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Editor

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Foreword

Terrestrial ecosystems are driven by microbial guildes. We are, however, severely hampered by our limited understanding of the diversity and function of such microbial ecosystems. Growing on mineral particles and decaying organic matter, and living in the vicinity of or within plant roots, are a cast of hundreds of competing versatile and diverse fungal species, including soil decomposers, pathogens, endophytes and obligate symbionts (Frankland 1998). Amongst the extensive intermingling webs of hyphae permeating the soil, those of mycorrhizal fungi play a crucial role in ecosystem sustainability through their role in biogeochemical cycles. These mycorrhizal species are no marginal oddity, having been shown to account for half of the fungal species in most temperate, montane and boreal forests. The hyphae of mycorrhizal fungi and plant short roots form a novel composite organ, the so-called mycorrhiza, which is the site of nutrient and carbon transfer between the two symbiotic partners. This mutualistic interactions allow terrestrial plants to grow efficiently in suboptimal environments (Read and Perez-Moreno 2003). They are key drivers of ecosystem function, receiving 15–25% of carbon net productivity and providing most of the host plant nitrogen and phosphorus. The symbiotic relationship between roots and these fungi is undoubtly one of the most prevalent associations in all terrestrial ecosystems. Knowing which processes these soil fungi are responsible for, and how, is thus increasingly important for understanding the inputs and outputs in forest ecosystems under global change. In this book, many of those at the forefront of the research field integrate and comment on recent developments and ideas on the molecular biology, physiology, and ecology of the mycorrhizal symbioses. All of the major types of mycorrhiza are considered. By taking a broad perspective, they show how new information on mycorrhizal fungi, but also on interactions involving endophytes, nitrogen-fixing bacteria and mycorrhiza helper bacteria, may contribute to concepts and ideas of biology and ecology as a whole. Just as important, they contribute to further invigoration of mycorrhizal research by illuminating the field with new ideas and concepts, derived in part from other fields of plant biology and mycology. Attempts to improve productivity of ecosystems by inoculation with more effective fungal symbionts are also described.

The work described here confirms that the ecological performance of mycorrhizal fungi is a complex phenotype affected by many different traits and by environmental factors. In this Foreword, I will look to future challenges that lie ahead.

Diversity, Function and Potential Applications of the Root-Associated Endophytes

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1 Diversity of Fungal Root Endophytes

Both mycorrhizal fungi and systemic fungal endophytes in the Order Clavicipitales have been extensively studied. Compared to these groups, root-associated fungal endophytes have received very little attention, even though they seem common in many ecosystems. Based on published reports, comparisons between host colonization by the root endophytes and mycorrhizal fungi from various habitats suggest that endophytes are possibly as abundant as mycorrhizas (Mandyam and Jumpponen 2005). As more reports that document the abundance of root endophytes in different habitats become available, a better understanding of the ecology and functions of these endophytes seems not only logical but critical.

The term 'endophyte' is used to describe either bacterial or fungal intracellular symbionts of plants that do not cause any visible signs of tissue damage or adverse effects on the host (Petrini 1991; Wilson 1995; Stone et al. 2000; Schulz and Boyle 2005). Fungal root endophytes are a paraphyletic group primarily occurring in the Ascomycota, although some examples also exist for Basidiomycetous endophytes (see Verma et al. 1998; Barazani et al. 2005). In this group, we usually include all root-inhabiting fungi that are considered non-mycorrhizal based on the morphology of the colonized host roots and on fungal structures produced in colonized roots typically considered indicative of dark septate endophytes (DSE). We also include fungi that produce hyaline structures when colonizing hosts intracellularly (O'Dell et al. 1993; Barrow and Aaltonen 2001; Ohki et al. 2002; Narisawa et al. 2003), but do not form typical DSE structures. These hyaline fungi can routinely be isolated from the roots of many plant species. Well-studied systemic and foliar endophytes of grasses, such as *Acremonium* sp., *Epichoë* sp. and *Neotyphodium* sp., will be excluded from this discussion.

Many of the studies of fungal root endophytes have either made no effort to identify the fungi or have focused on one fungus isolated at a single site. This gives the impression that the species diversity of fungal root endophytes is low. *Phialocephala*

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A. Varma (ed.) *Mycorrhiza*,

fortinii is probably the best-known fungal root endophyte (Addy et al. 2005). Much of what is known about these organisms has been extrapolated from studies conducted with *P. fortinii*. As sampling effort increases, it is becoming obvious that the diversity of fungal root endophytes may be much higher than previously thought. In this chapter, we address the resident diversity of root-associated fungi through a case study, and present data on the colonization by those fungi and on the host responses produced under laboratory conditions. We then continue with a discussion on the potential function of these endophytes beyond growth promotion, and conclude with a brief discussion on the possible applications of these endophytes.

2 The Shortgrass Steppe: A Case Study of Fungal Root Endophyte Diversity and Function

As a part of an as yet unpublished research effort that is still largely under way, we sampled five grassland and meadow sites in the Long Term Ecological Research (LTER) network in the western United States. The focus of these studies has been to gain a better understanding of the diversity of fungal root endophytes. The sampled LTER sites were Cedar Creek in Minnesota, HJ Andrews in Oregon, Jornada Range in New Mexico, Konza Prairie in Kansas, and the Shortgrass Steppe in Colorado. As a part of that research effort, the fungal cultures obtained from the roots of dominant plants at each site were divided into macromorphological groups, whose conspecificity was tested by Restriction Fragment Length Polymorphisms (RFLP) of the PCR-amplified Internal Transcribed Spacer (ITS) region of the nuclear rRNA gene repeat, and further refined by sequencing. The preliminary data analyses indicate that the communities of putative fungal endophytes were unique at each site and overlapped only marginally. We have selected one of the five field sites – Shortgrass Steppe in Colorado – for a detailed discussion, and present those findings here as a case study.

The Shortgrass Steppe is an arid grassland situated on the high plains of northeastern Colorado (1,650 m above sea level). This LTER site is dominated by *Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths and *Buchloe dactyloides* (Nutt.) Engelm. For more information on the site and its vegetation, see http://sgs.cnr.colostate.edu/. We sampled whole plants (*B. gracilis* and a dominant forb in Asteraceae, *Gutierrezia sarothrae* (Pursh) Britt. & Rusby) in order to be able to collect roots belonging to the target plants. The sampling was performed twice: early and late in the growing season in 2004. At each sampling occasion, roots from three individuals of each of the two species were washed free of soil, surface sterilized in hydrogen peroxide and plated out on low-nutrient media to isolate culturable, root-associated fungi. This culturing effort yielded a total of 54 isolates of filamentous fungi from this site. We extracted DNA from each isolate, and PCR-amplified the ITS region with primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990) for an ITS1-5.8S-ITS2 amplicon flanked by the small and large subunits of the rRNA gene repeat. To approximate the conspecific groupings, the ITS amplicons were digested with two endonucleases (*Alu* I and *Hind* III) and the fungal isolates were grouped based on these RFLP

phenotypes. To provide an approximate taxon affinity for the most commonly occurring RFLP phenotypes, the ITS region was also sequenced for 23 isolates using the ITS1F and ITS4 primers. The sequences were queried against GenBank using BLAST (Altschul et al. 1997) and the closest matches (Table 1) aligned in Sequencer v. 4.6 (GenCodes, Ann Arbor, Michigan). The taxon affinities were approximated using Neighbor Joining and Maximum Parsimony analyses in PAUP* 4.0 (Swofford 2002) in combination with the GenBank queries. The taxon affinities that we use here represent bootstrap supported clades (Fig. 1) and the greatest similarity to confirmed and identified accessions in GenBank.

