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# Seeking the elusive function of the root-colonising dark septate endophytic fungi

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**Abstract:** A comparison of published estimates of mycorrhizal and dark septate endophyte (DSE) colonisation from various ecosystems suggests that DSE may be as abundant as mycorrhizal fungi as judged by the proportion of host plants colonised in mixed plant communities, or by the extent of colonisation in sampled root systems. While many strides have been made in understanding the ecological significance of the mycorrhizal fungi, our knowledge about the role of DSE fungi is in its infancy. In order to provide a framework of testable hypotheses, we review and discuss the most likely functions of this poorly understood group of root-associated fungi. We propose that, like mycorrhizal symbioses, DSE-plant symbioses should be considered multifunctional and not limited to nutrient acquisition and resultant positive host growth responses. Admittedly, many mycorrhizal and endophyte functions, (e.g. stress tolerance, pathogen or herbivore deterrence) are likely to be mediated by improved nutritional status and increased fitness of the host. Accordingly, it is pivotal to establish whether or not the DSE fungi are involved in host nutrient acquisition, either from inorganic and readily soluble sources, or from organic and recalcitrant sources. Facilitation by DSE of the use of organic nitrogen, phosphorus and sulphur sources by plants is a topic that warrants further attention and research. Even in the absence of a clear nutrient uptake function, the extensive DSE colonisation that occurs is likely to pre-emptively or competitively deter pathogens by minimising the carbon available in host rhizosphere environment. The DSEs' high melanin levels and their potential production of secondary metabolites toxic or inhibitory to herbivores are also likely to be factors influencing host performance. Finally, the broad host ranges speculated for most DSE fungi thus far suggest that they are candidates for controlling plant community dynamics via differential host responses to colonisation. We emphasise the need for simple experiments that allow unravelling of the basic biological functions of DSE fungi when they colonise their hosts.

**Key words:** abundance, dark septate endophytes (DSE), multifunctional symbioses, mutualism, mycorrhiza.

## INTRODUCTION

Vascular plants host a great variety of fungi. In addition to being susceptible to soil-borne pathogens, plant roots are also colonised by non-pathogenic or mutualistic fungi like arbuscular mycorrhizae (AM), ectomycorrhizae (EM) and dark septate endophytes (DSE). A vast majority of terrestrial plant species form mycorrhizal associations (Harley & Smith 1983, Smith & Read 1997). The AM fungi comprise about 150 species of zygomycetous fungi, while EM fungi include about 6000 species that are primarily basidiomycetes, along with a few ascomycetes and zygomycetes. The AM fungi are associated with most herbaceous plants and with various woody plant families, while the EM fungi are confined chiefly to a limited number of woody plant families. It is now evident that the mycorrhizal fungi have many significant functions in ecosystems. To list a few important functions for which there is convincing evidence, they absorb non-mobile nutrients from the soil and translocate them to host plants, sequester potentially harmful heavy metal ions, facilitate interplant transfer of nutrients, and beneficially modify plant water relations (Smith & Read 1997).

In contrast to the plethora of knowledge about the EM and AM fungi, very little is known about the DSE fungi. The DSE are broadly classified as conidial or sterile septate fungal endophytes that form melanised structures such as inter- and intracellular hyphae and microsclerotia in the plant roots and that have known or likely affinities within ascomycetes (Jumpponen & Trappe 1998). It has also been suggested that hyaline septate hyphae may be associated with DSE colonisation (Haselwandter & Read 1982, Newsham 1999, Yu *et al*. 2001).

DSE are found worldwide and coexist often with different mycorrhizal fungi. They have been reported from 600 plant species including plants that have been considered non-mycorrhizal (Jumpponen & Trappe 1998). In this paper, we review recent literature to evaluate the abundance of DSE across various ecosystems. Based on available information, we conclude that DSE colonise a great diversity of plant species and parallel mycorrhizal fungi, AM fungi in particular, in the proportion of plant species they colonise as well as in their frequency of occurrence in root systems. We then discuss the possible functions

of DSE. Admittedly, the available data are scanty at best. However, rather than to provide a comprehensive review, our goal is to present a framework of testable hypotheses that may serve as a starting point for experimental testing of possible DSE functions.

**Abundance of the dark septate endophytes in various ecosystems**

In this section we review studies that have quantified both mycorrhizal and DSE colonisation and infer the potential global abundance of the latter (Table 1). The DSE fungi have been reported from various habitats the world over. They do not seem to exhibit any host specificity and have been isolated from plants that are non-mycorrhizal or that form well-defined mycorrhizal associations, including arbuscular, ericoid, orchid and ectomycorrhizal associations (Jumpponen & Trappe 1998, Addy *et al*. 2000). While many reports on the abundance of mycorrhizal fungi from different habitats exist, only a few studies have quantified root colonisation or systematically recorded the proportion of taxa hosting DSE. Jumpponen and Trappe (1998) emphasised that detection of DSE colonisation in most studies was incidental. Most of the available DSE abundance data have been collected from the arctic, alpine, antarctic, and temperate habitats, while next to nothing is known about the abundance of DSE in boreal and tropical ecosystems.

*Alpine habitats*: Read & Haselwandter (1981) studied mycorrhizal fungi of the dominant and sub-dominant plants in the Central and Northern Calcareous Alps of Austria and recorded the colonisation of typical AM fungi, fine endophyte, DSE, and EM fungi at two different sampling times at five Austrian alpine sites located at a range of altitudes. They concluded that more than half of the observed plant species had typical AM colonisation, with colonisation rates ranging up to 100 % (Table 1). Nearly one third of the plants were colonised by DSE, with colonisation frequencies ranging from non-existent to very high within a root system. Interestingly, these authors were able to infer that AM and DSE colonisation were correlated with altitude. The lowest AM colonisation was recorded at the highest altitudes and in fertilised meadows, whereas the most intense colonisation was found in the low-elevation species-rich grasslands. In contrast, although plants through the entire range of altitudes were colonised by DSE, the most intensive colonisation was recorded on mountain peaks at an altitude of 3100–3200m. This study suggested that DSE are more prevalent than AM in high-elevation, stressed environments.

In a broad study focusing on five ecoregions in Alberta, Currah & van Dyk (1986) confirmed that DSE did indeed occur mainly in alpine areas (see Table 1). Similarly, Treu *et al*. (1996) examined the mycorrhizal status of 40 taxa of vascular plants in montane interior Alaska within the Denali National Park and Reserve. The AM fungi were least common while ecto- and ericoid mycorrhizae as well as DSE occurred relatively frequently (Table 1). Treu *et al*. (1996) concluded that their results on the common occurrence of DSE agreed with Read and Haselwandter (1981): DSE appeared more frequently in stressed environments. However, the elevation-related patterns proposed by Read & Haselwandter (1981) were not observed in Finnish oroarctic and subalpine regions (Ruotsalainen 2003).

Trowbridge and Jumpponen (2003) recorded shrub willow (*Salix* spp.) colonisation by AM, EM and DSE fungi on a receding glacier forefront in a subalpine region of the Cascades Mountain Range, Washington, U.S.A. They found that < 1 % of root length was colonised by AM, 25 % of root tips and 19.4 % root length by EM while 25.6 % of root length was colonized by DSE. DSE colonisation varied widely in their study. Melanised hyphae and microsclerotia occurred in up to 80 % of the root length. In comparison, EM structures (Hartig net or pseudoparenchymatous tissue) were observed never to exceed 40 % of the root length.

*Arctic habitats*: AM colonisation in arctic and alpine regions is highly variable (Gardes & Dahlberg 1996). In arctic tundra, it is negligible in some cases ( Bledsoe *et al*. 1990, Kohn & Stasovski 1990, Väre *et al*. 1992), while in others it is more common, with studies of some areas showing frequent colonisation of a large proportion of any root system observed (Strelkova 1956, Katenin 1962, 1972 in Bledsoe *et al*. 1990). Kohn and Stasovski (1990) concluded that AM fungi were mainly absent in an arctic oasis at Ellesmere Island, Canada, as only one plant species (the fragrant wood fern *Dryopteris fragrans* Schott.) of the 24 observed was colonised by aseptate hyphae (see Table 1). This study also detected septate fungal symbionts in some plant species but they were not considered to be DSE. Similarly, Bledsoe *et al*. (1990) examined 55 herbaceous and woody plant species in the Canadian high arctic for mycorrhizae. These authors concluded that AM associations at their sites were absent as neither vesicles nor arbuscules were observed. They further confirmed the absence of AM by showing that spore isolation attempts were unsuccessful and that bioassay seedlings produced no AM structures in greenhouse when soils from the test site were used as the only potential source of AM inoculum. Nonetheless, both of these reports concluded that EM and ericoid mycorrhizas (ERM) were present in plants with such known affiliations.

Väre *et al*. (1992) studied the root colonisation of 76 different plant species from 19 families in west Spitsbergen in the middle-northern arctic zone.

