Mycorrhiza (2008) 18:103–110
DOI 10.1007/s00572-007-0157-y

SHORT NOTE

# The co-occurrence of ectomycorrhizal, arbuscular mycorrhizal, and dark septate fungi in seedlings of four members of the Pinaceae

**Cameron Wagg · Michael Pautler · Hugues B. Massicotte · R. Larry Peterson**

Received: 18 September 2007 / Accepted: 7 November 2007 / Published online: 19 December 2007


**Abstract** Although roots of species in the Pinaceae are usually colonized by ectomycorrhizal (EM) fungi, there are increasing reports of the presence of arbuscular mycorrhizal (AM) and dark septate endophytic (DSE) fungi in these species. The objective of this study was to determine the colonization patterns in seedlings of three *Pinus* (pine) species (*Pinus banksiana*, *Pinus strobus*, *Pinus contorta*) and *Picea glauca x Picea engelmannii* (hybrid spruce) grown in soil collected from a disturbed forest site. Seedlings of all three pine species and hybrid spruce became colonized by EM, AM, and DSE fungi. The dominant EM morphotype belonged to the E-strain category; limited colonization by a *Tuber* sp. was found on roots of *Pinus strobus* and an unknown morphotype (cf. *Suillus–Rhizopogon* group) with thick, cottony white mycelium was present on short roots of all species. The three fungal categories tended to occupy different niches in a single root system. No correlation was found between the percent root colonized by EM and percent colonization by either AM or DSE, although there was a positive correlation between percent root length colonized by AM and DSE. Hyphae and vesicles were the only AM intracellular structures found in roots of all species; arbuscules were not observed in any roots.

C. Wagg · M. Pautler · R. L. Peterson (✉)
Department of Molecular and Cellular Biology,
University of Guelph,
Guelph, Ontario N1G 2W1, Canada
e-mail: lpeterso@uoguelph.ca

H. B. Massicotte
Ecosystem Science and Management Program,
University of Northern British Columbia,
3333 University Way,
Prince George, British Columbia V2N 4Z9, Canada

**Keywords** Conifers · Symbiosis · Fungal endophytes · Mycorrhiza

## Introduction

Arbuscular mycorrhizas (AMs) are typical of most vascular plants including species in all families of gymnosperms except the Pinaceae (Smith and Read 1997). Although genera in the Pinaceae are typically colonized by ectomycorrhizal (EM) fungi, there are reports of the presence of AM structures in field-collected roots of some species (Cázares and Trappe 1993; and papers cited therein). More recently, Horton et al. (1998) reported the presence of vesicles, hyphae, and arbuscules in *Pinus muricata* Dougl. *ex* D. Don (bishop pine) seedlings growing in postfire, disturbed soils.

Arbuscular mycorrhizas have also been found in seedlings of *Pseudotsuga menziesii* (Mirbel) Franco (Douglas-fir) and *Tsuga mertensiana* (Bong.) Carr. (mountain hemlock) when used as bait plants grown in soil collected from Douglas-fir plantations (Cázares and Smith 1996). In an experimental study, seedlings of *Abies lasiocarpa* (Hook.) Nutt. (subalpine fir), *Pinus contorta* Dougl. var. *latifolia* Engelm. (lodgepole pine), *Pinus ponderosa* Dougl. *ex* C. Lawson (ponderosa pine), *Tsuga heterophylla* (Raf.) Sarg. (western hemlock), and *Pseudotsuga menziesii* inoculated with spores of *Glomus intraradices* failed to become colonized (Smith et al. 1998). In contrast, however, roots of some Douglas-fir and ponderosa pine seedlings became colonized by vesicles and hyphae when grown in dual culture with AM hosts inoculated with spores of *G. intraradices* (Smith et al. 1998). Similar results were reported for *A. lasiocarpa* when grown with an AM host (Johnson 1998).