Table 1 Approximated taxon affinities and sequence similarities of the filamentous fungi isolated from roots of *Bouteloua gracilis* and *Gutierrezia sarothrae* at the Shortgrass Steppe LTER in Colorado

Order	KSU Culture number	BLAST identification	Percent similarity	Plant host	Time of sampling
Helotiales	20345	*Cadophora luteo-olivacea* (DQ404349)	97	*G. sarothrae*	Late
	20459	*Cadophora luteo-olivacea* (DQ404349)	97	*G. sarothrae*	Late
Hypocreales	20043	*Fusarium* sp. (AY729069)	99	*B. gracilis*	Early
	20299	*Fusarium* sp. (AY729054)	99	*G. sarothrae*	Late
Pezizales	20226	*Strumella griseola* (AF485078)	87	*B. gracilis*	Early
Pleosporales	20060	*Alternaria longissima* (AF229489)	96	*B. gracilis*	Early
	20062	*Alternaria longissima* (AF229489)	97	*B. gracilis*	Early
	20346	*Alternaria longissima* (AF229489)	99	*G. sarothrae*	Late
	20414	*Alternaria longissima* (AF229489)	99	*B. gracilis*	Late
	20303	*Dreschlera* sp. (AY336133)	98	*B. gracilis*	Late
	20055	*Leptosphaeria* sp. (DQ093682)	96	*G. sarothrae*	Early
	20104	*Leptosphaeria* sp. (DQ093682)	94	*G. sarothrae*	Early
	20463	*Lophiostoma* sp. (AJ972793)	93	*G. sarothrae*	Late
	20490	*Lophiostoma* sp. (AJ972793)	93	*G. sarothrae*	Late
	20050	*Ophiosphaerella herpotricha* (U04861)	98	*G. sarothrae*	Early
	20277	*Phoma herbarum* (AY864822)	89	*B. gracilis*	Late
	20309	*Phoma herbarum* (AY864822)	89	*B. gracilis*	Late
	20328	*Phoma herbarum* (AY864822)	89	*B. gracilis*	Late
	20329	*Phoma herbarum* (AY864822)	87	*B. gracilis*	Late
Xylariales	20023	*Microdochium* sp. (AJ279477)	95	*B. gracilis*	Early
	20082	*Microdochium* sp. (AJ279477)	89	*B. gracilis*	Early
	20084	*Microdochium* sp. (AJ279477)	86	*B. gracilis*	Early
	20030	*Microdochium* sp. (AJ246155)	91	*B. gracilis*	Early

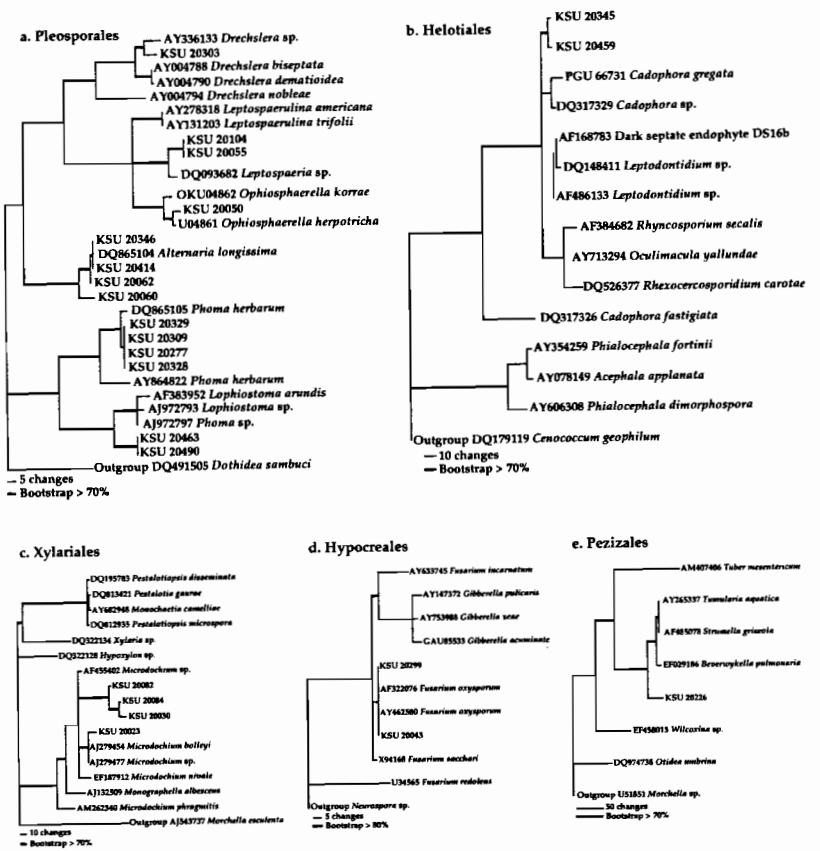

Fig. 1 Maximum parsimony bootstrap ITS trees of root endophytes isolated from the Shortgrass Steppe LTER, Colorado, USA, **a** Pleosporales, **b** Helotiales, **c** Xylariales, **d** Hypocreales, **e** Pezizales

The diversity of the root-acquired filamentous fungi was relatively high; the 54 isolates were distributed among 22 different RFLP groups, 12 of which occurred at the early season sampling and 10 at the late season sampling. The sequenced representatives of the most commonly occurring RFLP phenotypes were distributed across five orders (Helotiales, Hypocreales, Pezizales, Pleosporales, and Xylariales) and 12 genera (Fig. 1; Table 1), all within the Ascomycota. Sequence data closely correlated with the patterns observed with the RFLPs. Both datasets indicated that there were differences in the fungal communities isolated early and late in the growing season and with

regard to the plant host (Table 1). Our data suggest that roots of both *B. gracilis* and *G. sarothrae* host a different suite of fungi early and late in the growing season. We observed little overlap in RFLP groups or among sequences between the two seasons, suggesting a temporally dynamic community colonizing the roots of dominant plants at this site. Furthermore, with the exception of the most abundant RFLP groups—those with affinities to Pleosporales—most groups were limited to a single host suggesting some degree of host preference or specificity. For example, the sequenced fungal RFLP groups that represented the Pezizales or the Xylariales were exclusively obtained from *B. gracilis*, whereas the RFLP groups that represented the Helotiales or the Hypocreales were obtained from *G. sarothrae*. Only few of the isolates within the same clades (*Alternaria longissima*-like in Fig. 1a and *Fusarium*-like isolates in Fig. 1d) in our analyses were isolated from both hosts and during both sampling times.

Because of the possibility that many of the fungi isolated from plant roots may be pathogens or saprotrophs rather than true root endophytes, we screened a sub-sample of 20 isolates in a root-colonization experiment with *Allium porrum* L. (leek) in the laboratory. We grew leek plants on 1/10 strength Murashige and Skoog medium (Murashige and Skoog 1962), and inoculated 15 replicates with 20 isolates that represented the RFLP phenotypes with the highest frequencies. Each of the inoculations was compared to a paired, mock-inoculated control that received only a plug from the fungal media but no fungus. We examined roots 8 weeks after inoculation under the light microscope at 400× for the presence of intra- and intercellular hyphae and for the presence of melanized hyphae or microsclerotia. We also examined growth responses to inoculation with our isolates by measuring shoot biomass. A majority of the tested isolates failed to colonize leek roots under our experimental conditions. Furthermore, the majority of the host growth responses were either negative or neutral at the end of the eight-week incubation when compared to the paired, fungus-free control (Table 2). Inoculation with 2 of the 20 tested isolates, a *Cadophora luteo-olivacea*-like isolate and a *Phoma herbarum*-like isolate, yielded both significant and positive growth responses (Table 2) in leek when compared to the mock-inoculated controls. However, in both of these cases, only superficial or no colonization was observed. Four additional isolates, two with affinities to *Alternaria longissima*, and one to *Lophiostoma arudinis* and *Ophiosphaerella herpotricha*, produced marginally significantly ($p < 0.10$) negative effects on leek growth. Among these isolates, only the *A. longissima*-like isolate produced intracellular hyphae and microsclerotia. The remaining isolates had no visible or significant effect on host growth. Among those, the *Drechslera*-like isolate produced intracellular hyaline hyphae, a *Microdochium*-like isolate produced chlamydospores and intracellular hyphae, another *Microdochium*-like isolate produced mitospores and intracellular hyaline hyphae, and an *A. longissima*-like isolate produced microsclerotia and intracellular hyphae.

