the ericoid type, but until such time as the fungi involved can be isolated and the mycorrhizas resynthesized in aseptic culture, the physiological nature of the symbiosis will remain unclear.

From this survey it would appear that the exact number of taxa involved as ericoid endophytes and at what levels they differ is still uncertain. It is apparent that in the Ascomycota we have the main *Scytalidium–H. ericae* (liscomycetes) complex and a number of similar, as yet unidentified isolates, and the *Oidiodendron*–Myxotrichaceae complex whose teleomorphs are plectomycetes. In the Basidiomycota we probably have representatives of the Aphyllophorales and possibly the Tulasnellales in the Hymenomycetes. Clearly the number of different species involved as ericoid mycobionts is much smaller than the 130 or so which have been identified as vesicular-arbuscular mycorrhizal (VAM) mycobionts, but the diversity of their taxonomic relationships suggests that different fungal groups have been and perhaps still are able to evolve these associations under common selective pressures.

The phytobionts, on the other hand, seemingly represent a much more restricted group: members of the sub-families Vaccinioideae, Rhododendroideae and Ericoideae of the Ericaceae, the Empretaceae and the Epacridaceae, all of the order Ericales (Harley and Smith 1983; Read 1983). A few years ago, however, Duckett et al. (1991) observed fungi with simple septa and Woronin bodies as endophytes of the unicellular rhizoids of 16.2% of British liverworts. In these associa-

tions the fungi did not extend beyond the bases of the rhizoids into the medullary cells of the hepatic stems and formed typical hyphal loops characteristic of ericoid mycorrhizas. The extraordinary structural similarities of these associations with those of mycorrhizal hair roots of the Ericaceae prompted further investigations involving selective staining of the rhizoids and mycorrhizal ericaceous roots (Duckett and Read 1991) and isolation of the mycobionts and the resynthesis of the associations in vitro (Duckett and Read 1995). Axenic seedlings of the Ericaceae were readily colonized by fungal endophytes isolated from the liverworts and formed identical structures to those formed on colonization by *H. ericae*. Similarly, resynthesis of rhizoidal-fungal associations with the isolated endophytes produced structures identical to those formed when the liverworts were colonized by *H. ericae*. Liverworts from which the ascomycetous endophytes had been isolated could not be colonized by *Oidiodendron* isolates or orchid or ectomycorrhizal fungi, and neither *H. ericae* nor the hepatic isolates could penetrate living rhizoids of liverworts which in nature are either fungus-free or contain basidiomycetous or zygomycetous fungi. The results of these studies highlight two important issues: (a) the host range of *H. ericae* and associated fungi has been extended from a well-defined and specific group of families within the Ericales to members of the non-vascular Hepaticeae, and (b) the specificity that appears to exist between ericoid endophytes and their ericaceous hosts is paralleled in a similar specificity between the same endophytes and hepatic host families. From these issues arise two questions: (a) what is the molecular nature of specificity and compatibility between these fungi and such phylogenetically divergent host families and (b) what are the selective forces which might have guided the evolution of these associations in such divergent plant taxa?

Molecular specificity and compatibility

From the available evidence it would appear that molecular specificity and compatibility in ericoid mycorrhizas is determined at a number of levels of interaction, of which three can be immediately identified.

Recognition and attachment events between root and fungus which involve surface molecules

In terms of one of the conceptual models underlying the gene-for-gene relationship, specificity is determined by recognition signals involving elicitor and receptor molecules (Gabriel and Rolfe 1990). Because of the apparent specificity of ericoid mycorrhizal relationships, research at the molecular and cellular levels has had as one of its quests the identification and localization of signal molecules involved in recognition.