**Table 1**. Abundance of mycorrhizal and dark septate endophytes in various ecosystems. Number of species with fungal colonization over the total number of species examined, followed by the percentage of root length colonised (range in parentheses). AM = Arbuscular mycorrhiza, EM = Ectomycorrhiza, ERM = Ericoid mycorrhiza, DSE = Dark septate endophytes.

| Ecosystem type | AM | EM | ERM | DSE | Other | Reference |
|---|---|---|---|---|---|---|
| **Alpine** | | | | | | |
| | 63/89 (1–100) | 12/89 (NA)[a] | – | 33/89 (1–100) | 15/89 (FE) | Read & Haselwandter (1981) |
| | 2/40 | 6/40 | 7/40 | 11/40 | 1/40 (ArM) | Treu *et al*. (1996) |
| | 5/35 | – | – | 31/35 | 1/35 (Orc) | Currah & Van Dyk (1986) |
| **Arctic** | | | | | | |
| | –/55 (NA)[a,b] | 3/55 (NA)[a] | 2/55 (NA)[a] | Present[c] | | Bledsoe *et al*. (1990) |
| | 1/24 (+)[d] | 6/24 (+)[d] | 2/24 (+)[d] | – | | Kohn & Stasovski (1990) |
| | 5/6 (0–100) | – | – | 6/6 (10–50) | 5/6 (FE) | Ruotsalainen *et al*. (2002) |
| | –/76 | 3/76 | 2/76 | 30/76 | | Väre *et al*. (1996) |
| **Antarctic** | | | | | | |
| | 18/40 (NA)[a] | 0/40 (NA)[a] | | 21/40 (NA)[a] | | Laursen *et al*. (1997) |
| **Boreal forest** | | | | | | |
| | 4/6 | – | – | 2/6[e] | – | Currah & Van Dyk (1986) |
| | 4/25 | 6/25 | 5/25 | 14/25 | – | Thormann *et al*. (1999) |
| **Temperate grassland** | | | | | | |
| **Sandy grassland** | 60/89 (0–100) | 4/89 (NA)[a] | – | 63/89 (NA)[a] | – | Kovács & Szigetvári (2002) |
| **Short grass prairie** | 77/85 (NA)[a] | – | – | 0/85 (NA)[a] | – | Currah & Van Dyk (1986) |
| **Tropical rain forest** | 5/18 (26–75) | – | 12/18 (< 25–100) | 16/18 (< 25–75) | – | Rains *et al*. (2003) |

[a]NA = data not available.

[b]Although fine endophyte may have been observed, the absence or arbuscules and vesicles was determined to mean lack of AM associations.

[c]The taxa colonised were not listed.

[d]+ = high in most replicates; ± colonisation of few cells in less than 50% of the replicates.

[e]DSE was recorded only as superficial melanized hyphae.

ArM = Arbutoid mycorrhiza.

FE = Fine endophyte.

Orc = Orchid mycorrhiza.

They found that the DSE were the predominant fungi while EM and ERM fungi were seen in a minuscule number of plant species (Table 1). AM fungi were not observed in the examined roots, although 11 soil samples together yielded one AM spore. DSE fungi produced microsclerotia and hyaline hyphae in the roots. Väre *et al*. (1992), however, considered the hyaline septate hyphae to represent a group of root endophytes separate from the DSE (see discussion on melanised and hyaline structures in the "*Abundance of DSE – global inference*" section below). The non-melanised endophytic structures were recorded in seven plant species.

Ruotsalainen (2003) studied AM and DSE colonisation over one growing season across an altitudinal gradient in the subarctic meadows in northern Finland. The vegetation supported colonisation by AM and DSE fungi. Consistent with the observations of Read and Haselwandter (1981), Ruotsalainen found that DSE were common in the plant species studied. They colonised the host plants simultaneously with mycorrhizal fungi. Contrary to her initial hypotheses that DSE colonisation would increase and AM colonisation would decrease at higher altitudes, Ruotsalainen (2003) found no positive correlation between altitude and DSE colonisation, no negative correlation between altitude and AM colonisation. The fungal colonisation rates and patterns appeared to be species-specific during the growing season at a given altitude.

*Antarctic habitats*: AM have been suggested to be absent in the Antarctic, although many plant species that occur in the Antarctic form abundant AM when they grow in cool temperate and subantarctic regions (Christie & Nicholson 1983). In an antarctic study by Christie & Nicholson (1983), DSE were observed in only a limited number of samples. Subsequent studies gave contradictory results and even suggested that AM fungi are commonly present in Antarctica. Laursen *et al*. (1997) found that 18 of the 40 plant species studied in Antarctica harboured AM fungi. The majority of the sampled plants contained only vesicles; only three species were recorded to have both vesicles and arbuscules in their roots. In addition, Laursen *et al*. (1997) rated DSE as frequent in occurrence (Table 1). Of the 40 plant species studied, 21 possessed melanised septate hyphae and microsclerotia in addition to co-occurring AM colonisation. Laursen *et al*. (1997) found no EM, even though some plant species present belonged to the family *Rosaceae*, which have known EM representatives in the Northern Hemisphere.

*Boreal habitats*: Currah and van Dyk (1986) conducted a survey of fungal root colonisation over five ecoregions and 179 plant species. Four of the six species from the boreal sites were colonised by AM, whereas only two harboured DSE. Most studies conducted in boreal forest habitats tend to focus on EM colonisation. Therefore, studies reporting DSE from these habitats are rare or confined to marginal habitats. Thormann *et al*. (1999), for example, studied fungal colonisation of 25 plant species in boreal peatlands. Nearly half of the studied species contained melanised structures typical of DSE, whereas AM, EM, and ERM were less frequent (Table 1). Although it may be difficult to make conclusions about the relative abundance of the mycorrhizal and DSE fungi in boreal regions based on the limited available data, it appears that DSE can be commonly observed and may be frequent in some habitats.

*Temperate habitats*: Horton *et al*. (1998) did a five-month study of fungal colonisation of young post-fire seedlings of bishop pine, *Pinus muricata*, in scrub and forest sites. In the forest site, the frequency of seedlings with DSE colonisation was consistently greater than that of seedlings with either EM or AM colonisation except during the fourth month, when the number of seedlings with DSE and EM were comparable. In fact, mycorrhizal fungi were absent in the seedlings during the first two months of the study, while DSE were well represented. In the scrub site, the frequency of seedlings colonised by DSE was greater than that colonised by either AM or EM for the first three months. During the last two study months, DSE and EM fungi were found to have colonised a similar proportion of the seedlings. Horton *et al*. (1998) suggested that DSE may be pioneering colonisers of young tree seedlings in such secondary successional environments. However, other

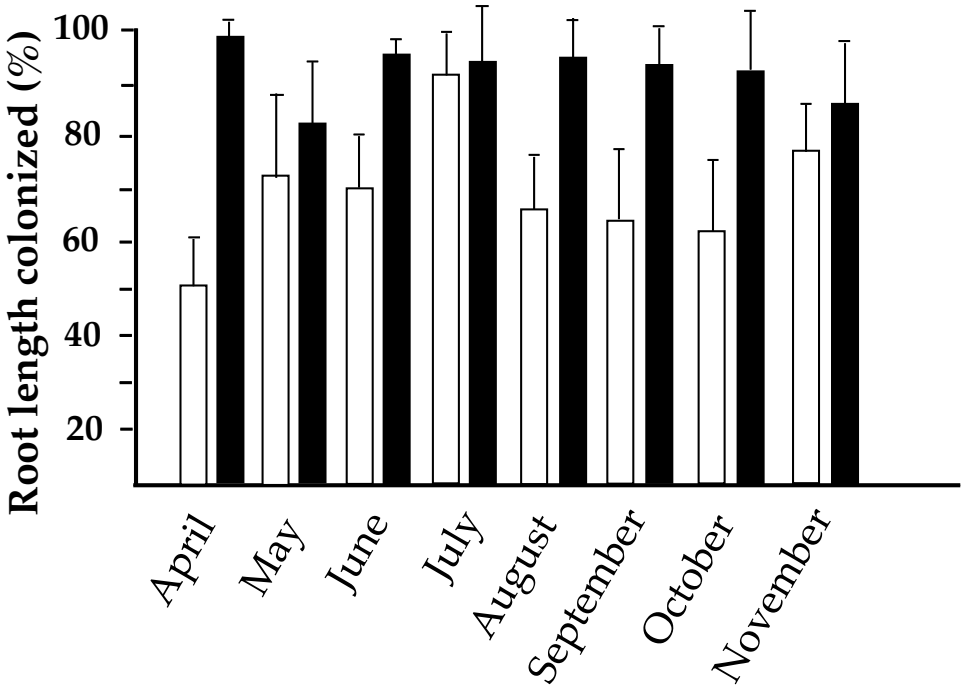


**Fig. 1.** Root colonisation (percent root length colonised, gridline intercept method) by arbuscular mycorrhizal (AM) fungi (open bars) and dark septate endophytes (DSE; filled bars) in mesic mixed grassland communities at the Konza Prairie Long-Term Ecological Research site near Manhattan, Kansas, U.S.A. DSE had higher colonisation levels than AM ($F_{1,80} = 132.7$, $P < 0.001$). Colonisation varied significantly among months ($F_{7,80} = 3.5$, $P = 0.002$). AM and DSE had different seasonal dynamics ($F_{7,80} = 6.5$, $P < 0.001$ for the interaction term fungus * time).

research indicates that it is not only young seedlings that host DSE. Ahlich and Sieber (1996) estimated the frequency of DSE by isolating them from non-ectomycorrhizal roots of adult EM plants (*Abies alba*, *Fagus sylvatica* L., *Picea abies* and *Pinus sylvestris*) at various temperate forest sites in Europe. They also considered additional EM host species from Asia and the U.S.A. They estimated that about 70–100 % of the fine roots of individual plants from Europe were colonised by DSE while 20–100 % of the additional samples were infected by these fungi.

In addition to occurring in temperate forests, DSE seem to occur frequently in temperate grassland ecosystems. In a recent year-long study of root endophytes in sandy grasslands of the Great Hungarian Plain, Kovács and Szigetvári (2002) found 67 % of the studied plant species to be colonised by AM. Most of these AM-colonised plants (78 %) had high colonisation levels ranging from 50 to 100 %. Kovács and Szigetvári (2002) also confirmed five plant species normally considered ectomycorrhizal hosts to be colonised by EM fungi. On the whole, plants were colonised by DSE as frequently as by mycorrhizal fungi (Table 1). A total of 63 plant species supported DSE colonisation while only 60 plant species were mycorrhizal. Within the mycorrhizal group, 56 species harboured only AM and four species had both AM and EM. Colonisation by DSE hyphae was found in about 75 % of the mycorrhizal plants, while one third of these plants also had microsclerotia. Interestingly, of the 29 non-mycorrhizal plant species, 18 were colonised by intra- and intercellular septate hyphae or by microsclerotia. This study was the first to suggest that DSE colonisation may be as abundant as AM colonisation in the types of habitats occurring in sandy Hungarian grasslands.