In this case study, we isolated a diverse array of fungi from roots of *B. gracilis* and *G. sarothrae*. Many of these fungi colonized the leek roots either superficially or failed to produce intra- and intercellular fungal structures indicative of typical root endophyte symbioses. Isolates that were placed in the Pleosporales with matches in GenBank and phylogenetic analyses were the most frequently observed fungi among the 54 isolates acquired from our sampling at the Shortgrass Steppe.

Table 2 Root colonization by fungi isolated from the Shortgrass Steppe LTER in Colorado and *Allium porrum* growth responses to inoculation. The growth responses were determined via comparisons among paired inoculated plants and non-inoculated controls. Non-significant host responses are considered neutral in our discussion and those that were significant according to a non-parametric median test as implemented in SAS were considered either positive or negative if inoculated hosts were larger or smaller than the controls that were mock inoculated with a inoculum from a fungus-free sterile plate with Corn Meal Agar on which the fungus was grown

BLAST identification	Isolate	Season	Colonization	Host response
Alternaria longissima (AF229489)	20060	Early	Microsclerotia, hyphae	Negative **
Alternaria longissima (AF229489)	20062	Early	Microsclerotia, hyphae	Negative**
Alternaria longissima (AF229489)	20346	Late	Microsclerotia, hyphae	Positive ^{ns}
Alternaria longissima (AF229489)	20414	Late	Superficial hyaline hyphae	Positive ^{ns}
Fusarium sp. (AY729054)	20299	Late	None	Positive ^{ns}
Cadophora luteo-olivacea (DQ404349)	20345	Late	None	Positive*
Leptosphaeria sp. (DQ093682)	20055	Early	Superficial hyphae	Negative ^{ns}
Lophiostoma sp. (AJ972793)	20463	Late	None	Negative ^{ns}
Lophiostoma sp. (AJ972793)	20490	Late	Superficial hyphae	Negative (*)
Microdochium sp. (AJ279477)	20082	Early	Spores, hyaline hyphae	Positive ^{ns}
Microdochium sp. (AJ279477)	20084	Early	Superficial spores, penetrating hyphae	Positive ^{ns}
Ophiosphaerella herpotricha (U04861)	20050	Early	None	Negative**
Phoma herbarum (AY864822)	20277	Late	None	Negative ^{ns}
Phoma herbarum (AY864822)	20309	Late	None	Positive*
Strumella griseola (AF485078)	20226	Early	None	None ^{na}

na Inoculation tests were not completed; ns $P > 0.10$; (*) $0.05 < P \leq 0.10$; * $0.01 < P \leq 0.05$; ** $P \leq 0.01$

Several of these isolates produced both melanized hyphae and microsclerotia in *A. porrum*. However, even among the isolates that produced fungal structures indicative of endophyte symbiosis, there was considerable variation. Three of the four studied *A. longissima*-like isolates were capable of colonization, whereas the fourth colonized the host only superficially. It remains uncertain whether the observed patterns indicate that true endophytes are relatively few among the root-associated fungi or that the artificial laboratory conditions preclude fungal colonization in a common host studies such as the one described here. The paucity of intracellular colonization by any particular isolate in the roots of *A. porrum* may not indicate lack of endophytic capacity in this trial, given the potential host preference that was observed among the RFLP phenotypes. Some fungal endophytes such as *P. fortinii* may be generalists and colonize a variety of hosts, whereas others—such as those examined in this case study—may exhibit some degree of host preference.

While our data may be limited in its scope and extent, they do provide some interesting background for discussion. The isolates producing fungal structures indicative of fungal root endophytes in leek roots were few in number, and those that did produce the indicative structures produced either adverse or neutral rather than positive responses as judged by the host biomass. While one must be cautious in interpreting these data, it seems that plants in their natural environment may host a greater variety of neutral and antagonistic fungi than mutualistic endophytes. Measuring biomass is only one way of examining the effect of endophytes on host plants. However, it is one of the simplest methods for screening a large number of isolates. The following section of this chapter illustrates a variety of other ways in which fungal root endophytes may affect their hosts.

Our results at the Shortgrass Steppe with *B. gracilis* and *G. sarothrae* indicate that host preference can be a factor in determining endophyte colonization. While not tested in the presented case study, the effects of the endophytes may vary among the host species. Further testing is warranted for our isolates with additional plant hosts, including native species. In addition, sampling throughout the growing season should be utilized in order to capture the full range of the root-associated fungus diversity. Finally, plant roots and soil host a diverse assemblage of organisms in natural and agricultural systems that may interact in ways that are difficult to reproduce in a laboratory setting. The effects of fungal root endophytes on plant hosts in nature may be the result of interactions with a variety other root-associated and soil-borne organisms.

3 Functions of Root Endophytes

The potential functions of root endophytes have not been as clearly defined as those of mycorrhizal fungi or clavicipitaceous grass endophytes. Our case study suggests that potential endophytes selected from a random collection of root-associated fungi are more likely to have adverse rather than positive effects on host biomass. In contrast, based on the review of limited number of published reports on the possible roles of endophytes, Mandyam and Jumpponen (2005) argue that endophyte–plant symbioses may be considered 'multifunctional.' In other words, the endophyte functions may not be limited to growth promotion or facilitation of host nutrient acquisition. For example, similarly to mycorrhizal fungi, endophytes may improve host resistance to pathogens or herbivores and enhance host stress tolerance. In the section below, we summarize briefly suggested and reported endophyte functions, and present some of our own unpublished data in support of some of the proposed potential functions.

3.1 Role of Endophytes in Host Growth and Nutrient Uptake

One and possibly the most pivotal function of mycorrhizal fungi is the facilitation of plant nutrient uptake and resultant growth stimulation. Improved nutrition and

growth may also indirectly affect the other well-known functions of mycorrhizas, such as greater stress tolerance or pathogen resistance in plants. Endophytes are also able to enhance the growth of many plant species with or without concomitant nutrient uptake (Table 3). The importance of endophyte colonization on host nutrient uptake has remained unresolved, and clear results of endophyte effects on host nutrient status are few. Inoculation of *Vulpia ciliate* ssp. *ambigua* with *Phialophora graminicola* increased P and root N levels in its roots and shoots (Newsham 1999). In an experiment with *P. fortinii* and *Pinus contorta*, Jumpponen and Trappe (1998) showed that inoculation, similarly, can enhance host nutrient acquisition from the substrate. However, even such facilitation of nutrient uptake can be variable among strains of endophytic fungi. Vohnik et al. (2003) used two strains of *P. fortinii*, neither of which had any significant effect on the shoot growth of a *Rhododendron* cultivar. However, one of the two *P. fortinii* strains increased root biomass and P levels compared to the control and to the other strain (Vohnik et al. 2005). Co-inoculation of *Rhododendron* cv. Azurro with *Oidiodendron maius* and *P. fortinii* altered N uptake and resulted in the highest foliar P concentrations (Vohnik et al. 2005).

The mechanisms of this proposed facilitation of host nutrient uptake have remained elusive. The arguments often used in support of mycorrhizal nutrient uptake may apply: extramatrical mycelium extending from the host roots may increase the surface area and therefore increase host access to soil nutrients. Barrow and Osuna (2002) present another interesting possibility. In a root exclusion experiment that controlled sources of P in the substrate, they showed that *Atriplex canescens* inoculated with *Aspergillus ustus* may have gained access to phosphate otherwise unavailable to the host plant.