An exocellular fibrillar sheath is characteristically associated with the cell wall of ericoid mycobionts and components of both structures have been biochemically identified using cytochemical and affinity techniques (Gianinazzi-Pearson et al. 1986; Bonfante-Fasolo 1988; Bonfante-Fasolo and Perotto 1988; Perotto et al. 1990). PATAg-positive 1,4-polysaccharides, alcian blue mucopolysaccharides and cysteine-containing proteins were localized on the fibrillar sheath and outer layer of the hyphal cell wall. Moreover, a strong correlation has been established between the production of fibrillar material and the ability of an ericoid isolate to infect a host root (Gianinazzi-Pearson and Bonfante-Fasolo 1986; Gianinazzi-Pearson et al. 1986). Lectin fluorescent and gold labelling has shown chitin chains to be present on septa and inner longitudinal walls of hyphae of both infective and non-infective strains (Bonfante-Fasolo et al. 1987). However, mannose sugar residues are scarce on the surface of non-infective strains but abundant within the fibrillar sheath of infective strains. In addition, the surfaces of low-infective strains contain WGA (wheat germ agglutinin)-binding sites rather than the concanavalin A-binding sites of the high-infective strains. The hyphal cell wall and fibrillar sheath are also sites of active acid phosphatase and acid invertase activity (Straker et al. 1989; Lemoine et al. 1992; Straker et al. 1992; Hughes and Mitchell 1995). These enzymes are high-mannose glycoproteins, and this has led to speculation that it is the sugar components of these enzymes which act as recognition or signal molecules. However, attempts to correlate the abundance of surface mannose sugars with differences in the enzyme activities of high- and low-infective strains have been unsuccessful, as both strains contain similar levels of the active wall-bound enzymes (Straker 1990). Monoclonal antibodies, raised against hyphae of *H. ericae*, have also been used to gain a better insight into the role of hyphal surface molecules (Perotto et al. 1987). An antibody recognised cell surface molecules in the extracellular material of the immunizer and other high-infective strains but not low-infective strains, which confirmed the presence of surface molecules specific to the high-infective strains.

However, the fibrillar network emanating from ericoid fungal hyphae is not especially unusual for fungi. Similar arrangements of fibrils have been observed in the yeast-like sporidial cells of the anther smut fungus, *Ustilago violacea* (Pers.) Fuckl. and other yeasts (Day and Poon 1975; Day et al. 1975; Gardiner et al. 1981, 1982), as well as wood-decay hymenomycetes (Palmer et al. 1983). It has also been demonstrated in the ascomycetous scleroderris canker agent of conifers, *Ascocalyx abietina* (Lagerberg.) Schlaepfer-Bernhard (Benhamou and Ouellette 1986, 1987). These workers established the presence of *N*–acetylglucosamine, *N*–acetylgalactosamine and d-mannose in the cell walls, whilst the fibrillar sheath contained mainly b-glucopyranosides and sialic acid, and felt that these negatively charged groups were probably involved in the process of attachment between host and fungus.

A complementary interest in the root cell surface molecules of the host plants has also been strong. Bonfante-Fasolo (1988), Bonfante-Fasolo and Perotto (1988) and Peretto et al. (1990) have used lectin and immuno-affinity techniques to characterize the surfaces of hair roots of *Calluna vulgaris* and *V. myrtillus*. These roots typically consist of a layer of epidermal cells surrounded by a thin cortex of one or two cell layers and a narrow stele and are the components of the root systems habitually colonized by ericoid mycorrhizal fungi. The walls show a helicoidal organization and may be surrounded by a luxurious layer of mucilage, easily visualized after Alcian blue fixation or the periodic acid–Schiff reaction, which detects glycoproteins. FITC (fluorescein isothiocyanate)-labelled lectins are able to recognize the soluble sugars, glucose and mannose, along the whole root surface but in particular the cap region, and galactose, which is more predominant in the differentiated zone behind the meristematic cap. *N*–Acetylglucosamine residues can be detected, and xylose, arabinose and rhamnose also form a major component of the mucilage, but not fucose or polygalacturonic acids. Insoluble carbohydrates such as b-1,4-glucans form the insoluble fibrillar skeleton of the mucilage layer. The relative precision in distribution of the carbohydrate components was seen by Peretto et al. (1990) as providing the molecular basis for the understanding of specificity in ericoid mycorrhizas.