Barrow and Aaltonen (2001) conducted an intense sampling of fungal colonisation in the roots of native plants in temperate semi-arid rangelands of New Mexico, U.S.A. Their study was conducted over a span of one year and plants were sampled two or three times per month. The native vegetation was dominated by the four-wing saltbush, *Atriplex canescens* (Pursh) Nutt. Although *A. canescens* is known to form AM (Barrow *et al*. 1997), Barrow and Aaltonen (2001) concluded that the root systems were nearly exclusively colonised by DSE. They attributed the low AM colonisation and the prevalence of DSE to extended drought in the region, thus supporting the hypothesis of Read and Haselwandter (1981) that DSE occur most frequently in extreme environments and stressed conditions. In a later study involving a weekly sampling of native grama grasses in the genus *Bouteloua*, Barrow (2003) concluded, similarly, that DSE colonisation exceeded that of AM.

These observations from temperate grass- and rangelands are supported by our own unpublished results and may indicate that DSE are widely prevalent in temperate grassland ecosystems. In a sampling of mixed tallgrass prairie plant communities at a mesic prairie site at Konza Prairie Long Term Ecological Research site in Kansas, U.S.A., we observed that DSE colonisation exceeded that of AM (Fig. 1). In our study, however, a statistically significant interaction involving the type of colonisation (AM vs. DSE) established that whilst DSE colonisation remained rather stable throughout the growing season, AM colonisation was lower than DSE colonisation early in the season but reached comparable levels later. Clearly, temporal dynamics need to be taken into account in studies of root colonisation, particularly when conclusions are made about patterns of fungal abundance.

*Tropical habitats*: Tropical ecosystems may be the least well understood in terms of the status of fungal root endophytes. We are aware of only one study that systematically quantified DSE and mycorrhizal colonisation. Rains *et al*. (2003) assessed the mycorrhizal status of epiphytes and terrestrial plants in neotropical rain forests in Costa Rica. They surveyed 18 species based on a total sample size of 43 plants, including 23 canopy epiphytes, 16 terrestrial plants and four *Disterigma humboldtii* (*Ericaceae*) plants rooted in coarse woody debris. AM colonisation was recorded in only a few plant species while the ericaceous plants possessed typical ERM structures (hyphal coils). Nearly all the species observed were colonised by DSE (Table 1). However the extent of DSE colonisation was highly variable ranging from a low or moderate to a high level of occurrence (< 25–75 %) of melanised hyphae or microsclerotia. Consistently with Read and Haselwandter (1981), Rains *et al*. (2003) attributed the frequent occurrence of DSE to the stressful habitat occupied by the epiphytic plants.

*Abundance of DSE – global inference*: Only a very limited number of studies so far have attempted to evaluate and quantify root colonisation by both mycorrhizal and DSE fungi. Based on the information available, however, it appears that DSE may be as abundant as mycorrhizal fungi. In many studies, comparable proportions of the plants within the communities studied were observed to be colonised by the non-mycorrhizal DSE as well as by mycorrhizal fungi. The actual rates of colonisation, although highly variable, seem to fall into similar ranges.

The DSE fungi, when present, seem to occur in large proportions of any examined root system. Grünig *et al*. (2002) isolated DSE from a small (3 × 3 m) field plot in Austria. These fungi were obtained from over 80 % of root segments. Studies by Grünig *et al*. (2002)

and Ahlich & Sieber (1996) clearly indicate that these fungi occur very frequently (see above). If we presume that most roots of EM plants are colonised by EM, DSE colonisation in those studies appears to be at nearly comparable levels. Similarly, in a study relying on direct PCR amplification from roots of EM nursery trees, a DSE fungus, *Phialocephala fortinii* Wang & Wilcox, or closely related taxa, were among the three most commonly found sequence types (Kernaghan *et al*. 2003). Clearly, the jury is still out on determining DSE abundance across different ecosystems, since ecosystems and habitats show differing patterns of root colonisation (see Read & Haselwandter 1981). We must emphasise the need for further studies with a focus not limited just to mycorrhizal fungi, but rather broadened to include consideration of the overall pattern of root colonisation by various typically root-inhabiting fungi.

DSE colonisation may be even more abundant than is reported in the studies cited above, since these mainly relied on observing melanised intra- and intercellular hyphae and microsclerotia. Firstly, as has been known now for several decades (Girlanda *et al*. 2002), DSE frequently co-occur with mycorrhizal fungi. Girlanda *et al*. (2002) designed a study focused on isolating the dark, sterile mycelia from ecto- and endomycorrhizal roots. Up to nearly 60 % of the isolates obtained belonged to the target group and possessed melanised hyphae. Observing DSE colonisation may be difficult when it occurs in ectomycorrhizal roots, since many inter- and intracellular structures may be hidden under an ectomycorrhizal mantle or a Hartig net. Also, Haselwandter and Read (1982), Newsham (1999) and Yu *et al*. (2001) all reported the formation of non-melanised, hyaline hyphae by these fungi in the plant host. The hyaline structures were continuous with melanised hyphae and were clearly produced by the same DSE fungus. Yu *et al*. (2001) suggested that the hyaline hyphae produced by melanised DSE fungi often went unnoticed in microscopic studies and that this resulted in an underestimation of the true abundance of DSE. Supporting these observations, Barrow and Aaltonen (2001) and Barrow (2003) found that hyaline hyphae were extremely common in *A. canescens* and *Bouteloua* spp., but were usually not visible with ordinary light microscopy or staining. They suggested that the hyaline hyphae could only be visualised by careful observation with differential interference contrast (DIC) microscopy at high magnification (400–1000×). The hyaline hyphae did not stain with the Trypan blue often used in root studies, suggesting poor chitinisation or poor development of the fungal cell wall during host colonisation (Barrow & Aaltonen 2001). Staining with Sudan IV, a lipid-specific stain, followed by DIC microscopy was shown to be necessary for visualising the hyaline component of fungal colonisation. Although further confirmation based on inoculated plant growth in well-controlled aseptic conditions is required, it can be preliminarily stated that DSE produce a variety of morphological structures, including not just melanised hyphae and microsclerotia but also hyaline hyphae and vesicles, when occupying host tissues.

We concur with Yu *et al*. (2001) that the accurate observation and quantification of non-melanised DSE structures may be impaired by the poor visibility of these structures and their low affinity for chitin-targeting stains such as Trypan blue. In addition, we have observed that the lipid bodies stained efficiently by Sudan IV stains are highly variable in their occurrence in field-collected samples (unpubl. data). Barrow and Aaltonen (2001) suggested that the occurrence of these lipids within the hyaline fungal tissues varies seasonally and may be associated in resource translocation in the host plant. Given the unknown seasonal dynamics affecting the chemistry of the hyaline structures, their true frequency remains difficult to estimate. However, we observed that when DSE colonisation is high, the melanised and hyaline structures co-occur and often occupy the same tissues (unpubl. data). Under such conditions, the risk of serious underestimation of DSE occurrence is very limited.

In summary, it is emphasised that DSE fungi a) are ubiquitous in occurrence b) co-occur with different types of mycorrhizae c) are most prevalent in stressed environments d) can be as abundant as mycorrhizal fungi and e) can be underestimated when hyaline structures are formed in the absence of melanized structures.

**Potential functions of DSE in natural ecosystems**

In this section we briefly review the commonly proposed functions for mycorrhizal fungi and hypothesise as to which are most likely to be performed by DSE. The dependence of plants on their mycorrhizal symbionts is fairly well known. Mycorrhizal fungi confer several benefits on their host plants, and these are especially significant in stressed environments (Smith & Read 1997). While mycorrhizal functions may be relatively well understood, very little is known about the function of DSE. We concluded above that in ecosystems where DSE fungi have been studied, they have generally been found to colonise a high proportion of the plants present. Often this colonization occurs at a fairly high density. The great abundance and the apparent broad host ranges (Jumpponen & Trappe 1998) of DSE suggest that they have an important, albeit unknown, function in ecosystems.

While it may be difficult to estimate the abundance of various types of DSE (see above), understanding the relevant ecological roles of these fungi is even more time-consuming and difficult. The role of DSE in nutrient

capture has been studied to a limited extent. However, in discussing the positive contributions of DSE to plant vitality, we cannot limit ourselves to consideration of the nutritional effects of the symbioses. Mycorrhizal fungi have been shown to fulfil a variety of different types of functions (Newsham *et al*. 1995, Smith & Read 1997). Dark septate endophyte (DSE) may also have various functions within plant communities. Although many of the mycorrhizal functions are unquestionably related to improved host nutrient acquisition, some rely on the production of inhibitory metabolites or on exploitation competition exerted against rhizosphere-inhabiting microorganisms. The non-nutritional effects of the symbioses, such as protection from soil-borne pathogens or herbivores, modification of environmental tolerance, and involvement in plant community dynamics, can also be of great relevance.

The current understanding of the abundance of DSE and their co-existence with conventional mycorrhizas raise interesting questions. Are DSE more efficient than mycorrhizal fungi in foraging for nutrients from organic sources? Do the functions of DSE complement those of the mycorrhizal fungi? Before we can answer such questions, we must gather experimental evidence. It is our intention to give these investigations some direction by putting forth proposals for the most likely functions of DSE. We argue that the host responses to these fungi fall within the range of the mutualism-parasitism continuum proposed for the mycorrhizal fungi (Johnson *et al*. 1997). Currently, only limited evidence exists for many of the functions discussed here. Consequently, we use examples from studies in mycorrhizal systems, and propose a framework that can assist in further development of hypotheses and future experiments.

*Facilitation of host mineral nutrient uptake by DSE*: The involvement of mycorrhizal fungi in plant nutrition has been the most studied function of mycorrhizae. In particular, a great many reports deal with mineral nutrient uptake (see Smith & Read 1997, Allen *et al*. 2003). Mycorrhizal roots are capable of uptake of P, N, Zn, Cu, Ni, S, Mn, B, Fe, Ca, and K (Marschner 1994, Smith & Read 1997, Clark 2000, Liu 2000). The result of this improved nutrient acquisition is often that host growth improves in relation to the extent of mycorrhizal fungal colonisation, though this does not occur in all cases.