Regardless of whether or not the host nutrient uptake is enhanced by the endophytes, the results from inoculation assays are variable and depend on choices of host species, endophyte taxa or strains and experimental conditions. For example, Fernando and Currah (1996) studied the effects of two DSE fungi, *Leptodontidium orchidicola* and *P. fortinii*, on host plants both under axenic resynthesis conditions and in pot cultures using monocultures of four host species or combination of these species. The results were variable depending on the growth conditions, the fungal endophyte and the host species. In the axenic resynthesis system, *L. orchidicola* damaged the host stele indicating a pathogenic interaction; in pot cultures, no such tissue damage was observed. Different strains of *L. orchidicola* also resulted in a range of growth responses from neutral to positive and negative. In the same study, *P. fortinii* did not cause any marked changes in host performance in the axenic resynthesis system. In the pot studies, however, monocultures of *Potentilla fruticosa* responded negatively to *P. fortinii*. Our unpublished studies (Mandyam and Jumpponen, unpublished) with native tallgrass prairie endophytes also suggest that growth responses are variable among the different combinations of endophyte strains and host species. *Periconia macrospinosa* is an endophyte that has been repeatedly isolated from native tallgrass prairie plants in North America (Mandyam and Jumpponen 2005). This fungal endophyte forms typical microsclerotia in the host roots. When *Andropogon gerardii*, a dominant C_4 grass, and *Elymus canadensis*, a C_3 grass, were inoculated with *P. macrospinosa* in an axenic resynthesis system,

Table 3 Effects of fungal root endophytes on plant growth and nutrition

Fungal endophyte	Host	Growth response	Nutrient uptake	Other effects	Source
Aspergillus ustus	*Atriplex canescens*	Increased root biomass, equivalent shoot biomass when plant unavailable P is provided	-	Root exclusion system used	Barrow and Osuna 2002
Cladorrhinum foecundissimum	*Gossypium hirsutum*	Increased biomass at blossom stage	Increased foliar P under P deficient condition	-	Gasoni and Gurfunkel 1997
Cryptosporiopsis sp.	*Larix decidua*	Increased root length	-	-	Schulz et al. 2002
Fusarium sp.	*Hordeum vulgare*	-	-	-	Schulz et al. 1999
L. orchidicola	*S. glauca*	Neutral in axenic system	NA	Stele damaged	Fernando and Currah 1996
Periconia macrospinosa	*Andropogon gerardii*	Increased biomass	-	-	Mandyam and Jumpponen unpublished
	Elymus canadensis	Decreased biomass	-	-	
	Brassica campestris, *Raphanus sativus*	Increased root growth	-	Culture filtrate used at low concentration	Shin et al. 2005
	Dryas octopetala, *S. glauca*, *Picea glauca*	Variable based on host and strain	NA	NA	
P. fortinii	*Carex* sp.	Increased biomass and levels of P in shoots	-	-	Haselwandter and Read 1982
P. fortinii	*Larix decidua*	Increased root length	-	-	Schulz et al. 2002
P. fortinii	*Pinus contorta*	+ in axenic system	Lower foliar N, P	-	Jumpponen and Trappe 1998
		Neutral in pot system	No effect	-	

(continued)

Table 3 (continued)

Fungal endophyte	Host	Growth response	Nutrient uptake	Other effects	Source
P. fortinii	*P. contorta*	Neutral with added N in pot system	Increased N	-	Jumpponen et al. 1998
		Increased with N and organic matter	-	-	
		Root biomass increased	Increased P	-	
P. fortinii	*Betula platyphylla*	Decreased growth	-	-	Hashimoto and Hyakumachi 2001
P. fortinii	*Rhododendron* sp.	Neutral	-	-	Vohnik et al. 2003
P. fortinii	*Rhododendron* sp.	Increased root biomass	Increased P	-	Vohnik et al. 2005
Phialophora graminicola	*Vulpia ciliate*	Increased short, root, total biomass; Increased root length, tillers	Increased root N Decreased shoot N Increased shoot, root and total P	-	Newsham 1999
Piriformospora indica	*Zea mays, Nicotiana tobaccum, Bacopa monniera, Artemisia annua, Petroselinum crispum, Populus tremula*	Increased growth, early rooting in tobacco calli	-	-	Varma et al. 1999
	Nicotiana tobaccum	Increased growth, seed germination and stalk elongation	No change in total N and P	-	Barazani et al. 2005
	Oryza sativa, Sorghum vulgare, Triticum sativm, Glycine max, Cicer arientinum, Solanum melongera, Dactylorhiza purpurella, D. inacrnata, D. majalis, D. fuchsia	Increased growth, greater survival rate of orchid seeds	Increased P uptake, mobilization of insoluble P	-	Singh et al. 2000 and references therein
	Spilanthes calva, Withania somnifera	Increased growth, yield, basal stem and leaf area, number of flowers and fruits, NPP	-	-	Rai et al. 2001
	Brassica oleracea, Spinancia oleracea, Brassica junacea, Arabidopsis thaliana	Increased growth, early fruiting and flowering	-	-	Kumari et al. 2003
	Adhaloda vasica	Increased biomass and root proliferation	-	-	Rai and Varma 2005
	Hordeum vulgare	Doubled biomass and increased grain yield	-	-	Waller et al. 2005
	N. tobaccum, A. thaliana	Growth increased	Total protein and N content increased in aerial parts	Increase in nitrate reductase activity	Sherameti et al. 2005
Sterile red fungus (basidiomycete)	*Triticum vulgare*	Increased shoot, root biomass and root length in non sterilized soil	-	Culture filtrate had similar effect	Sivasithamparan 1998
	Lolium rigidum	Increased shoot and root biomass in sterilized and non sterilized soils	-	-	
	Rotational crops	Increased growth	-	-	Dewan and Sivasithamparan 1989

A. gerardii growth was enhanced while *E. canadensis* growth was reduced (Fig. 2). Experimental conditions as well as the choice of hosts and/or fungal strains are clearly important drivers of the outcomes of endophyte-host interaction.

Most of the outlined examples have used fungi that form typical DSE morphologies in the roots including microsclerotia and melanized hyphae. In addition to these fungi, a number of asco- and basidiomycetes that do not form microsclerotia, but colonize host roots inter- and intracellularly, have been shown to positively affect host growth. *Cladorrhinum foecundissimum* isolated from healthy roots of *Agropyron* spp. was inoculated onto *Gossypium hirsutum* cv. Guazuncho in pot cultures (Gasoni and Gurfunkel 1997). The fungus colonized the host roots intercellularly and developed dense infection cushions in the cortex and in the root hairs. This endophyte enhanced *G. hirsutum* growth by 50% at blossom stage. Additionally, in P-deficient soils, the inoculation doubled the foliar P levels. However, similarly to many mycorrhizal experiments growth enhancement or increase in foliar P levels were not evident in high P soils.

Recently, a new basidiomycetous endophyte, *Piriformospora indica*, has gained substantial attention as a potential growth-promoting agent. This Hymenomycete colonizes the roots both inter- and intracellularly and forms coils or round bodies

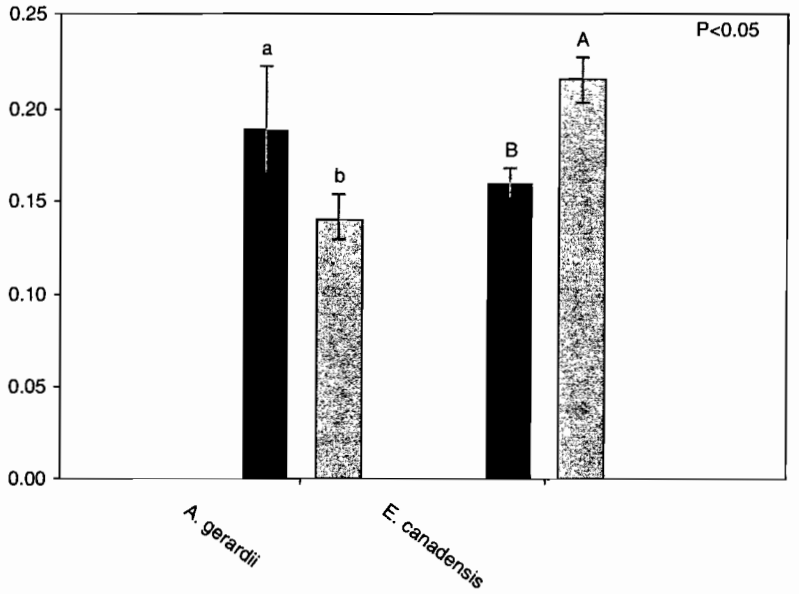

Fig. 2 Effect of *Periconia macrospinosa* on the shoot dry weight of *Andropogon gerardii* and *Elymus canadensis*. *Black bars* represent *Periconia macrospinosa* inoculated plants and *grey bars* represent control plants. Pair-wise differences ($P<0.05$) in *Andropogon* are indicated by *lowercase letters* and *uppercase letters* in *Elymus*, respectively. Treatments are significantly different within a species if they do not share a letter. *Bars* indicate standard error