The chemical composition of primary cell walls of higher plants is fairly consistent and comprises cellulose, arabinogalactan, xyloglucan and rhamnogalacturonan polymers and associated sugars; of these, glucose and arabinose are the most abundant, with moderate levels of galactose, polygalacturonic acids and xylose and lower amounts of rhamnose, fucose and mannose (Albersheim 1976). Ericaceous hair roots do, however, differ from this pattern in the lack of fucose and polygalacturonic acid residues and the presence of WGA-binding *N*–acetylglucosamine. In another model based on the surface molecules associated with cell membranes, Hohl and Balsiger (1986, 1988) were unable to find any qualitative or quantitative differences in sugar determinants between soybean isolines susceptible to different races of the fungal pathogen *Phytophthora megasperma* f. sp. *glycinea*. On the basis of agglutination experiments, using soybean protoplasts and germinated cysts, these authors proposed five potential linkages between lectin glycosyl receptors on the host membrane and terminal glucosyl residues on the fungus which, as lectin-ligand bonds, are likely to be involved in adhesion and attachment ('docking') processes but are not necessarily determinants of recognition and therefore compatibility or incompatibility. If one recalls the ericoid mycorrhizal condition, one can easily conceive of the interaction of components of the mucilage layer of the host root and the fibrillar sheath of the fungus as being essential for successful 'docking'. This would explain why the so-called low-infective strains with minimal fibrillar development are less able to colonize host roots (Gianinazzi-Pearson and Bonfante-Fasolo 1986) and why high-infective strains with an abundant fibrillar network are able to colonize non-host roots (Bonfante-Fasolo et al. 1984). The fact that the low-infective strains develop a normal mycorrhizal condition if successful adhesion does occur with host roots, but that the high-infective strains do not develop a normal mycorrhizal association in non-host roots, suggests that the interaction between surface molecules only governs broad compatibility and that molecular specificity lies at different levels of interaction.

Regulation of fungal behaviour by host cells

Bonfante-Fasolo et al. (1984) observed striking differences between the infection process in host and non-host plants when colonized by *H.ericae*. In non-host clover, the fibrillar sheath normally associated with the ericoid endophyte when external to the host root, but not with intracellular growth, did not disappear but remained evident around intracellular hyphae. Infected clover cells showed no increase in cytoplasmic content normally associated with cell invasion in a host, but instead degeneration of the cytoplasm and organelles occurred, accompanied by rupture of the host cell tonoplast and plasmalemma. *H. ericae* also developed in the intercellular spaces of the cortex, destroying the middle lamella, and eventually colonized the endodermis and parenchyma and sometimes xylem vessels of the vascular cylinder. All in all, colonization of non-host was reminiscent of uncontrolled invasion by a necrotroph. The authors argued that because the fibrillar sheath was produced by the fungus in the presence of both host and non-host roots, it could only be involved in unspecific recognition events and attachment of the fungus to the cell wall, but that the host plasmalemma may be the site of specific recognition.

Although similar observations have been made in the non-host tobacco (Lemoine 1992), studies have not been repeated in naturally growing non-hosts so we do not know how prevalent this type of behaviour by *H. ericae* is. Nor do we know whether ericoid endophytes of different taxa behave similarly with non-hosts. Nevertheless, the hyphal fibrillar sheath and associated surface molecules of *H. ericae* and the surface molecules of host roots have continued to be candidates as components of recognition and/or specificity events. In a recent review, however, Perotto et al. (1995) suggest that cell wall components other than surface sugars underlie the successful establishment of a mutualistic association by the fungus in a host plant. These authors conclude that the plasticity of the ericoid endophyte in being able to move from a saprotrophic to a mutualistic or a necrotrophic life style is regulated by the production of cell wall-degrading enzymes whose induction is dependent on the levels of substrate which are components of the plant cell wall. In vitro, ericoid isolates have been induced to produce two isoforms of polygal-