Our knowledge of DSE involvement in host nutrient acquisition is limited, and existing reports have concluded variously that DSE exert positive, negative or negligible effects on host performance (see Jumpponen 2001). Haselwandter and Read (1982) isolated DSE fungi from *Carex* species from the European Alps. When inoculated on the same species of *Carex*, these fungi resulted in increased dry weight of roots, shoots and whole plants along with an increase in shoot P content. Jumpponen *et al*. (1998) evaluated the role of the common DSE fungus *P. fortinii* in nutrient uptake by *Pinus contorta*. Inoculation with the endophyte alone did not enhance growth but increased the foliar P concentration. A combination of N amendment and fungal inoculation increased host biomass by more than 50 % beyond that obtained via N amendment alone. Jumpponen *et al*. (1998) speculated that the removal of N limitation allowed *P. fortinii* to exhibit mycorrhizal behaviour, *i.e.*, enhancement of plant growth and nutrient uptake.

Newsham (1999) found that the DSE fungus *Phialophora graminicola* (Deacon) J. Walker, currently called *Harpophora radicicola* (Deacon) W. Gams, was beneficial to the grass *Vulpia ciliata* Dumort. ssp. *ambigua* . Grasses inoculated with *P. graminicola* had more tillers than controls had, and also had increased root, shoot and total biomasses. The inoculated seedlings also had increased root length and root N content plus increased root, shoot and total P compared to controls, but had reduced shoot N. The mechanisms underlying these growth responses remained unknown. Barrow and Osuna (2002) concluded that *Aspergillus ustus* (Bainier) Thom & Church, which they considered a DSE fungus, had a mutualistic association with fourwing saltbrush (*A. canescens*). In pure culture, the fungus was shown to be able to hydrolyse P sources unavailable to the plant, like rock- and tricalcium phosphates. It also improved seedling nutrition by supplying P from these sources. The improved P nutrition derived from the recalcitrant sources resulted in a typical mycorrhizal response, that is, increased shoot and root biomass. It was emphasised that the plants colonised by *A. ustus*, with their access to immobile P sources, were more efficient in P use than were uninoculated plants that could only obtain P only from readily soluble sources.

Although the examples cited here seem to support DSE involvement in plant nutrient acquisition, a great number of studies have failed to show positive effects from DSE inoculation [see Table 1 in Jumpponen (2001)]. The lack of any growth or nutritional benefits under experimental conditions does not negate the possibility of important functions in natural systems. It is important to note that colonisation by fungal hyphae is likely to allow access to soluble nutrient sources otherwise unavailable to the host plant. The relatively small diameter of DSE hyphae, especially when compared to root diameter, allows penetration of soil micropores and acquisition of resources from a soil volume impenetrable to the plant roots. The fulfillment of such functions by DSE under natural conditions remains open to speculation, as many aspects of the basic biology of DSE fungi are still unknown. For example, we are unaware of any reports

on extramatrical DSE mycelium and soil volumes occupied by such mycelia, although Barrow and Osuna (2002) were able to demonstrate that *A. ustus* mycelium extended into the root exclusion chamber in their experiment.

Overall, the range of the observed DSE associations fall within a continuum ranging from mutualism to parasitism (Johnson *et al*. 1997). Although the DSE involvement in host nutrient acquisition appears unclear, innovative studies such as that of Barrow and Osuna (2002) suggest that DSE function may be more complex than simple enhancement of foraging for nutrients in soluble soil pools. For example, use of organic nutrient sources (see below) is one of the areas where DSE may complement the functions of mycorrhizal fungi.

*Utilisation of organic nutrient pools by DSE*: In most terrestrial ecosystems N is the nutrient most limiting for plant growth (Aerts & Chapin 2000). Current data suggest that in many soils organic N is in greater abundance than inorganic forms (Aerts 2002 and references therein). The most common forms of organic N in the soil are various simple amino acids. A proportion of the total amino acid content is readily available in the soil solution (Lipson & Näsholm 2001). Similarly, less than 1 % of soil P is in solution in soil, while large pools of organic P are found in the form of inositol phosphates, phospholipids and nucleic acids. Conversion of these organic nutrient forms into inorganic forms for easy uptake by plants is dependent on extracellular enzymes derived from microbes, including fungi and bacteria (Smith & Read 1997).

The nutrient foraging strategies utilized by mycorrhizal fungi differ. While AM fungi may mainly absorb soluble sources of P and N, EM and ERM fungi have an array of extracellular enzymes available for the degradation of complex organic material (Olsson 2002). DSE fungi, like EM and ERM fungi, produce arrays of hydrolytic enzymes and can access sources of C, N and P in detritus. Table 2 lists the hydrolytic capabilities reported so far in the DSE fungi.

One promising avenue of research yet to be explored is the question of whether DSE can mobilize nutrients from the amino acids that are abundant in the soil environment (Lipson & Näsholm 2001). The exploitation of soil amino acids by EM fungi is well known ( Read *et al*. 1989, Finlay *et al*. 1992). Smith and Read (1997) suggest that mycorrhizal fungi are more efficient than saprobic fungi in obtaining N from organic sources, since they are not dependant on soil organic C but have access to recently fixed photosynthate C. Our preliminary experiments suggested that most DSE isolates from Konza Prairie in Kansas were able to utilise a great variety of N sources (Mandyam & Jumpponen, unpublished). In a liquid culture system similar to that of Finlay *et al*. (1992), amino acids including alanine, glycine and arginine were utilised as efficiently as $NH_4^+$ when they were provided to the DSE isolates as the sole source of N.

Caldwell and Jumpponen (2003a) have also investigated the ability of DSE fungi and EM fungi to utilise heterocyclic organic N. The heterocyclic compounds used were guanine and uric acid, which are excretion products of mites and many other invertebrates commonly found in the soil environment. DSE and ERM fungi were capable of utilising guanine and uric acid as sole source of N in pure culture. Interestingly these fungi attained greater growth yields on heterocyclic N than on $NH_4^+$.

The enzymatic capabilities discussed here demonstrate the potential of DSE fungi to access detrital C, N and P. Caldwell and Jumpponen (2003b) have also established the ability of DSE fungi to hydrolyse organic sulphate. Organic sulphur compounds may be important sources of sulphur for many mycorrhizal plants. Ester-sulphate is an organic form of sulphur and may contribute significantly to the total soil sulphur (Autry & Fitzgerald 1990). *Phialocephala fortinii* and other DSE species along with ERM fungi were able to produce aryl sulphatase and to hydrolyse aryl sulphate esters. In contrast, the EM fungi studied seemed unable to hydrolyse these organic sulphur compounds. This suggests a potential for DSE fungi to transfer sulphur from organic pools to their host plants.

As a next step, it is necessary to determine if the nutrient use "*in vitro*" will prove to be significant when DSE fungi occur in symbiosis. Simple experiments can be designed to show whether complex organic molecules provided as a sole source are made available by DSE, for example when roots are excluded from a soil compartment and only fungal access to the nutrient sources is allowed. We find it very likely that DSE will allow direct host access to recalcitrant nutrients. Barrow and Osuna (2002) have already confirmed that root-inhabiting *A. ustus* improved plant growth when insoluble P sources were placed in a root exclusion compartment. Similar experiments are required to determine whether fungi known to solubilize other complex nutrient sources *in vitro* will do so in a way that improves plant growth *in vivo*.

*Alteration of host water uptake and environmental tolerance by DSE*: Mycorrhizal fungi can alter the environmental tolerance of host plants in various ways. Inoculation with heavy-metal-tolerant mycorrhizal fungi often improves survival and longevity of hosts in contaminated sites (Jones & Hutchinson 1986, Meharg & Cairney 2000, Sharples *et al*. 2000, Cairney *et al*. 2001, Malcová *et al*. 2001, Hall 2002, Turnau & Haselwandter 2002, Cairney & Meharg 2003). Similarly, colonisation by well-adapted mycorrhizal

**Table 2.** Reported enzymatic capabilities of DSE fungi.

| Enzyme | DSE fungus | References |
|---|---|---|
| Cellulases | Unidentified DSE isolates | Bååth & Söderström (1980) |
| | *Cadophora* (*Phialophora*) *finlandica* (C.J.K. Wang & H.E. Wilcox) T.C. Harrington & McNew and isolates similar to *Phialocephala fortinii* | Caldwell *et al*. (2000) |
| | *Periconia* species and other DSE isolates | Mandyam and Jumpponen, unpubl. |
| Laccases | *P. fortinii* | Currah & Tsuneda (1993) |
| Amylases | *C. finlandica* and isolates similar to *P. fortinii* | Caldwell *et al*. (2000) |
| Lipases | *C. finlandica* and isolates similar to *P. fortinii* | Caldwell *et al*. (2000) |
| Pectinases | *C. finlandica* and isolates similar to *P. fortinii* | Caldwell *et al*. (2000) |
| Xylanases | *C. finlandica* and isolates similar to *P. fortinii* | Caldwell *et al*. (2000) |
| Proteolytic enzymes | Unidentified DSE isolates | Bååth & Söderström (1980) |
| | *C. finlandica* and isolates similar to *P. fortinii* | Caldwell *et al*. (2000) |
| Tyrosinases | *Periconia* ssp. and other DSE isolates | Mandyam & Jumpponen, unpubl. |
| Polyphenol oxidases | *P. fortinii* strains | Currah & Tsuneda (1993) |
| | *Leptodontidium orchidicola* | Ferrnando & Currah (1995) |
| | *Periconia* ssp. and other DSE isolates | Mandyam & Jumpponen, unpubl. |

strains on saline sites often improves host performance (Hirrel & Gerdemann 1980, Al-Karaki & Hammand 2001, Feng *et al*. 2002). Although such functions clearly are critical in stressed environments, we focus here mainly on another topic, the moderation of host water relations and drought tolerance. We consider these to be the functions most likely to be altered by DSE fungi.