and branches in the cortex (Verma et al. 1998; Varma et al. 1999) without any colonization of the host stele. This endophyte appears to have a broad host range. It has been shown to colonize and enhance growth of, for example, *Zea mays*, *Nicotiana tobaccum*, *Bacopa monniera*, *Artemisia annua*, *Petroselinum crispum*, *Populus tremula*, *Oryza sativa*, *Sorghum vulgare*, *Triticum sativum*, *Glycine max*, *Cicer arientinum*, *Solanum melongera*, and terrestrial orchids like *Dactylorhiza purpurella*, *D. inacrnata*, *D. majalis* and *D. fuchsia* (Singh et al. 2000; Varma et al. 1999). Barazani et al. (2005) confirmed the growth increase in *N. tobaccum* and showed that the growth promotion may be associated with improved fitness, as the inoculated plants produced more seed; similar results were also obtained in inoculation assays using *Spilanthes calva* and *Withania somnifera* (Rai et al. 2001) as well as in *Hordeum vulgare* (Waller et al. 2005).

Piriformospora may serve as a clever model system to elucidate the mechanisms of host growth and fitness promotion. A number of studies have tested its role in nutrient uptake and assimilation in symbiosis with host plants. It seems that *P. indica* is capable of mobilizing plant unavailable P by excreting extracellular phosphatases, as well as mediating uptake and translocation of labeled P via an energy dependent process (Singh et al. 2000 and references therein). It is also possible that *P. indica* is involved in N accumulation in the shoots of *N. tobaccum* and *A. thaliana* (Sherameti et al. 2005). N content in *N. tobaccum* was increased by 22%, indicating a transfer of about 60% substrate N into the plants. This N content increase was correlated with a 50% increase in nitrate reductase activity, a key enzyme in nitrate assimilation, in *N. tobaccum* and a similar 30% increase in *A. thaliana* (Sherameti et al. 2005). Whether the enhanced enzyme activity resulted in growth enhancement remains to be tested.

Endophytes may enhance growth by producing phytohormones without any apparent facilitation of host nutrient uptake or stimulation of host nutrient metabolism. The endophytic fungi may enhance biomass by producing growth hormones or inducing the host hormone production (Petrini 1991; Schulz and Boyle 2005). Simple experiments using culture extracts indicate that soluble culture extracts may stimulate host growth similarly to the actively growing fungi. The mycelial culture extract of *P. fortinii* induced a similar increase in *Larix decidua* shoot and root biomass as did the fungus itself (Römmert et al. 2002, in Schulz and Boyle 2005). Most likely the growth promotion was attributable to indole acetic acid (IAA) as the fungus synthesized the hormone *in vitro*. A similar effect has also been observed with *P. indica*. When a fungal filtrate (1% w/v) was added to maize seedlings three times a week for 4 weeks (Varma et al. 1999), shoot biomass increase was similar to that observed in inoculation experiments with living cultures of the fungus.

To summarize, many root-associated endophytes may be involved in nutrient transfer and growth enhancement in at least some cases. However, as exemplified by the case study presented above, the diversity of endophytes and their interactions with the hosts complicate generalizations, as any given combination of hosts and endophyte species or strains can behave differently. With this, we are limited to conclusions that are often presented for mycorrhizal systems (Johnson et al. 1997): the host-endophyte symbioses tend to be idiosyncratic and context dependent. In other words, the endophyte symbioses may be best judged on a case-by-case

basis without attempting overarching generalizations. As we become aware of a greater number of fungi that colonize native plants as endophytes, it appears that many common soil saprobes or benign parasites may behave like facultative endophytes.

3.2 *Role of Endophytes in Resistance to Pathogens and Pests*

Mycorrhizal fungi and clavicipitaceous grass endophytes can protect their hosts from pathogens and pests (Table 4). The systemic and foliar endophytes have received particular attention and can reduce herbivory by producing alkaloids toxic to insects and vertebrates (Schardl 2001). Mycorrhizal fungi are also capable of inducing resistance, and a number of mechanisms have been proposed for this resistance induction (Azcon-Aguilar and Barea 1996). Many such mechanisms of mycorrhiza-induced resistance are related to the nutritional status of the host, often correlated with mycorrhizal colonization, although some non-nutritional alternatives have also emerged (Borowicz 2001). Mycorrhizas can also mitigate the effects of herbivores, although these effects are highly variable (Gehring and Whitham 2002). To a large extent, endophytes may also be capable of improving host resistance to pathogens and pests. We will briefly review the sparse available data below and present a brief synthesis on the possible roles and mechanisms that may attribute to the altered resistance.

3.2.1 Protection from Pathogens

In the recent past, a number of reports have suggested that some endophytes can improve plant resistance to pathogens. A summary of these reports with possible mechanisms is listed in Table 4. There are at least three primary mechanisms by which endophytes can improve host resistance to pathogens (Mandyam and Jumpponen 2005).

The first mechanism is based on preemptive resource utilization by endophytes and endophyte and pathogen competition for the same resources (Lockwood 1992). This is well-illustrated in a *Fusarium oxysporum* system. A non-pathogenic *F. oxysporum* Fo47 inhibits the pathogenic *F. oxysporum* f. sp. *radicis-lycopersici* and reduces the tomato foot and root rot symptoms (Bolwerk et al. 2005). In this study, Fo47 inoculum load was 50-fold greater than that of the pathogen. The difference in inoculum loads ensured that more Fo47 spores competed with the pathogen for the same C source, thereby reducing nutrient availability to the pathogen. Both of these *Fusarium* strains exhibit similar colonization strategies. Accordingly, Fo47 can occupy and reduce the number of suitable sites for spore attachment and subsequent colonization resulting in fewer symptomatic lesions. Similar mechanisms of pathogen resistance and fewer pathogen symptoms may be applicable in other asymptomatic endophyte systems. *Phialophora radicola* var. *graminicola* may

Table 4 Role of fungal root endophytes in improving host resistance to pathogens and pests

Endophyte	Host plant	Pathogen/pest	Effects on host	Possible mechanism	Source
Piriformospora indica	*Hordeum vulgare*	*Blumeria graminis* (Powdery mildew)	Decrease of disease by 58%; hypersensitive reaction- host cell death, CW associated defense; enhanced GSH and GR activity	Systemic induction of resistance by unknown mechanism	Waller et al. 2005, 2006
	O. sativa	*Cochliobolus sativus, Fusarium culmorum*	Significant improvement of biomass in Infected plants; higher ascorbate levels in roots	Higher antioxidant levels protects from cell death	
	Spilanthes calva	*F. oxysporum, Trichophyton mentagrophytes*	Antifungal alkaloid production was enhanced	Anti microbial compounds	Rai et al. 2002
Sterile red fungus	*Triticum vulgare cv. Guntha responded the best*	*Gaeumannomyces graminis*	Lesion length and rate of lesion development reduced; results seen in field as well, root rot absent when very high inoculum densities used; culture filtrate also has similar effect	No thickening of endodermis	Sivasithamparan 1998, Kurtboke et al. 1993, Dewan and Sivasithamparan 1989
	Triticum vulgare	*Rhizoctonia solani, Pythium irregulare*	-	-	Sivasithamparan 1998
	Telopea speciosissima	*Pythophthora cryptogea*	No reduction in disease symptoms	-	
Phialophora graminicola	*Triticum vulgare*	*Gaeumannomyces graminis*	Corticular colonization by *Phialophora*, thickened endodermis; prevents pathogen entry into stele	Preemptive action, mechanical barrier; induced resistence	Deacon W 1981 in Sivasithamparam 1998, Speakman and Lewis 1978

(continued)

Table 4 (continued)