acturonase (PG) enzyme in the presence of pectin substrate (Peretto et al. 1993) as well as b-1,4-glucanase and b-1,3-glucanase in the presence of carboxymethylcellulose substrate (Varma and Bonfante 1994). In fact, total cellulase activity in culture can be as high as that of such highly cellulolytic fungi as *Trichoderma koningii* Oudem. and *Pythium sylvaticum* Campbell & Hendrix. (Straker et al. 1994). Yet, there is little evidence of extensive enzymatic degradation at sites of wall penetration during the establishment of the mycorrhizal symbiosis (Perotto et al. 1995). Peretto et al. (1993) used an antibody raised against PG enzyme of *Fusarium* sp. which cross-reacted with an ericoid isolate to demonstrate the almost complete absence of labelling of intracellular hyphae but strong labelling of extraradical hyphae. These authors' work provided a clue to the repression of PG activity inside the root: when they used monoclonal immunogenic probes to non-esterified and methyl-esterified pectin, the results indicated that pectin may be absent from the tangential wall of differentiated epidermal cells of *Calluna vulgaris*, the normal sites of penetration points. Peretto et al. (1993) also suggested that the helicoidal structure of cellulose in the walls makes it unsuitable as a substrate for the appropriate wall-degrading enzymes. In non-host clover, however, immunolabelling of infected roots with the same anti-PG antibody revealed a strong labelling of enzyme in hyphae both inside and outside the root. The use of the anti-pectin monoclonal antibodies also revealed that non-host clover contains high levels of pectin in cell walls, which would explain the induction of high levels of PG activity in the fungus and the susceptibility of the non-host wall to degradation by the fungus (Perotto et al. 1995).

From the work discussed above it seems clear that in host roots what prevents the ericoid fungus from establishing a necrotrophic relationship is the regulation of the fungal enzymes by host cell wall components. It is not clear whether this regulation occurs as a response to invasion (i.e. through activation of host genes) or represents a spontaneous biochemical response to the physical and chemical nature of the host root cell wall. In this context it may be significant that it is only the hair roots of ericaceous plants, which may be unique components of the root system with their low pectin walls and helicoidal cellulosic structure, which are colonized. It would be of interest to probe the structure and chemical composition of walls of root cells other than hair roots, as well as determine their array of surface molecules, to find clues as to why they are not invaded by these fungi. It is clear, though, that the suppression of fungal wall-degrading enzymes, by itself, does not maintain the association of internal functional and physiological compatibility so evident in TEM studies of the symbiosis (Gianinazzi-Pearson 1986). The functional mutualism must also involve the activation of other host genes involved in regulation of internal fungal activity and the suppression or modulation of host defence genes.

Modulation of fungal metabolism and cell compatibility

First evidence for regulatory control of internal fungal activity by ericaceous hosts came from Gianinazzi-Pearson (1986) and Gianinazzi-Pearson et al. (1986). These authors observed that in compatible host associations the fibrillar sheath evident around external hyphae disappeared once hyphae had entered cortical cells, as did the intense acid phosphatase activity associated with these fibrils. In non-host associations neither the fibrils nor the acid phosphatase activity was affected upon entry of the hyphae into the root cells. Confirmation of host regulation of fungal metabolism was given by Lemoine et al. (1992), who used a probe in the form of polyclonal antiserum to a low-molecular-weight acid phosphatase of *H. ericae*. Immunogold and cytochemical labelling demonstrated that the decline in intraradical fungal acid phosphatase activity was associated with an inhibition of activity rather than enzyme synthesis. It was suggested that this enzyme control mechanism involved conformational modifications of the glycosidic portion of the enzyme molecule; the glycosides had been shown essential for the enzyme's biological activity (Lemoine et al. 1992). There is, as yet, no evidence as to whether this control is exerted through the transcription of new host gene products or through biochemical factors such as pH or phosphate concentration. Research into the genetical control of the arbuscular mycorrhizal (AM) symbiosis is more advanced than that into the ericoid mycorrhiza and has led to the demonstration of specific symbiosis host genes which regulate the internal functioning of the association (Gianinazzi-Pearson et al. 1995). Compelling evidence for the regulatory action of these genes involves their modulation of host defence gene expression so that AM fungi first develop a transient incompatible-like interaction with their hosts accompanied by the induction of defence-related genes, followed by a general systemic repression of these genes which facilitates the formation of a longer-term compatible interaction (Gollotte et al. 1993; Lambais and Mehdy 1995).