The occurrence of both AM and EM colonization is also common in several angiosperm tree genera (see review by Molina et al. 1992). Although AM and EM can occur within the same root system (Chilvers et al. 1987), the dominance of each category of mycorrhiza may change over time (Lapeyrie and Chilvers 1985; Bellei et al. 1992), perhaps reflecting different roles for the symbioses (Chen et al. 2000). In *Populus deltoids* Bartr. Ex Marsh. (eastern cottonwood), Lodge and Wentworth (1990) found a negative association between AM and EM colonization within the same root system.

In addition to AM and EM, root systems of both conifers and angiosperms may host other fungal associations, including dark septate endophytic fungi (DSE; Ahlich and Sieber 1996; Jumpponen and Trappe 1998), illustrating the complex nature of interactions that can occur between roots and soil biota.

The objective of this study was to assess the co-occurrence of AM, EM, and DSE fungal colonization in the roots of *Pinus contorta* (lodgepole pine), *Pinus banksiana* Lamb. (jack pine), *Pinus strobus* L. (eastern white pine), and *Picea glauca* (Moench) Voss × *Picea engelmannii* Parry ex Engelm. (hybrid spruce) in the presence and absence of a typical AM host plant, *Trifolium pratense* (red clover), when seedlings were grown in soil collected from a disturbed forest site. Red clover was chosen because it is common in open forests and along forest margins in Central Canada.

## Materials and methods

### Site description and soil sampling

The field soil (classified as Grey-Brown Podzolic; Soil Survey of Wellington County) used as inoculum was collected in the summer of 2006 from a mixed conifer forest dominated by *Pinus resinosa* Ait. (red pine) and *Thuja occidentalis* L. (eastern white cedar), located at the Vance Tract conservation area near Guelph, Wellington County, Ontario, Canada (N 43°30′52.6″ W 80°13′10.8″ to N 43°27′50.6″ W 80°13′02.7″). This area had been disturbed by prescribed thinning between December 2004 and January 2005, and by herbicide (Release ™, DowAgroSciences; active ingredient triclopyr [(3,5,6-trichloro-2-pyridinyl) oxyacetic acid] present as a butoxy ethyl ester) application to control *Rhamnus cathartica* L. (European buckthorn) saplings in the fall of 2004 and the summer of 2006 (Grand River Conservation Authority, personal communication). The herbicide was applied directly to the *R. cathartica* plants either as a 30% solution in mineral oil to stems (fall 2004) or as the same treatment with the addition of a 5% solution foliar application (summer 2006). Soil was collected several weeks after the latter treatment.

The forest had a moderately open canopy with an understory consisting of several herbaceous species including *Hieracium* spp. (hawkweed), *Taraxacum officinalis* (dandelion), and *Pteridium aquilinum* (bracken fern). As well, seedlings of *Acer* spp. (maple) and *P. banksiana* were present.

Soil was collected every 3 m along a transect approximately 30 m long. Leaf litter was removed, and soil containing root fragments was collected to a depth of approximately 10 cm. Soil was bagged for transport to the lab and subsequently combined (approximately 20 l total). Root fragments were cut into <5 cm lengths, and soil and root fragments mixed 4:1 with sterilized 1:1 sand/Turface (calcined montmorillonite clay, Applied Industrial Materials, Buffalo Grove, IL, USA).

### Experimental design

Seeds of *P. banksiana*, *P. strobus*, *P. contorta*, and *P. glauca* x *engelmannii* were surface sterilized in 15% $H_2O_2$ for 45 min, soaked in $dH_2O$ for 24 h, and planted as monocultures with a minimum of three seeds per round plastic pot [each having approximately 300 ml of rooting space (ITML Horticultural Products, Brantford, Ontario)]. Twenty replicates were set up for each species; half (ten) of these pots also contained sterilized seeds of *T. pratense*; these were sterilized using the same procedure as for the Pinaceae seeds. The presence and absence of *T. pratense* was used to test for the effects of a known AM host on the fungal colonization of the Pinaceae species. All pots were then watered to field capacity and covered with 9 cm diameter Petri dish lids for 10 days to allow for seed germination and seedling establishment. After the Petri dish covers were removed, seedlings were watered as needed (usually every 2–3 days) with $dH_2O$. Plants were maintained at 24°C/16-h days and 20°C/8-h nights and grown for 3 months under both cool white fluorescent and incandescent light receiving approximately 180 μmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux (ppf).