Endophyte	Host plant	Pathogen/pest	Effects on host	Possible mechanism	Source
Phialophora sp.	*Triticum vulgare*	*Gaeumannomyces graminis*	Increased seedling dry weight, increased grain yield, decreased root disease	Fast root colonization and competition for resources	Zirba et al. 1999, Mathre et al. 1998
P. fortinii	*Solanum melongena*	Verticillium dahliae	Pathogen suppression		Narisawa et al. 2002
DSE taxon LtVB3	*Brassica campestris*	*Verticillium longisporum*	External and internal symptoms reduced by 84 and 88%; CW appositions and thickenings	Indirect; DSE mycelium form mechanical barriers	Narisawa et al. 2004
Periconia macrospinosa	*A. thaliana*	*Botrytis cinerea*	Three fold reduction in disease symptoms	A systemic induced resistance like mechanism	Mandyam et al. unpublished
	No host	Bacteria	Biocidal effect	Antibacterial compounds	Kim et al. 2004, McGahren et al. 1969
Fusarium oxysporum	*Lycopersicum esculentum*	*Meliodogyne incognita*	Reduction of infection by 60%; culture filtrate toxic to adults and juveniles	Anti-microbial compounds	Hallman and Sikora 1994, 1996
Fusarium oxysporum Fo47	*Lycopersicum esculentum*	*F. oxysporum* f. sp. *radicis-lycopersici*	Pathogen colonization reduced; increased concentration of Fo47 arrested initial attachment of pathogen	Competition for same nutrients and niches; induced resistance	Bolwerk et al. 2005
Fusarium oxysporum	No host	Fungal cultures like *Phytophthora cactorum*, *Pythium ultimum*, *Rhizoctonia solani*	Radial growth reduced	Culture filtrate used at 75% concentration	Hallman and Sikora 1996
	Cucumis sativus	Virulent *F. oxysporum* f. sp. *cucumericum*	Decreased pathogen inoculum, disease suppression	Reduction of pathogen chlamydospores, competition of infection sites, induced systemic resistance	Mandeel and Baker 1991
Acremonium strictum	*Lycopersicum esculentum*	*Helicoverpa armigera*	Growth rate of larve reduced, increased developmental time, smaller pupae and suppressed moulting, reduced efficiency of food conversion	Fungal alteration of phytosterol composition	Jallow et al. 2003
Acremonium alternatum	*Brassica oleracia* var. *gemmifera*	*Plutella xylostella*	Growth rate of larvae reduced, change in female feeding preference, increased mortality, reduced efficiency of food conversion	Fungal alteration of phytosterol composition	Raps and Vidal 1998

pre-emptively reduce the colonization of *Gaeumannomyces graminis* var. *tritici* as suggested by Sivasithamparan (1998).

The second possible mechanism of pathogen control may result and stem from the chemical inhibition of root pathogens. Colonization by benign and asymptomatic endophytes may enhance the host's ability to produce biocidal compounds as in the case of *Spilanthes calva* when inoculated with *P. indica* (Rai et al. 2002). *Spilanthes calva* produces a range of antifungal compounds. Plants inoculated with *P. indica* produced extracts that were inhibitory to soil-borne pathogens (*F. oxysporum* and *Trichophyton mentagrophytes*) suggesting induction of antifungal chemical production in the host. While only scant evidence supports endophyte induction of host production of biostatics or biocides, there are many reports of endophyte culture filtrates with anti-microbial properties. A sterile red fungus, a basidiomycete, was found to produce exudates capable of lysing *G. graminis* hyphae (Sivasithamparam 1998). Pathogen exposure to the exudates reduced the size of host lesion and slowed the lesion development. *Chaetomium globosum* isolated from a barnyard grass controlled plant pathogenic fungi, including *Magnoporthe grisea* and *Puccinia recondite* (Park et al. 2005). Schulz et al. (2002), showed that, of the tested endophytes, 43% expressed antimicrobial activities while only 27% were phytopathogenic. Additionally, *Taxus cuspidate*-inhabiting *Periconia* sp., a taxon likely congeneric to the root-inhabiting *P. macrospinosa* (Mandyam and Jumpponen 2005), inhibited *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhimurium* with an inhibitory range that was similar to that of the commonly used antibiotic gentamycin (Kim et al. 2004). Similarly, Hallman and Sikora (1994) found that the culture filtrate of non-pathogenic *F. oxysporum* reduced the radial growth of pathogens such as *Rhizoctonia solani*, *Pythium ultimum* and *Phytophthora cactorum*. While these examples suggest that some endophytes may be capable of producing antimicrobial compounds and protect their hosts from pathogens, there is little evidence in support of this mechanism for a broader range of endophytic fungi.

The third possible mechanism in improving host resistance to pathogens by endophytes is the role of induced defense responses. This mechanism is often encountered in mycorrhizal plants where weak resistance is induced locally (Koide and Schreiner 1992) or transiently during early mycorrhizal colonization (Gianinazzi-Pearson et al. 1996). Structural modifications and induction of defense signaling can similarly result from endophyte colonization. An unidentified root-associated endophyte known as LtVB3 restricted the spread of *Verticillium longissima* in *Brassica campestris* by forming mechanical barriers, cell wall appositions and thickenings (Narisawa et al. 2004). As a result, external and internal pathogen symptoms were reduced by over 80%. Narisawa et al. (1998) also observed inhibition of *Plasmodiophora brassicae*-caused clubroot in *B. campestris* by 5% of endophytes that were isolated from the field. These endophytes included *Heteroconium chaetospira*, *Mortierella elongate*, *Westerdykella* sp. as well as three unknown hyaline and melanized species. Narisawa et al. (1998) proposed that superficial (*M. elongata*), cortical (hyaline and DSE fungi, *Westerdykella* sp.), or superficial and cortical (*H. chaetomium*) colonization created a mechanical barrier to the pathogens. Another example of localized and

systemic induction of host resistance is a study that used *P. indica* and barley (Waller et al. 2005). *Fusarium culmorum* KF 350 and *Cochliobolus sativus* disease severity in inoculated plants was reduced in *P. indica*-inoculated hosts. Similarly, biomass loss of the pathogen-infected plants was also drastically reduced. These positive effects correlated with higher levels of the antioxidant compound ascorbate in the roots. The antioxidants were thought to protect the cells from hypersensitive reactions. In that study, the effect of *P. indica* on a powdery mildew pathogen, *Blumeria graminis* f. sp. *hordei*, was also studied. Similar to the effect on the root pathogens, foliar pathogenesis was reduced by over 50% and hypersensitive reactions were elicited. The authors concluded that *P. indica* inoculation induced a systemic resistance. It is likely that many tissue-penetrating endophytes may induce pathogen resistance. Our preliminary results (Mandyam et al., unpublished) indicate that *P. macrospinosa*, a common root endophyte in plants at the Konza Prairie in Kansas, USA, can reduce *Botrytis cinerea*-caused leaf spot symptoms three-fold *A. thaliana* (Fig. 3), a response most likely attributable to induced systemic resistance resulting from endophyte–host interaction.

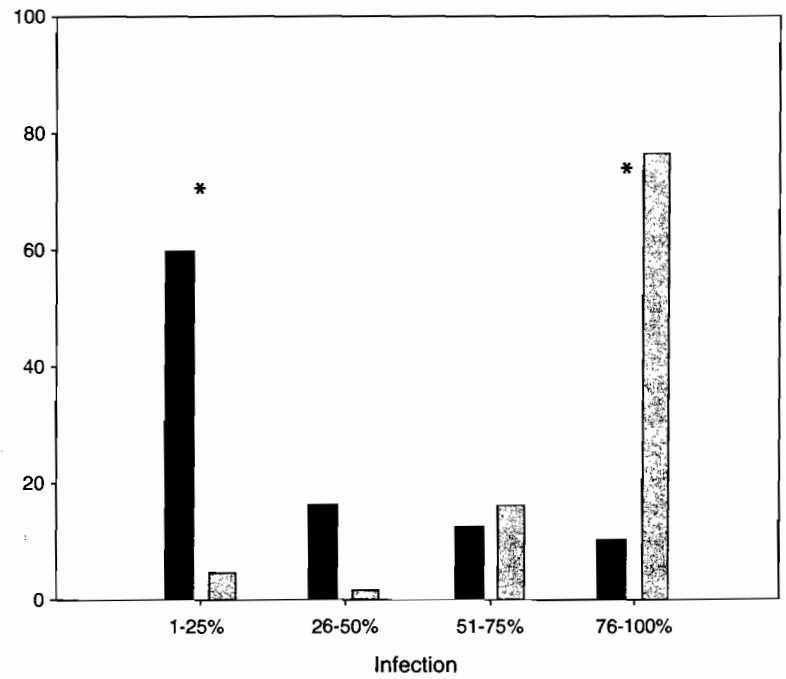

Fig. 3 Effect of *Periconia macrospinosa* on *Botrytis cinerea* necrosis in *Arabidopsis thaliana*. The *X-axis* indicates the percent leaf area necrotized by *Botrytis cinerea*. The *Y-axis* indicates the percentage of inoculated leaves. *Black bars* represent *Periconia* pre-inoculated *Arabidopsis* and *grey bars* represent control *Arabidopsis* (pre-inoculated with broth). * Indicates a three-fold difference between the treatments