Pertinent to these observations is a study in which 22 plants of endemic or indigenous *Vaccinium* spp. of the Hawaiian Islands were found to have ericoid mycorrhizas in their hair roots but AM in other parts of their root systems (Koske et al. 1990). These authors suggested that ancestral members of the Ericales were all probably AM but in some, more recent, forms this dependence was replaced by one on ericoid mycorrhiza. The fact that these Hawaiian forms have not yet lost their ability to form AM may be because the ericoid interaction has evolved much later, due to the geographical isolation of Hawaii and the later terrestrial colonization by the appropriate ascomycetous fungi. One could postulate that in ericaceous plants which have lost their dependence on AM, the same symbiosis genes that control compatible AM interactions control the internal compatibility of the ericoid mutualism, but

that it is the complementarity of the surface molecules of the hair roots with those of the ericoid endophytes which determines that the hair root component becomes colonized by ericoid fungi. Thus, the AM symbiosis genes may model those of ericoid associations.

Whatever the genetic control mechanisms which determine compatibility and specificity in ericoid mycorrhizas are, the new-found ability of hepatic rhizoids to be colonized by ericoid endophytes (Duckett and Read 1995) suggests that there may be a potential for a much wider diversity of plants to form mycorrhiza of the ericoid type. This potential is only realized, though, when the appropriate genetic determinants are naturally selected for under pressure from defined selective forces.

Ecological specificity

It has been proposed that the uniformity of the root structure of those members of the Ericales which form ericoid mycorrhizas is paralleled by a similar uniformity in the ecological conditions of the soils in which these plants are dominant, and that the characteristic mycorrhiza has evolved in response to the specific stresses imposed by these soils (Read 1983, 1985). Support for this view is given by the recent study by Duckett and Read (1995), which shows a close overlap in the habitats of the hepatics which form ericoid-type mycorrhizas and ericaceous hosts. An enormous investigative effort, mainly from Read's laboratory, has been focussed on testing this proposal, mainly in vitro. However, this work has concentrated predominantly on the fungus *H. ericae* and the host plants *Calluna, Rhododendron* and *Vaccinium*, and the ecological context has been that of a mor-humus upland *Calluna* heathland. The remainder of this review will discuss the body of knowledge which has emerged from these studies, not only within the context of ecological specificity, but also to look at how well it serves as a model for other ecosystems where ericaceous plants are dominant, in particular the fynbos in South Africa and the kwongan in southwestern Australia.

Calluna heathlands

In northern European *Calluna* heathlands net rates of mineralization are low (Read 1985); they may be even lower at higher altitudes in the acid soils of alpine dwarf scrub heath, where the total release of inorganic nitrogen may be less than 25 ppm in 3 months (Haselwandter 1987). These low rates would be mediated by low rates of microbial decomposition caused by the inhibitory effects of both cool temperatures and the extremely high levels of extractable phenolics and lignin in the sclerophyllous leaf litter (Read and Mitchell 1983), and the toxic phenolics and aromatic and aliphatic organic acids in *Calluna* heathland soils (Jalal and Read 1983a,b). In addition, levels of total nitrogen and phosphorus in fallen heath litter may be only 25% of that of deciduous mesophytes, producing high C:N ratios which also contribute to negative correlations with rates of decomposition. The accumulation of high levels of organic matter leads to the release of carboxylic ions, which contribute to the acidic nature of the soil, inhibiting nitrification so that NH_4^+ becomes the main source of inorganic nitrogen (Read et al. 1989). Given these low rates of mineralization one can expect most nitrogen to be in the form of organic molecules. This expectation has been validated by Abuarghub and Read (1988a), who measured significant levels of 'free' amino acids in the upper parts of the soil profile of an upland *Callunetum* and major fluctuations in patterns throughout the year. The quantities of these, mainly neutral, amino acids often exceeded those of extractable ammonium, and that of histidine was found to be surprisingly high (Abuarghub and Read 1988b). The levels of free orthophosphate in soils dominated by *Calluna* are also extremely low, while the levels of organic phosphorus may reach as much as 90% in alpine dwarf-scrub humus (Cosgrave 1967). Penta- and hexaphosphate salts of iron and aluminium would form a major proportion of this organic fraction in acidic soils (Anderson 1967). Another major source of organic phosphorus would be in the form of phosphodiesters, like nucleic acids and phospholipids, whose slow decomposition under heathland conditions would allow it to contribute a much higher proportion to the organic pool than under cultivated conditions where its recycling is extremely rapid (Leake and Miles 1996).