*Host water uptake*: Augé (2001) comprehensively reviewed the potential mechanisms involved in mycorrhizal modification of plant water relations and drought tolerance. Many of the proposed mechanisms were related to the size of the host plant and the nutrition of the host. Since the jury is still out on determining whether or not DSE enhance host growth and improve host nutritional status, as discussed above, we must limit our discussion of parallel mechanisms here. Clearly, if DSE colonisation improves plant nutrient status, the same uptake mechanisms may also affect water relations. Here, however, we mainly concentrate on mechanisms not related to nutrient uptake, as water relations have been shown to be affected by mycorrhizal colonisation independent of nutritional changes (Bethlenfalvay *et al*. 1988, Davies *et al*. 1993).

Factors that can affect water absorption by mycorrhizal or DSE-colonized roots tend to be features that affect water movement into the plant (Hardie & Leyton 1981, Allen 1982, Brownlee *et al*. 1983, Landhäusser *et al*. 2002) or through the plant (Johnson *et al*. 1982, Kucey & Paul 1982). Hormonal control of host physiology by root-colonising fungi may also be a factor (Allen *et al*. 1980, Levy & Krikun 1980). The extent to which plant water absorption may be mediated by DSE hyphae is uncertain. As stressed earlier, many aspects of the basic biology of the DSE fungi are unknown. For example, efficient water scavenging from soil matrix and transportation into the host roots would require extramatrical mycelium. Although soil is likely to contain vast quantities of melanised fungal hyphae, distinction of the extramatrical mycelium of root-inhabiting melanised fungi from melanised hyphae of saprobic soil fungi is almost impossible. Even if plentiful mycelium can be observed in aseptic resynthesis experiments (Mandyam & Jumpponen, unpublished), it is uncertain whether such structures can be inferred to occur in natural systems.

It is likely that extensive DSE colonisation of host plants under field conditions (see Barrow & Aaltonen 2001 and Fig. 1) is indicative of altered water conductance within host tissues. According to Boyer (1971) and Black (1979), root system resistance

accounts for most of the total resistance to water flow through the plant. It has been suspected that mycorrhizal colonisation alters radial or axial resistance to water flow in roots (Safir *et al*. 1972). Similarly, extensive DSE colonisation may alter root water dynamics.

In addition to experiencing altered resistance of roots to water flow, mycorrhizal and non-mycorrhizal plants often exhibit altered transpiration rates and stomatal conductances (for reviews see e.g. Allen & Allen 1986, Koide 1993, Smith & Read 1997, Augé 2001). Although the primary physiological drivers for mycorrhizal control of host stomatal conductance have not been identified, hormonal effects seem most likely (Augé 2001). Since the growth promotion of hosts by mycorrhizae often involves production of hormones and since growth promotion by DSE fungi is not consistently observed under experimental conditions, it is unlikely – yet still possible – that DSE could hormonally control host stomatal conductance. Overall, comparisons of stomatal conductances and transpiration rates between inoculated and DSE-free plants would be likely to provide cues to whether or not DSE fungi are involved in host water uptake. Although such experiments are easy to perform, they have not been conducted for the DSE fungi in spite of their prevalence in stressed conditions.

*Host drought and heat tolerance*: Augé (2001) classified drought tolerance mechanisms as nutritional or non-nutritional. Clearly, DSE involvement in host drought tolerance and the control of water dynamics would depend on the overall fitness of the host plant and on the growth or nutritional benefits that DSE colonisation may confer. Again, we underline that basic physiological questions about DSE need to be addressed before the involvement of these fungi in plant water relations can be assessed. For example, it would be essential to conduct experiments assessing whether DSE fungi are involved in host water acquisition or involved in the control of stomatal conductance or of transpiration rates. At present, however, there are two lines of evidence suggesting DSE may be involved in modifying host environmental tolerance, especially in relation to drought and heat tolerance.

Firstly, the nearly exclusive DSE colonisation seen in native plants in an arid ecosystem of New Mexico has been suggested to help plants overcome the severe drought conditions typical of that ecosystem. This was studied by Barrow (2003), who suggested that nearly systemic root colonisation by septate endophytes, along with the presence of abundant mucilaginous hyphae extending over 300 µm from the root matrix, aid nutrient and water transport under extended drought conditions. He also proposed that the continuous fungal network seen linking the vascular sieve elements to the root surface and rhizosphere is probably linked to water uptake.

Second, a fungal endophyte isolated from woolly panic grass, *Dichanthelium lanuginosum* (Elliott) Gould (now generally synonymized with *Dichanthelium acuminatum* in geothermal soils in Lassen Volcanic and Yellowstone National Parks in the U.S.A. was shown to increase host thermotolerance (Redman *et al*. 2002). Grass seedlings inoculated with the root- and foliage-inhabiting endophyte, provisionally identified as *Curvularia* sp., were able to withstand constant high soil temperature of 50 °C for 3 d while the non-symbiotic plants shrivelled and were chlorotic. When inoculated and endophyte-free plants were exposed to intermittent soil temperatures of 65 °C for 10 d, all non-inoculated seedlings died, whereas the inoculated seedlings survived. Interestingly, neither the fungus nor the grass survived the increased temperature regimen separately. In further inoculation studies, the same fungus conferred improved heat and drought tolerance on various agricultural and horticultural plants (Pennisi 2003). For example, wheat plants were able to withstand substantially longer periods of drought when inoculated with the endophyte. The *Curvularia* endophyte possessed melanised cell walls but was not considered to be a DSE.

The mechanisms for the altered environmental tolerance have only been hypothesised. The endophytes produce melanised cell walls when colonising the host. Redman *et al*. (2002) suggested that the fungal melanin may play a role in heat dissipation or may form complexes with oxygen radicals formed during stress. If this is true, then the DSE that produce melanised hyphae and microsclerotia, already shown by Read & Haselwandter (1981) and Barrow & Aaltonen (2001) to occur abundantly in stressed environments, may perform similar functions, which may be essential to plant survival and growth in those environments.

Experiments designed to study the role of DSE fungi in drought tolerance are necessary. As mentioned above, preliminary experiments reported by Pennisi (2003) suggest that endophyte colonisation can increase drought tolerance of various agricultural cultivars. As many of the experiments required to confirm such important functions are fairly simple, a project to screen multiple hosts and DSE strains would seem to be timely. If the DSE do turn out to assist plants in water uptake, or to increase drought-tolerance and thermal resistance, the agricultural implications will be far-reaching. Since these DSE fungi are easily cultured, unlike the obligately symbiotic AM fungi, they may have important uses as biofertilizers, and may allow us to manage agricultural systems more efficiently.

*Protection from herbivores*: Mycorrhizal fungi can mitigate the effects of herbivory on the host plants

(Gehring & Whitham 2002). Although the effects of mycorrhizas on herbivores are highly variable and no consensus on overall trends has been reached, we assume that mycorrhizal and DSE colonisation mainly act to reduce rather than increase the negative impacts of herbivores on plant fitness and performance. We propose three possible mechanisms that may limit herbivory or decrease its impact. First, by improving overall plant performance, fungal symbionts may improve plant tolerance of herbivory and thus increase the plant's ability to sustain herbivore damage without incurring visibly reduced productivity (Borowicz 1997, Gehring & Whitham 2002). Second, fungal symbionts can alter carbon-to-(mineral)-nutrient ratios, thus allowing an increased investment in carbon-based antiherbivore defences (Jones & Last 1991). Third, symbiotic fungi themselves may produce antiherbivore compounds, thus reducing the overall herbivory (Clay 1990, Clay 2001). Because the first mechanism we have listed depends on the improvement of host growth or nutrient uptake by the symbiont, and because those effects are uncertain in the DSE-host association, we will only consider the last two possibilities, i.e., those based on production of anti-herbivory compounds by plants or DSE.

No experimental evidence exists for induction of strong plant defences by DSE colonisation. The sole piece of potential direct evidence to date was provided by Yu *et al*. (2001), who observed irregular wall thickening in asparagus cells colonized by *P. fortinii*. Apart from such physical changes, biochemical changes also need to be investigated in connection with DSE. It is possible that DSE may produce or induce production of herbivore feeding deterrents that are unrelated to the compounds hosts produce in conventional pathogen resistance mechanisms. With AM fungi, Gange and West (1994) have shown that colonisation of *Plantago lanceolata* increased tissue concentrations of iridoid glycosides, an insect feeding deterrent. As a result, the performance of a generalist herbivorous insect, the garden tiger moth *Arctia caja*, was negatively affected. EM plants can also differ from non-mycorrhizal plants in their chemical composition. However, changes in host chemical composition as a result of colonisation by EM fungi may be minimal, and the effects of EM on insect herbivores are highly variable. For example, only one of several herbivorous insect species was found to be affected by EM colonisation of Scots pine (Manninen *et al*. 1998, 1999a, 1999b). Similar to mycorrhizal fungal colonisation, colonisation by DSE may alter host metabolism and increase plant production of general deterrents against herbivores, although the effects of these deterrents may depend on the individual herbivore species and their sensitivity to the mechanisms elaborated.

Another interesting question is whether or not the DSE fungi themselves can produce herbivore deterrents. Foliar clavicipitaceous endophytes such as the *Neotyphodium* inhabitants of certain grasses have been shown to inhibit herbivory by producing toxic secondary metabolites (Clay 1988, 1990, 2001). Systemic endophyte colonisation and resultant herbivore resistance was suggested to represent a new type of defensive mutualism (Clay 1988). What is the likelihood of DSE fungi producing compounds that inhibit herbivory? There are two main lines of reason that lead us to consider it likely that the DSE chemically inhibit herbivory, even though the compounds involved may not be similar to those reported for clavicipitaceous fungi.