In many cases, more than one of the three discussed mechanisms can act simultaneously. For example, root colonization by *P. graminicola* can pre-emptively reduce the growth of the pathogen *G. graminis* by competition for space and resources. However, it can also form mechanical barriers resulting from thickening of endodermis that inhibits colonization of the stele by the pathogen (Speakman and Lewis 1978; Deacon 1981, in Sivasithamparan 1998). Similarly, any tissue-colonizing benign organism reduces available carbon to pathogens and can occupy likely colonization sites resulting in fewer possible sites for pathogen penetration.

3.2.2 Protection from Pests and Herbivores

Mycorrhizae, and especially systemic and foliar Clavicipetalean grass endophytes, are widely known to reduce herbivory. Clavicipitaceous fungi produce toxic alkaloids against insect and vertebrate herbivores. Endophytic fungi may similarly play a role in protection of hosts from pests and herbivores. Mandyam and Jumpponen (2005), suggested three possible mechanisms by which root-associated endophytes can improve resistance of host plants to herbivores and pests. The first mechanism is based on overall improvement of plant performance by endophytes, which helps plants tolerate herbivory and sustain damage without visible effects on productivity (Gehring and Whitham 2002). Table 3 lists the instances where root endophytes can improve growth, enhance nutrient levels and improve plant fecundity whereas Table 4 lists instances where host tolerance and/or resistance to pathogens and pests has been shown to be altered.

The second possible mechanism is the alteration of plant nutritional chemistry both qualitatively and quantitatively, by altering the carbohydrate and nitrogen contents, C:N ratio and phytosterol composition (Jones and Last 1991; Bernays 1993; Schulz and Boyle 2005). The endophytes are capable of altering nutrient levels and content in host plants as discussed above. This, coupled with altered carbohydrate metabolism, can affect the host herbivore susceptibility. A few examples exist for endophytes that alter phytosterol composition in host plants and decrease herbivory. Raps and Vidal (1998) and Jallow et al. (2004) studied the interaction of non-specific endophyte with host plants to reduce insect infestation. Inoculation of *Lycopersicon esculentum* with *Acremonium strictum* reduced infestation by the tomato grub, *Helicoverpa armigera* (Jallow et al. 2004). Similarly, *Acremonium alternatum* inoculation of *Brassica olerecia* var. *gemmifera* inhibited the cabbage moth, *Plutella xylostella*, before the fungus had colonized the green foliage of the plant (Raps and Vidal 1998). In both cases, larval mortality increased and the larval growth rate was reduced among the survivors. On endophyte-inoculated *L. esculentum*, molting was also suppressed. In case of endophyte-inoculated *B. olerecia*, the moth females seemed more sensitive to the inoculation treatment, suggesting sexual differences in feeding. In both illustrated examples, the insects showed decreased efficiency in converting ingested food to biomass. While the endophytes did not appear to produce any feeding deterrents, they appeared to change the host plant phytosterol composition. Jallow et al. (2004) provided

supporting evidence and showed that *A. alternatum* can affect and alter tomato phytosterols both quantitatively and qualitatively. Raps and Vidal (1998) hypothesized that when endophytes and herbivores occupy discreet and different plant parts, the competition for nutrients will result in host-mediated differences in herbivore preferences or performance. Based on the 'sink competition hypothesis' by Larson and Whitham (1997), Raps and Vidal (1998) suggested that the greater the spatial disjunction between endophytes and herbivores, the more important will be the impact of sink build-up by endophytes on the nutritional value of food.

The third possible mechanism of host herbivore resistance is the production of feeding deterrents by the endophytes themselves. Toxic alkaloids are produced by foliar endophytes of grasses (Clay and Holah 1999; Clay 1990). Non-pathogenic *F. oxysporum*, a common root endophyte in *L. esculentum*, produces soluble toxic metabolites that are present in culture filtrates (Hallman and Sikora 1996). The filtrate has been shown to be toxic to *Meloidogyne incognita*, a root nematode. These toxic metabolites reduce nematode mobility, inactivate juveniles and are lethal within a 24-h exposure. The effects of the endophyte filtrates were reproducible in pot experiments (Hallman and Sikora 1994), indicating that the fungus also produces the metabolites *in vivo*. Mandyam and Jumpponen (2005) suggest that extensive endophyte colonization may also prevent grazing on roots. Many root-associated endophytes produce abundant melanized structures. Melanin discourages microbial grazing (Kuo and Alexander 1974; Bell and Wheeler 1986; Griffith 1994). *P. macrospinosa* extensively colonizes native grasses in the tallgrass prairie (Mandyam and Jumpponen, unpublished). As mentioned earlier, *Periconia* spp. congeneric to those from native prairies are known to produce chlorine-containing compounds that may have antibiotic properties.

4 Potential Applications of Root-Associated Endophytes

In the sections above, we have briefly reviewed the potential diversity and distribution of endophytes as well as their possible and potential functions. We continue by hypothesizing and discussing some possible applications of these root-associated fungi. Although we are unaware of any presently available commercial, agricultural and/or horticultural applications, we argue that the observed and proposed diverse functions provide a marketable base for an application development. As we outline above, some—although possibly few—endophytes are capable of the growth stimulation of many economically important species. Furthermore, the examples of induced resistance against plant pathogens and herbivores suggest potential applications as biopesticides. While use of arbuscular mycorrhizal fungi may have been limited by their obligate biotrophism, many of the endophytic fungi are readily isolated and easily maintained in pure culture facilitating their mass production. This is especially the case if conidial microfungi could be utilized for these applications. We will complete this section with precautionary notes and outline some pitfalls of these applications.

4.1 Need for Microbial Solutions in Sustainable Agriculture

In the course of the past few decades the human population has doubled. Food production has similarly increased. Use of man-made fertilizers has enabled much of the increase in the crop production. This has resulted in a 9-fold increase in N fertilization and a 4-fold increase in P fertilization (Vance 2001). Concurrent with the escalating use of commercial fertilizers, the intensity of agricultural practices has increased and a wide variety of fungicides, bactericides and pesticides are utilized in large-scale crop production. The widespread use of chemical pest-control agents can contribute to ground- and surface-water pollution.

The goals of yield-focused, large-scale agriculture remain valid, but additional priorities have emerged (Cook 1992). While industrialized countries are considering ways to reduce the environmental costs of intensive agriculture, and are seeking alternatives to traditional pesticides and fertilizers, the priorities in developing countries are somewhat different. Lack of affordable fertilizers as well as affordable biocides do not allow for intensive agriculture. Documented benefits of the arbuscular mycorrhizal symbiosis on plant performance and crop protection have fanned discussions on their use in agriculture (Menge 1983) as potential solutions for both developed and developing nations. If efficient production systems for inoculum were available, and the results were as predictable as with man-made fertilizers and biocides, sustainable microbial solutions would be more marketable. However, mycorrhizal inoculation practices are rarely compatible with industrial scale agriculture. Mycorrhizal inoculation applications are more feasible in either smaller scale agricultural and horticultural operations (Ryan and Graham 2002) or in organic farming systems (Prakash and Adholeya 2006). Further complications include the unculturability and obligate biotrophy of arbuscular mycorrhizal fungi (Wood and Cummings 1992). The production of inoculum requires growing it in symbiosis with living host plants or in cumbersome root cultures. Such inoculum production systems are a possibility, but are hindered by high costs, slow turnover and difficulty of selecting against root pathogens in long-term maintenance.