The European ericoid endophyte *H. ericae* demonstrates an array of physiological attributes which confer on its host distinct selective benefits under heathland conditions. The fungus is not only able to utilize amino acids, but also peptides and proteins as sole nitrogen sources, and the assimilated nitrogen can be transferred to infected plants (Stribley and Read 1980; Bajwa and Read 1985; Bajwa et al. 1985). The fungus achieves this by production of an extracellular proteinase which operates within a narrow acid pH range outside that of the intracellular fluid of the host plant (Leake and Read 1989, 1990a,b) and for which histidine acts as a powerful inducer (Leake 1992). Moreover, the breakdown products of protein are amino acids, not ammonium, which in terms of energetics provides the most efficient metabolic pathway for nitrogen acquisition and assimilation (Langdale and Read 1989). The ability to release nitrogen from otherwise recalcitrant complexes demonstrates the essential role that ericoid associations play in nutrient cycling (Read et al. 1989). A similar strategy by *H. ericae* for the acquisition of phosphorus from organic sources has very recently been unveiled by Leake and Miles (1996). The endophyte gave a greater yield on DNA as a sole phosphorus source than on orthophosphate. The phosphodiesterase activity which contributes to this ability, was achieved through the action of an exonuclease 5′-nucleotide diesterase

which shows a pH optimum of 3.0–5.5, entirely suited to the soils in which the host plant grows. No evidence of mineralization of the DNA was demonstrated, which suggests that the nucleotide was transferred intact or dephosphorylated in the cell wall matrix. Myers and Leake (1996) used salmon sperm nuclei as a sole source of phosphorus (but also carbon and nitrogen) for mycorrhizal and non-mycorrhizal *Vaccinium* plants and found that the mycorrhizal effect by way of greater yield and plant phosphorus content and concentration on this source of phosphorus was equal to that on orthophosphate as a source of phosphorus. Apart from its ability to use organic sources of nitrogen and phosphorus, *H. ericae* shows its potential as a saprotroph by its facility for degradation of lignin and the dehydropolymer of coniferyl alcohol (Haselwandter et al. 1990) and cellulose (Straker et al. 1994; Varma and Bonfante 1994) as sole carbon sources and chitin as a carbon and nitrogen source (Mitchell et al. 1992).