Firstly, the DSE fungi produce large amounts of melanin in their cell walls. The melanins are known to provide rigidity to the cell wall, resistance to microbial grazing, and protection from desiccation and radiation damage (Kuo & Alexander 1967, Bell & Wheeler 1986, Griffith 1994). Typical DSE root colonisation, featuring the extensive presence of melanised superficial, intercellular and intracellular hyphae (Currah & Van Dyke 1986, O'Dell *et al*.1993, Newsham 1999), may protect the belowground tissues from foraging insects. Curiously, recent observations have shown that structures suggestive of melanised microsclerotia and variously pigmented and stained hyphae are present in the apoplastic spaces in leaves of black grama grass, *Bouteloua eriopoda*, a native grass in arid southwestern rangelands of U.S.A. (Aaltonen & Barrow 2003). Similarly, *Periconia* spp. isolated from roots of mixed grassland communities at the Konza Prairie Biological Station, a native tallgrass prairie reserve in northwestern Kansas, were also able to produce melanised microsclerotia in the leaves of *Allium porrum* in an *in vitro* resynthesis system. However, this occurred only when multiple strains were inoculated on the host roots (Mandyam & Jumpponen, unpublished). When only a single strain of *Periconia* was inoculated, colonisation was confined to the root tissues. It remains to be seen if the pattern seen with these *Periconia* isolates is widespread. The contribution of foliar colonisation by root-associated fungi to herbivore resistance requires further study.

Some species of *Periconia*, presumably conspecific with endophytes commonly isolated from roots in the tallgrass prairie ecosystem (Mandyam & Jumpponen, unpublished), are able to produce toxic chemical compounds. Giles and Turner (1967) showed that *Periconia macrospinosa* Lefebvre & Johnson was able to produce a chlorine-containing compound. Such compounds have been shown to be bioactive (McGahren *et al*. 1969), although the biocidal properties of the *P. macrospinosa* metabolite have not been investigated (Giles & Turner 1967).

The involvement of DSE in controlling herbivory, whether attributable to induction of host metabolites or to toxic molecules and melanins produced by DSE themselves, can be tested in simple feeding trials. For example, preliminary studies could assess herbivore performance when only DSE-colonised and endophyte-free tissues are made available. Alternatively, herbivore preferences for DSE-colonised vs. uncolonised plants can be evaluated by measuring plant tissue losses in both conditions.

*Protection from plant pathogens*: AM associations have long been thought to play a role in control of root pathogens. Azcón-Aguilar & Barea (1996) reviewed this topic and outlined the possible mechanisms of root pathogen control by AM fungi. These included general improvement of plant nutritional status, compensatory supply of materials to damaged roots, activation of plant defences, modification of microbial communities in the rhizosphere, promotion of morphological or anatomical changes in roots, and competition with pathogens for host photosynthates. It is very likely that multiple mechanisms are involved in the reduction of pathogen impact on mycorrhizal plants. Borowicz (2001), in a meta-analysis of data collected over 30 yr on AM-pathogen interactions, concluded that both non-nutritional mechanisms and facilitation of phosphorus uptake contributed to the observed inhibition of pathogens. As in our section above on protection from herbivory, we will concentrate here on non-nutritional mechanisms.

There are three particularly likely mechanisms through which DSE may inhibit pathogens, or minimise their impact on plant growth and performance. Firstly, mycorrhizal fungi and rhizosphere-inhabiting pathogens may compete for the plant photosynthates or for sites of colonisation. Secondly, compounds inhibitory to pathogens may be produced. Finally, the DSE colonisation may have prophylactic value by inducing plant defence responses to subsequent pathogen infection. These topics are detailed below.

Competition between symbiotic fungi and pathogens may be localised in small spatial scales within root systems (Dehne 1982, Linderman 1994). If DSE fungi and root pathogens depend on similar mechanisms of accessing host photosynthates or on similar host entry and colonisation sites, the presence of one or the other fungal type would result in pre-emptive resource utilisation, a form of exploitation competition (Lockwood 1992). Our data from Konza Prairie (Fig. 1) shows a great abundance of DSE in mixed grassland communities. The sheer abundance of DSE fungi colonising the root tissue is likely to consume significant amounts of available carbohydrates, limiting their availability to pathogens and thereby inhibiting pathogen establishment.

With regard to the chemical inhibition of pathogens, we have already mentioned that *Periconia* strains possibly conspecific with those common in tallgrass prairie grasses have been shown to produce chlorine-containing compounds (Giles & Turner 1969) that may be biocidal (McGahren *et al*. 1969). Similarly, *Periconia* sp. from *Taxus cuspidata* in Korea produced two compounds (Periconicins A and B), which were antibacterial against *Bacillus subtilis* (Ehrenberg) Cohn, *Staphylococcus aureus* Rosenbach, *Klebsiella pneumoniae* (Schroeter) Trevisan and *Salmonella typhimurium* (Loeffler) Castellani & Chalmers with a minimum inhibitory concentration (MIC) in the range of the well-known antibiotic gentamicin (Kim *et al*. 2004). Although it remains open to discussion whether or not DSE fungi are capable of production of antibacterial or antifungal compounds, the presence of such compounds in congeneric strains warrants further investigation.

With regard to induced plant defenses, it has generally been concluded that AM colonisation results in only weak and localised induction of these mechanisms (Koide & Schreiner 1992). However, transient activation of plant defences during early mycorrhizal formation (Gianinazzi-Pearson *et al*. 1996) or induction of low levels of defences (Benhamou *et al*. 1994) may occur. As mentioned above, Yu *et al*. (2001), when studying asparagus roots during colonisation by *P. fortinii*, observed irregular wall thickening adjacent to *P. fortinii* hyphae in certain root cells, specifically, long exodermal cells. They suggested that this was a weak defence reaction. These weak host responses to DSE colonisation are somewhat similar to those attributed to AM colonisation and suggest that the DSE fungi may be able to effectively induce a degree of host defence.

There are several ways in which DSE fungi may inhibit root-associated pathogens. As a first step, we suggest that simple experiments based on exposing strongly DSE-colonised host plants to an array of pathogens may be most profitable. Selection of an array of root endophytes and pathogens is necessary in such studies. Arnold *et al*. (2003) showed that many antagonistic effects among foliar endophytes and pathogens of a tropical tree (*Theobroma cacao* ) were direct and appeared to be species-specific. Similarly, competitive and antagonistic interactions among non-pathogenic root endophytes and root pathogens must be analysed within the relevant ecological context: the niche requirements among the component organisms assessed must be sufficiently similar (for discussion see Janisiewicz 1996). Whether the mechanism involved is competitive exclusion, induction of plant defences, or DSE production of biocides, experiments can readily be designed to show whether or not pathogen control by DSE is likely to occur under natural conditions.

*Impact on plant community dynamics*: AM fungi can influence plant community composition and determine plant diversity levels. Van der Heijden *et al*. (1998) showed that AM fungi enhanced plant diversity in European calcareous grasslands. They also showed for the first time that AM species richness and community composition were very important in determining the primary productivity in mixed grassland plant communities. In contrast, Hartnett and Wilson (1999) concluded that AM fungi actually reduced plant species diversity while bringing about no significant change in productivity. They suggested that a fungicidal treatment reducing AM fungal colonisation allowed dominance by weakly mycotrophic plant species while causing no decrease in the productivity of the system. Van der Heijden (2002) suggested that the conflicting effects of AM fungi on plant diversity depend upon the degree of mycorrhizal dependency of the plants involved, as well as the diversity of AM fungi and the nutritional status of the ecosystem. We concur that plant species must express differing responses to colonising fungi before any changes will be seen in community composition in experiments manipulating symbiotic fungal diversity or abundance.

Although studies evaluating the responses of different hosts to DSE are few, we propose that DSE can impact plant community composition if the plant species in the community respond differently to DSE colonisation. In fact, there is a great deal of variation in plant responses to DSE colonisation (see Jumpponen & Trappe 1998, Jumpponen 2001). Fernando and Currah (1996) conducted inoculation experiments with several hosts and DSE strains. They concluded that the observed responses were specific to the individual hosts and fungi involved. Although determining the net effects of multiple inocula in soils of mixed plant communities may be a nearly impossible task, it appears that DSE fungi with broad host ranges (see Jumpponen & Trappe 1998) and differing impacts on host performance would be able to have impacts on community composition. Clearly, to investigate our predictions about the role of DSE fungi in structuring plant communities, more experiments are needed. Microcosm studies need to be designed to evaluate the role of various DSE fungi after the appropriate fungi and host plants have been carefully selected. Such studies would allow direct experimental assessment of the effects of single DSE strains in mixed plant communities.

**Conclusions**

Information on the abundance and possible function of DSE is scanty at best. Based on the limited number of studies available, we conclude that DSE fungi are prevalent in various habitats and colonise a substantial proportion of the species present in mixed plant communities. This group of fungi cannot be overlooked while assessing the fungal communities of any ecosystem, as their abundance may equal or even exceed that of the AM fungi.

There is a conspicuous gap in our understanding of the ecological relevance of these root-associated endophytes. We propose that DSE, like many mycorrhizal fungi, are multifunctional. Even in the absence of clear consensus about positive impacts on host fitness, growth or performance, DSE can be said to be likely to perform functions similar to those attributed to mycorrhizal fungi. Although experimental evidence is limited and experimental results may conflict, DSE are likely to be involved in host nutrient uptake, especially from recalcitrant or complex organic sources. Ample production of melanised tissues may indicate a function of altering environmental tolerances, or deterring insect and mammalian herbivores. Simple pre-emptive resource use and competition for host root exudates may be adequate for reducing host susceptibility to soil-borne pathogens. Finally, broad DSE host ranges and the reported diversity of host responses to DSE colonisation suggest that these fungi may drive plant community dynamics via differential host responses and resource capture. Determining the impact of these functions under natural conditions is desirable but may be exceedingly difficult. We propose that simple, controlled preliminary experiments may be the most efficient way to obtain clues to the functions of DSE. Our intent in this contribution was to provide a framework of testable hypotheses to be used in designing such experiments. The bottom line is that the DSE fungi are abundant and their ecological significance needs to be understood.

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## REFERENCES

Aaltonen RE, Barrow JR (2003). A method of evaluating systemic colonization of leaves by dark septate fungal endophytes in *Bouteloua eriopoda* (Torr.) Torr. a native grass in arid southwestern U.S.A. rangelands (abstract). In: *Fourth International Conference on Mycorrhizae*, Montreal, Canada. 10–15th August 2003: 128.