Given that adoption of inoculation practices might be of interest in both developing and developed countries, but the use of arbuscular mycorrhizal fungi is complicated by biological constraints or lack of suitable practices that would allow large scale application, we will discuss whether fungal endophytes may be a potential solution. Many of the endophytes that we discussed above are easily cultured, maintained, and manipulated.

4.2 Potential of Endophytes for Production Agriculture Applications

In the sections above, we visited the diversity of fungal root-associated endophytes. Data from our preliminary studies (Kageyama et al., unpublished) suggest that grassland ecosystems, possibly other ecosystems as well, host a diversity of known and

unknown fungi that inhabit the roots of native plants. Many of these fungi were not previously considered root-associated and/or putative endophytes. In our survey of root-associated fungi isolated at the five LTER sites, we have thus far collected a vast number of isolates distributed across over 50 taxa (Kageyama et al., unpublished). We point out that, as a result of our limited and superficial understanding of the diversity of root-inhabiting fungi, any terrestrial ecosystem has the potential of hosting taxa and/or strains that hold potential for agricultural applications. Although the numbers of the truly beneficial endophytes may be low and their discovery rare, such endophytes are likely to exist. The best available example to date is possibly *P. indica* (Varma et al. 1999; Singh et al. 2000; Shende et al. 2006). Isolated relatively recently (Verma et al. 1998), this root-associated fungus has received substantial attention as it seems to possess a broad host range, tolerates a broad range of environmental conditions, and stimulates vegetative growth as well as seed production of many economically important plants (Singh et al. 2000; Shende et al. 2006).

Plant host responses to root-inhabiting endophytes have admittedly remained somewhat unpredictable. However, our initial screenings of the larger pool of isolates from the unpublished studies, including the case study presented above, suggest that possibly up to 5–7% of the obtained root-associated fungal strains may be considered either benign or mutualistic endophytes whose mechanisms of growth stimulation remain unknown. Further studies are necessary to test if the observed colonization and the growth responses can be reproduced for agricultural plants. Given the large and unexplored diversity of these endophytes, it is likely that the natural environments host a diversity of fungi that may find an application in production systems. One should, however, exercise caution in considering using isolated fungi for horticultural and/or agricultural applications: twice as many isolates were either clearly pathogenic or antagonistic in our screening.

In addition to growth promotion, the induction of host resistance or inhibition of pathogens presents another interesting possibility for endophyte application as biopesticides, as has been proposed for some plant growth promoting rhizobacteria (Benerjee et al. 2006) and arbuscular mycorrhizal fungi (Azcon-Aguilar and Barea 1996; Maia et al. 2006). A number of mechanisms can result in the biocidal or resistance-inducing properties of root-associated micro-organisms as described above. Our preliminary experiments indicate that *P. macrospinosa* strains isolated from oak savannah and tall grass prairie often stimulate host growth simultaneously, but also induce systemic resistance to fungal pathogens (Mandyam et al., unpublished). Based on preliminary analyses of the host transcriptome, it appears that this resistance induction is mainly attributable to systemic induction of plant defense signaling pathways. Although the resistance induction is an interesting and exciting possible application, one should bear in mind that these defense reactions likely are host's responses to what it considers an attack, and result likely from penetration into the host tissue. It is important to acknowledge that these reactions may bear a carbon cost to the host. In sum, if biopesticides are to be considered, one should also maintain host growth stimulation to provide an economically viable application.

To be able to provide a viable alternative as biological fertilizer or biocide, the product should provide greater or, at the very least, a comparable yield increase or crop protection as can be obtained via conventional means when the costs of using these different approaches are accounted for. Although the endophytes may provide a variety of benefits, including increased resistance to pathogens and/or herbivores in addition to growth and yield promotion, we are not aware of a reliable cost benefit analysis that would provide a solid economical basis for selecting the growth promoting endophytes over more widely considered mycorrhizal or bacterial alternatives.

4.3 *Precautionary Notes*

Although the endophytes may bear a promise as biofertilizers and biopesticides, no marketable applications have emerged thus far. There are a number of complications that make product development difficult. We have previously pointed out that, while a number of records suggest arbuscular mycorrhizal benefits to many crop plants, their applications have been hindered by the difficulty of producing inocula. Selection of suitable species or strains can also be difficult: no fungal species or strain may be applicable across diverse environmental conditions and hosts. While arbuscular mycorrhizal fungi may have limited host specificity (Eom et al. 2000; Helgason et al. 2002), host specificity as well as differential growth stimulation among taxa and strains (van der Heijden et al. 1998) underlines the importance of strain and taxon selection. Because thus far only a limited number of fungi have been tested for applications under field conditions, we use *Pisolithus tinctorius*, an ectomycorrhizal basidiomycete, as an example. Strains of *P. tinctorius* selected for early conifer seedling growth promotion in southeastern United States did not perform as well as local strains and species when tested in northwestern United States (Perry et al. 1987). Similarly, strains that can be easily applied and readily colonize hosts under nursery conditions may not provide favorable effects once the seedlings are planted in the field (le Tacon et al. 1992; Jackson et al. 1995). Furthermore, it is difficult to predict how the inoculated fungi compete with the ubiquitous microbial flora present naturally in soil. If the inoculants are quickly competitively excluded, the initial growth promotion of the biofertilizer fungi may be short-lived.

Among our precautionary notes we also wish to express our concern for nation-wide and international commerce using fungal inocula. It is likely that antropogenic factors have contributed to the global spread of plant pathogens and invasive weeds. An issue that often receives little attention in considerations of biofertilizer applications is the impact that imported and possibly invasive microbes may have on the endemic communities. The inoculated fungi may persist and threaten endemic strains and species via competition (El Karkouri et al. 2006). Presently, our understanding of such dynamics is crude and no evidence exists for competitive exclusion within soil

and rhizosphere microbial communities. However, introduced aggressive and invasive strains may homogenize endemic populations and communities.

5 Conclusions

Natural ecosystems are likely to host a diversity of root-associated fungi, and some of them may be considered true endophytes. The nature of these symbioses remains unclear, although we feel confident that no single unifying generalization can be applied across the wide spectrum of these associations. Although the examples outlined indicate that some endophytes stimulate host growth and some may be involved in the facilitation of host nutrient uptake, a variety of additional host responses emerge as well. Our laboratory screening measuring shoot biomass in leeks inoculated with isolates from the Shortgrass Steppe LTER suggests that fungi that fit the definition of endophyte may be few in number and not necessarily beneficial to the host vegetative biomass accumulation. Although most studies use host growth responses as a measure of mutualism, many endophytes may also affect their hosts in other ways, such as inducing host defenses, either distally or locally, and benefit them by reducing host susceptibility to pathogens. This is natural: endophytes penetrate through the cell walls and colonize their hosts intracellularly, resulting in early host response similar to pathogen attacks. These systemic and local responses may also be analogous to those induced by mycorrhizal mutualists. However, there is still some question as to whether or not this upregulation of host defenses presents a carbon cost to the host and whether or not the induced resistance to plant pathogens allows maintenance of greater or comparable growth rates and/or greater or comparable fitness and fecundity.

The ease of culturing fungal root endophytes and their potential positive effects on hosts invites speculation on their use as biostimulants or as biopesticides. Although some endophytes, *P. indica* in particular, bear a substantial promise with their broad host ranges and documented multiple positive effects on many economically important crop species, we note that development of marketable endophyte bioproducts is difficult. At the same time, we advise caution and extensive background testing to avoid possible negative outcomes of wide applications of such endophytes. Even if such bioproduct applications are considered safe to crops and cause no adverse effects on tested plants, the responses among endemic plant and soil communities remain unknown. Widespread applications may result in homogenization of the local and endemic populations and communities of the soil- and rhizosphere-associated micro-organisms. Consequently, wide-spread applications have a potential to result in unknown losses in local and global biodiversity.

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