H. ericae possesses other physiological characteristics which show it to be superbly selected for the northern European heathland environment. When grown in a medium containing a mixture of the highly toxic phenolic acids identified by Jalal and Read (1983a), the fungus showed a high sensitivity to only two of the eight acids and an ability to assimilate those not inhibitory to growth (Leake et al. 1989). The inhibitory effect of the phenolics on root yield and extension of *Calluna* seedlings was also removed when the seedlings were mycorrhizal. The endophyte shows a remarkable resistance to aluminium, which did not inhibit growth even at a concentration of 800 mg l^{-1} Al^{3+} (30 mM) (Burt et al. 1986). At concentrations in excess of 400 mg l^{-1} Al^{3+} (15 mM), mycorrhizal plants were protected from root growth inhibition and stilt root formation, which are normally found in non-mycorrhizal plants growing at these levels of aluminium (Leake et al. 1989). *H. ericae* also demonstrates a greater resistance to high levels of iron (Fe^{3+}) than its host plant, being able to tolerate concentrations up to 144 mg l^{-1} (2.6 mM) (Shaw et al. 1990), and provides protection to the host shoots from toxic levels by a process of exclusion whereby Fe^{3+} ions are retained in the mycorrhizal roots. At the same time, however, the fungus possesses a mechanism which enhances uptake at low levels of available Fe^{3+} ions. There is strong evidence that this scavenging ability is conferred by a hydroxymate siderophore whose production is optimal at pH 4.5, which requires l-ornithine as a precursor and which is sensitive to levels of Fe^{3+} ions higher than those required for optimal production (Schuler and Haselwandter 1988; Federspiel et al. 1991; Dobernigg and Haselwandter 1992). Siderophores were also demonstrated in other ericoid mycorrhizal isolates, namely *Oidiodendron griseum* and the ericoid endophyte of a calcicolous ericaceous host (Dobernigg and Haselwandter 1992) and have been identified (Haselwandter et al. 1992): *H. ericae* and *O. griseum* produce ferricrocin as principal siderophore, whereas the isolate of the calcicolous host produces a different molecule, fusigen. These mechanisms provide a fine system of regulation in a situation where certain ions may be made more soluble in acidic soils but may also be immobilized by high levels of organic material.

Although these experimental results are from studies involving the cultured fungus and synthesized mycorrhizal host seedlings growing aseptically, most have used nutrient levels which can be reasonably expected under the defined heathland conditions. Extrapolation to the field situation is, of course, always difficult, especially when one is unaware of the dynamics of nutrient flux and growth under conditions of competition which would exist for both the host and the mycobiont. Nevertheless, no matter to what degree they may be modulated in the field, the selective advantages that the ericoid endophyte brings to the association are a clear indication of what has driven the evolution of the mutualism.

Heathlands of the southern hemisphere

The heathland systems of South Africa (fynbos) and Australia (kwongan) share with the heathlands of the north the sclerophyllous nature of the vegetation, with its high lignin content, and the nutrient-poor status of the soils. The oligotrophic nature of the soils of the southern heathlands has now been well described. The soils on which fynbos vegetation grows are highly leached, acidic structureless sands, most with some degree of podzolization (Kruger 1979). Coastal soils of limestone hills and outcrops are neutral to slightly alkaline and in general there is little profile development, with A horizons containing less than 2% organic carbon. These sandy soils have very low levels of base saturation (20–40%), low levels of total phosphorus in the upper horizons (12–180 mg g^{-1} dry mass) and very low levels of extractable phosphorus, averaging less than 2 mg g^{-1} dry mass (Mitchell et al. 1984; Witkowski and Mitchell 1987). In the acid soils much of the inorganic phosphorus is Fe-bound (2–88%) and organic phosphorus levels are high (58–77%). Levels of exchangeable nitrate and ammonium are low, and in a climatic zone of hot, dry summers soil moisture and temperature become critical factors in determining rates of nitrification, which tend to be low (Stock and Lewis 1982; Stock et al. 1988). No data is available on the organic nitrogen content of these soils. The soils of Australia as a whole are notoriously low in phosphorus (Wild 1958), with levels in the deep, highly leached, podzolized acid sands of the kwongan vegetation areas similar or even lower to those of fynbos soils (Specht and Rayson 1958; Groves 1983). Total nitrogen in these soils is also low.

Thus, we see many of the same selective pressures operating in the south as in the north, especially with regard to the recalcitrant nature of minerals locked away in organic polymers. We could, therefore, expect to find ericoid mycobionts isolated from endemic hosts