Addy HD, Hambleton S, Currah RS (2000). Distribution and molecular characterization of the root endophyte *Phialocephala fortinii* along an environmental gradient in the boreal forest of Alberta. *Mycological Research*

**104**: 1213–1221.
Aerts R, Chapin FS (2000). The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research* **30**: 1–67.
Aerts R (2002). The role of various types of mycorrhizal fungi in nutrient cycling and plant competition. In: *Mycorrhizal Ecology* (van der Heijden M, Sanders I, eds). Springer-Verlag, Berlin, Germany: 117–133.
Ahlich K, Sieber TN (1996). The profusion of dark septate endophytic fungi in non-mycorrhizal fine roots of forest trees and shrubs. *New Phytologist* **132**: 259–270.
Al-Karaki GN, Hammand R (2001). Mycorrhizal influence on fruit yield and mineral content of tomato grown under salt stress. *Journal of Plant Nutrition* **24**: 1311–1323.
Allen EB, Allen MF (1986). Water relations of xeric grasses in the field: interactions of mycorrhizae and competition. *New Phytologist* **104**: 559–571.
Allen MF (1982). Influence of vesicular-arbuscular mycorrhizae in water movement through *Bouteloua gracilis* (H.B.K.) Lag ex Steud. *New Phytologist* **91**: 191–196.
Allen MF, Moore TS, Christensen M (1980). Phytohormone changes in *Bouteloua gracilis* infected by vesicular-arbuscular mycorrhizae. I. Cytokinin increases in the host plant. *Canadian Journal of Botany* **58**: 371–374.
Allen MF, Swenson W, Querejeta JI, Egerton-Warburton LM, Treseder KK (2003). Ecology of mycorrhizae: a conceptual framework for complex interactions among plants and fungi. *Annual Review of Phytopathology* **41**: 271–303.
Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA (2003). Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences, U.S.A.* **100**: 15649–15654.
Augé RM (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* **11**: 3–42.
Autry AR, Fitzgerald JW (1990). Sulfonate S: a major form of forest soil organic sulfur. *Biology and Fertility of Soils* **10**: 50–56.
Azcón-Aguilar C, Barea JM (1996). Arbuscular mycorrhizas and biological control of soil-borne plant pathogens-an overview of the mechanisms involved. *Mycorrhiza* **6**: 457–464.
Bååth E, Söderström B (1980). Degradation of macromolecules by microfungi from different podzolic soil horizons. *Canadian Journal of Botany* **58**: 422–425.
Barrow JR (2003). Atypical morphology of dark septate fungal root endophytes of *Bouteloua* in arid southwestern U.S.A. rangelands. *Mycorrhiza* **13**: 239–247.
Barrow JR, Aaltonen RE (2001). Evaluation of the internal colonization of *Atriplex canescens* (Pursh) Nutt. roots by dark septate fungi and the influence of host physiological activity. *Mycorrhiza* **11**: 199–205.
Barrow JR, Havstad KM, McCaslin BD (1997). Fungal root endophytes in fourwing saltbrush, *Atriplex canescens*, on arid rangelands of southwestern U.S.A. *Arid Soil Research and Rehabilitation* **11**: 177–185.
Barrow JR, Osuna P (2002). Phosphorus solubilization and uptake by dark septate fungi in four wing saltbrush, *Atriplex canescens* (Pursh) Nutt. *Journal of Arid Environment* **51**: 449–459.
Bell AA, Wheeler MH (1986). Biosynthesis and functions of fungal melanins. *Annual Review in Phytopathology* **24**: 4111–451.
Benhamou N, Fortin JA, Hamel C, St-Arnaud M, Shatilla A (1994). Resistance responses of mycorrhizal Ri T-DNA-transformed carrot roots to infection by *Fusarium oxysporum* f. sp. *chrysanthemi*. *Phytopathology* **84**: 958–968.
Bethlenfalvay GJ, Brown MS, Ames RN, Thomas RS (1988). Effects of drought on host and endophyte development in mycorrhizal soybeans in relation to water use and phosphorus uptake. *Physiologia Plantarum* **72**: 565–571.
Black CR (1979). A quantitative study of resistances to transpirational water movement in sunflower (*Helianthus annuus* L.) *Journal of Experimental Botany* **30**: 947–953.
Bledsoe C, Klein P, Bliss LC (1990). A survey of mycorrhizal plants on Truelove Lowland, Devon Island, N.W.T., Canada. *Canadian Journal of Botany* **68**: 1848–1856.
Borowicz VA (1997). A fungal root symbiont modifies plant resistance to an insect herbivore. *Oecologia* **112**: 534–542.
Borowicz VA (2001). Do arbuscular mycorrhizal fungi alter plant-pathogen relations? *Ecology* **82**: 3057–3068.
Boyer JS (1971). Resistances to water transport in soybean, bean and sunflower. *Crop Science* **11**: 403–407.
Brownlee C, Duddridge JA, Malibari A, Read DJ (1983). The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in assimilate and water transport. *Plant and Soil* **71**: 433–443.
Cairney JWG, Meharg AA (2003). Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. *European Journal of Soil Science* **54**: 735–740.
Cairney JWG, van Leerdam DM, Chen DM (2001). Metal insensitivity in ericoid mycorrhizal endophytes from *Woollsia pungens* (*Epacridaceae*). *Australian Journal of Botany* **49**: 571–577.
Caldwell B A, Jumpponen A (2003a). Utilization of heterocyclic organic nitrogen by mycorrhizal fungi. In: *Fourth International Conference on Mycorrhizae*, Montreal, Canada. 10–15th August 2003: 311.
Caldwell BA, Jumpponen A (2003b). Arylsufatase production by mycorrhizal fungi. In: *Fourth International Conference on Mycorrhizae*, Montreal, Canada. 10–15th August 2003: 312.
Caldwell BA, Jumpponen A, Trappe JM (2000). Utilization of major detrital substrates by dark septate, root endophytes. *Mycologia* **92**: 230–232.
Christie P, Nicholson TH (1983). Are mycorrhizas absent from the Antarctic? *Transactions of the British Mycological Society* **80**: 557–560.
Clark RB, Zeto SK (2000). Mineral acquisition by arbuscular mycorrhizal plants. *Journal of Plant Nutrition* **23**: 867–902.
Clay K (1990). The impact of parasitic and mutualistic fungi on competitive interactions among plants. In: *Perspectives on Plant Competition* (Grace JB, Tilman D, eds). Academic Press, New York: 391–412.

Clay K (1988). Fungal endophytes of grasses: a defensive mutualism between plants and fungi. *Ecology* **69**: 10–16.

Clay K (2001). Symbiosis and the regulation of communities. *American Zoologist* **41**: 810–824.

Currah RS, Tsuneda A (1993). Vegetative and reproductive morphology of *Phialocephala fortinii* (Hyphomycetes, *Mycelium radicis atrovirens*) in culture. *Transactions of Mycological Society of Japan* **34**: 345– 356.

Currah RS, Van Dyk M (1986). A survey of some perennial vascular plant species native to Alberta for occurrence of mycorrhizal fungi. *Canadian Field Naturalist* **100**: 330–342.

Davies FT, Potter JR, Linderman RG (1993). Drought resistance of mycorrhizal pepper plants independent of leaf P concentration – response in gas exchange and water relations. *Physiologia Plantarum* **87**: 45–53.

Dehne HW (1982). Interaction between vesicular-arbuscular mycorhizal fungi and plant pathogens. *Phytopathology* **72**: 1115–1119.

Feng G, Zhang FS, Li XL, Tian CY, Tang C, Rengel Z (2002). Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza* **12**: 185–190.

Fernando AA, Currah RS (1995). *Leptodontidium orchidicola* (*Mycelium radicis atrovirens* complex): aspects of its conidiogenesis and ecology. *Mycotaxon* **54**: 287–294.

Fernando AA, Currah RS (1996). A comparative study of the effects of the root endophytes *Leptodontidium orchidicola* and *Phialocephala fortinii* (Fungi Imperfecti) on the growth of some subalpine plants in culture. *Canadian Journal of Botany* **74**: 1071–1078.

Finlay RD, Frostegård Å, Sonnerfeldt A-M (1992). Utilization of organic and inorganic nitrogen sources by ectomycorrhizal fungi in pure culture and in symbiosis with *Pinus contorta* Dougl. ex Loud. *New Phytologist* **120**: 105–115.

Gange AC, West HM (1994). Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects in *Plantago lanceolata* L. *New Phytologist* **128**: 70–87.

Gardes M, Dahlberg A (1996). Mycorrhizal diversity in arctic and alpine tundra: an open question. *New Phytologist* **133**: 147–157.

Gehring C A, Whitman T G. (2002). Mycorrhizae- herbivore interactions: Population and community consequences. In: *Mycorrhizal ecology* (van der Heijden M, Sanders I, eds). Springer-Verlag, Berlin, Germany: 295–320.

Gianinazzi-Pearson V, Dumas-Gaudot E, Golotte A, Tahiri-Alaoui A, Gianinazzi S (1996). Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. *New Phytologist* **133**: 45–47.

Giles D, Turner WB (1969). Chlorine-containing metabolites of *Periconia macrospinosa*. *Journal of Chemical Society, Perkin Transactions 1 (Organic and Bio-organic Chemistry)* **16**: 2187–2189.

Girlanda M, Ghignone S, Luppi AM (2002). Diversity of sterile root-associated fungi of two Mediterranean plants. *New Phytologist* **155**: 481–498.

Griffith G W (1994). Phenoloxidases in *Aspergillus nidulans*: 50 years on. In: *Progress in industrial microbiology,* Martinelli SD, Kinghorn JR, eds). Elsevier Science Publishers, Amsterdam. **29**: 763–788.

Grünig CR, Sieber TN, Rogers SO, Holdenrieder O (2002). Spatial distribution of dark septate endophytes in a confined forest plot. *Mycological Research* **106**: 832–840.

Hall JL (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany* **53**: 1–11.

Hardie K, Leyton L (1981). The influence of vesicular-arbuscular mycorrhiza on growth and water relations of red clover. I. in phosphate deficient soil. *New Phytologist* **89**: 599–608.

Harley JL, Smith SE (1983). *Mycorrhizal symbiosis*. Academic Press, New York.