of these biomes to show biochemical abilities in releasing nitrogen and phosphorus similar to those of *H. ericae*. However, there are some relevant differences between the soils of the northern and southern heathlands. The organic content of the top 100 mm of fynbos soils never reaches more than 10% (Witkowski and Mitchell 1987), whereas that of the top horizons of a *Callunetum* soil is as high as 85% (Jalal and Read 1983a). Litter production in fynbos is extremely low, as are rates of decomposition, and fire is regarded as the major means of nutrient release from the litter (Mitchell et al. 1986). Decomposition turnover rates in fynbos (up to 7 years) are longer than those of Australian heathlands (Witkowski and Mitchell 1987). The fynbos and kwongan are also floras of intense speciation, including the families Ericaceae (672 species) and Epacridaceae (117 species), but these plants co-exist with other taxa in complex communities, whereas in *Calluna* heathlands ericaceous plants form pure stands. The high levels of toxic organic acids produced by microbial conversion of fatty and phenolic acid-rich residues in *Calluna* heathland soils (Jalal and Read 1983a,b) can be detoxified and assimilated by *H. ericae* (Leake et al. 1989), and the latter authors suggest that this is one of the means by which ericaceous plants can exclude competitors and become so dominant. Given the low organic matter content and litterfall rates in the southern heathlands, one would not expect to find the same high accumulation of toxic organic acids in these soils. It would be of interest to establish whether ericoid endophytes endemic to these soils have the same physiological tolerance to these acids as *H. ericae*. There may be other genotypic or phenotypic variations between fungi from the north and south, such as tolerance to aluminium, levels of which are only moderate in fynbos soils (Mitchell et al. 1984). In this context it is interesting that the wall-bound polygalacturonases of South African mycorrhizal isolates have basic isoforms, whereas those of European isolates, including *Oidiodendron*, have acidic isoforms (Perotto et al. 1995). These differences may relate to differences in soil chemical characteristics, such as the lower cation exchange capacities and base saturation levels which would be associated with the low organic matter and clay content of fynbos soils.

From this brief survey it can be seen that there are undoubtedly similar ecological constraints which exist in heathland ecosystems of different climatic zones, although the causal mechanisms behind these constraints, such as pedological evolution and temperature and aridity gradients, will not be the same and therefore rates of processes in the systems will differ. It would be reasonable to propose that, given the presence of potential microbial symbionts supremely selected for existence in these soils through a longer evolutionary history than that of the Ericaceae and Epacridaceae (Lewis 1987), a symbiosis of mutual benefit could evolve by selection of compatible allelic determinants. To gain a clue as to what has powered this evolution, one could look again at the study of Koske et al. (1990), who observed typical ericoid mycorrhizal hair roots in endemic Hawaiian *Vaccinium* plants, but AM infection in the other parts of the root system. One could conjecture that the loss of dependence on AM by ericaceous plants and the development of a dependence on ericoid mycorrhizas has evolved because of the ericoid endophyte's superior physiological fitness for conditions where nutrients remain inaccessible in organic polymeric form. The ericoid mycorrhiza is evolutionarily more recent than either AM or ectomycorrhizas (Lewis 1987) and, in the future, we may find that the combination of appropriate genetic determinants and heathland-like selective forces has guided hosts other than vascular epacrids and ericads and non-vascular hepatics to acquire an ericoid-type mutualism.

Conclusions

The predominant conclusion one can draw from this review is the need for the basis of research into ericoid mycorrhizas to be extended from the *Hymenoscyphus–Calluna/Vaccinium/Rhododendron* model to include other, taxonomically different host and fungal partners. Only when we have analyzed the surface molecules of a wider group of hosts and mycobionts can we generalize with certainty about their role as determinants of host specificity. Similarly, to confirm propositions on the ecological determinants of specificity, we need to appraise the physiological and biochemical qualities of the fungi isolated from ericads and epacrids of the southern hemisphere within the context of their ecology and plant host range. Another feature of the floras of the fynbos and kwongan is their extraordinary species richness and high level of compositional turnover (beta diversity) between adjacent soils that display chemical and physical differences (Cowling et al. 1990). This observation, together with preliminary evidence of significant differences between calcicole and calcifuge strains of ericoid mycorrhizal fungi in tolerance to Ca^{2+} ions and high pH levels (Straker and Wilson 1993), has introduced a new challenge – that of exploring the function of ericoid mycobionts, by way of their own edaphic specializations, as determinants of host community composition and speciation.

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