Hartnett D, Wilson G (1999). Mycorrhizae influence plant community structure and diversity in tallgrass prairie. *Ecology* **80**: 1187–1195.

Haselwandter K , Read DJ (1980). Fungal associations of roots of dominant and sub-dominant plants in high-alpine vegetation systems with special reference to mycorrhiza. *Oecologia* **45**: 57–62.

Haselwandter K, Read DJ (1982). The significance of root-fungus association in two *Carex* species of high-alpine plant communities. *Oecologia* **53**: 352–354.

Heijden M van der (2002). Arbuscular mycorrhizal fungi as a determinant of plant diversity: in search for underlying mechanisms and general principles. In: *Mycorrhizal ecology* (van der Heijden M, Sanders I, eds). Springer-Verlag, Berlin, Germany: 243–261.

Heijden M van der, Klironomos J, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders I R (1998). Mycorrhizal fungal diversity determines plant diversity, ecosystem variability and productivity. *Nature* **396**: 69–72.

Hirrel MC, Gerdemann JW (1980). Improved growth of onion and bell pepper in saline soils by two vesicular-arbuscular mycorrhizal fungi. *Soil Science Society of America Journal* **44**: 654–655.

Horton TR, Caźares E, Bruns TD (1998). Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire. *Mycorrhiza* **8**: 11–18.

Janisiewicz W (1996). Ecological diversity, niche overlap, and coexistence of antagonists used in developing mixtures for biocontrol of post harvest diseases of apples. *Phytopathology* **86**: 473–479.

Johnson CR, Menge JA, Schwab S, Ting IP (1982). Interaction of photoperiod and vesicular-arbuscular mycorrhizae on growth and metabolism of sweet orange. *New Phytologist* **90**: 665–669.

Johnson NC, Graham JH, Smith FA. (1997). Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytologist* **135**: 575–583.

Jones CG, Last FT (1991). Ectomycorrhizal and trees: implications for aboveground herbivory. In: *Microbial mediation of plant-herbivore interactions* (Barbosa P, Krischik VA, Jones CG, eds). Wiley, New York: 65–103.

Jones MD, Hutchinson TC (1986). The effect of mycorrhizal infection on the response of *Betula papyrifera* to nickel and copper. *New Phytologist* **102**: 429–442.

Jumpponen A, Mattson K, Trappe JM (1998). Mycorrhizal

functioning of *Phialocephala fortinii* with *Pinus contorta* on glacier fore front soil: interactions with soil nitrogen and organic matter. *Mycorrhiza* **7**: 261–265.

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

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

Kernaghan G, Sigler L, Khasa D (2003). Mycorrhizal and root endophytic fungi of containerized *Picea glauca* seedlings assessed by rDNA analysis. *Microbial Ecology* **45**: 128–136.

Kim S, Shin D, Lee T, Oh KB (2004). Periconicins, two new fusicoccane diterpenes produced by an endophytic fungus *Periconia* sp. with antibacterial activity. *Journal of Natural Products* **67**: 448–450

Kohn LM, Stasovski E (1990). The mycorrhizal status of plants at Alexandra Fjord, Ellesmere Island, Canada, a high arctic site. *Mycologia* **82**: 23–35.

Koide R (1993). Physiology of the mycorrhizal plant. *Advances in Plant Pathology* **9**: 33–54.

Koide RT, Schreiner RP (1992). Regulation of the vesicular-arbuscular mycorrhizal symbiosis. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**: 557–581.

Kovács GM, Szigetvári C (2002). Mycorrhizae and other root-associated fungal structures of the plants of a sandy grassland on the great Hungarian Plain. *Phyton* **42**: 211–223.

Kucey RMN, Paul EA (1982). Carbon flow, photosynthesis, and N2 fixation in mycorrhizal and nodulated faba beans (*Vicia faba* L.). *Soil Biology and Biochemistry* **14**: 407–412.

Kuo MJ, Alexander M (1967). Inhibition of the lysis of fungi by melanins. *Journal of Bacteriology* **94**: 624–629

Landhäusser SM, Muhsin TM, Zwiazek JJ (2002). The effect of ectomycorrhizae on water relations in aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) at low temperatures. *Canadian Journal of Botany* **80**: 684–689.

Laursen G, Treu R, Seppelt RD, Stephenson SL (1997). Mycorrhizal assessment of vascular plants from subantarctic Macquarie Island. *Arctic and Alpine Research* **29**: 483–491.

Levy Y, Krikun, J (1980). Effect of vesicular-arbuscular mycorrhiza on *Citrus jambhiri* water relations. *New Phytologist* **85**: 25–31.

Linderman RG (1994). Role of VAM fungi in biocontrol. In: *Mycorhizae and plant health* (Pfleger FL, Linderman RG, eds). American Pathological Society, St. Paul, Minnesota: 1–26.

Lipson D, Nashölm T. (2001). The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia* **128**: 305–316.

Liu A, Hamle C, Hamilton RI, Ma BL, Smith DL (2000). Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. *Mycorrhiza* **9**: 331–336.

Lockwood JL (1992). Exploitation competition. In: *The fungal community – its organization and role in the ecosystem* (Carroll GC, Wicklow DT, eds). Marcel Dekker, New York: 243–263.

Malcová R, Albrechtová J, Vosátka M (2001). The role of the extraradical mycelium network of arbuscular mycorrhizal fungi on the establishment and growth of *Calamagrostis epigejos* in industrial waste substrates. *Applied Soil Ecology* **18**: 129–142.

Manninen A-M, Holopainen T, Holopainen JK (1998). Susceptibility of ectomycorrhizal and non-mycorrhizal Scots pine (*Pinus sylvestris*) seedlings to a generalist insect herbivore, *Lygus rugulipennis*, at two nitrogen availability levels. *New Phytologist* **140**: 44–63.

Manninen A-M, Holopainen T, Holopainen JK (1999a). Performance of grey pine aphid, *Schizolachnus pineti*, on ectomycorrhizal and non-mycorrhizal Scots pine seedlings at different levels of nitrogen availability. *Entomology Experimental Applications* **9**: 117–120.

Manninen A-M, Holopainen T, Lyytikäinen-Saarenmaa P, Holopainen JK (1999b). The role of low level ozone exposure and mycorrhizas in chemical quality and insect herbivore performance of Scots pine seedlings. *Global Change Biology* **5**: 1–11.

Marschner H, Dell B (1994). Nutrient uptake in mycorrhizal symbiosis. *Plant Soil* **159**: 89–102.

McGahren WJ, van den Hende JH, Mitscher LA (1969). Chlorinated cyclopentenone fungitoxic metabolites from the fungus, *Sporormia affinis*. *Journal of the American Chemical Society* **91**: 157–162.

Meharg AA, Cairney JWG (2000). Co-evolution of mycorrhizal symbionts and their hosts to metal-contaminated environments. *Advances in Ecological Research* **30**: 69–112.

Newsham KK (1999). *Phialophora graminicola*, a dark septate fungus, is a beneficial associate of the grass *Vulpia ciliata* ssp. *ambiqua*. *New Phytologist* **144**: 517–524.

O'Dell TE, Massicotte HB, Trappe JM (1993). Root colonization of *Lupinus latifolius* Agardh. and *Pinus contorta* Dougl. by *Phialocephala fortinii* Wang & Wilcox. *New Phytologist* **124**: 93–100.

Olsson PA, Jakobsen I, Wallander H (2002). Foraging and resource allocation strategies of mycorrhizal fungi in a patchy environment. In: *Mycorrhizal ecology* (van der Heijden M, Sanders I, eds). Springer-Verlag, Berlin, Germany: 93–110.

Pennisi E (2003). Fungi shield new host plants from heat and drought. *Science* **301**: 1466.

Rains KC, Nadkarni NM, Bledsoe CS (2003). Epiphytic and terrestrial mycorrhizas in a lower montane Costa Rican cloud forest. *Mycorrhiza* **13**: 257–264.

Read DJ, Haselwandter K (1981). Observations on the mycorrhizal status of some alpine plant communities. *New Phytologist* **88**: 341–352.

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

Redman R, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002). Thermo tolerance generated by plant/fungal symbiosis. *Science* **298**: 1581

Ruotsalainen AL (2003). *Mycorrhizal colonization and plant performance in arcto-alpine conditions.* Ph.D dissertation. Department of Biology, University of Oulu,

Finland.

Ruotsalainen AL, Väre H, Vestberg M (2002). Seasonality of root fungal colonization in low-alpine herbs. *Mycorrhiza* **12**: 29–36.

Safir GR, Boyer JS, Gerdemann JW (1972). Nutrient status and mycorrhizal enhancement of water transport in soybean. *Plant Physiology* **49**: 700–703.

Sharples JM, Meharg AA, Chambers SM, Cairney JWG (2000). Symbiotic solution to arsenic contamination. *Nature* **404**: 951–952.

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

Thormann MN, Currah R, Bayley SE (1999). The mycorrhizal status of the dominant vegetation along a peatland gradient in southern boreal Alberta, Canada. *Wetlands* **19**: 438–450.

Treu R, Laursen GA, Stephenson SL (1996). Mycorrhizae from Denali National Park and Preserve, Alaska. *Mycorrhiza* **6**: 21–29.

Trowbridge J, Jumpponen A (2004). Fungal colonization of shrub willow roots at the forefront of a receding glacier. *Mycorrhiza* **14**: 283–293.

Turnau K, Haselwandter K (2002). Arbuscular mycorrhizal fungi, an essential component of soil microflora in ecosystem restoration. In: *Mycorrhizal technology in Agriculture* (Gianinazzi S, Schüepp H, Barea JM, Haselwandter K, eds). Birkhauser Verlag, Geneva: 137–149.

Väre H, Vestberg M, Eurola S (1992). Mycorrhiza and root associated fungi in Spitsbergen. *Mycorrhiza* **1**: 93–104.

Yu T, Nassuth A, Peterson RL (2001). Characterization of the interaction between the dark septate fungus *Phialocephala fortinii* and *Asparagus officinalis* roots. *Canadian Journal of Microbiology* **47**: 741–753.