### Mycorrhizal assessment

Two to six seedlings of each Pinaceae species grown under each treatment (±*T. pratense*) were harvested at 1-, 2-, and 3-month intervals. Roots were gently rinsed in tap water, and the roots were viewed under a stereo dissecting microscope. For root tips showing EM fungal colonization, macroscopic characteristics were noted. The tips were then removed, mounted in 50% glycerin and gently squashed under a cover slip. Squashed root tips were viewed under high magnification (400–630x) using a compound light microscope (Leitz-Wetzlar), and details were noted for identifying ectomycorrhizal morphotypes using descriptions

by Agerer (1987–2006) and Ingleby et al. (1990). Tips lacking root hairs but having a Hartig net, either with very little mantle structure or fully developed mantle structures, were scored as EM.

The remaining portion of the root system for each seedling was cleared in 10% *w/v* KOH in a water bath at 80 °C for 2 h, followed by bleaching with 10% *v/v* sodium hypochlorite (household bleach) for approximately 10 min to remove phenolics and pigments from the roots. The roots were then stained with 0.05% Trypan blue in lactoglycerol (1:1:1 lactic acid: glycerol: $dH_20$) for approximately 1 h at 80°C, rinsed in $dH_20$, and stored in 50% glycerin. The entire root system was cut into approximately 1 cm lengths, and these were mounted on microscope slides in 50% glycerin under a cover glass. One hundred 1-cm pieces were mounted for each seedling. These were viewed using a compound light microscope at 100–400x, and the number of 1 cm root fragments exhibiting AM colonization was counted. The total number of vesicles and arbuscules was also counted for each AM colonized centimeter of root. *Trifolium pratense* seedlings were harvested at the same time as the Pinaceae seedlings, cleared in 5% *w/v* KOH for 30 min at 80°C, and stained with 0.05% Trypan blue to determine AM colonization.

The Pinaceae roots were also scored for total centimeter of root length colonized by DSE based on the presence of characteristic darkly pigmented, septate hyphae and, when present, microsclerotia (Peterson et al. 2004).

All colonized root characteristics and fungal structures of AM, EM, and DSE were photographed with a Nikon Coolpix 4500 digital camera.

### Statistical analysis

One-way analysis of variance (ANOVA) was used to determine significant effects of harvest month and host species as sources of variation for each measure of EM, DSE, and AM colonization. The distribution of residual error was assessed for homogeneity and normality (univariate procedure, SAS Institute, Inc. 2002–2003). To improve the residual error distribution, an arc-sine square-root transformation was used on the percent EM, DSE, and AM colonization data. Mean comparisons were tested using the Tukey–Kramer HSD test ($\alpha$=0.05). A Student's *t* test was used to determine overall differences in colonization between seedlings grown in the absence and presence of *T. pratense*. Spearman's rank correlation analysis was used to determine significant relationships between the levels of colonization of EM, DSE, and AM. All statistical analyses were done using SAS version 9.1 for Windows (SAS Institute Inc. 2002–2003). A Type 1 error rate of $\alpha<0.05$ was considered to be significant for all statistics. Means and SEs back-transformed to the original scale are reported. Back-transformed SEs are expressed asymmetrically as the amount of error above and below the mean on the original scale.

## Results

For all host species examined, from pots with and without *T. pratense*, approximately 50% of all root tips were colonized by EM fungi (Table 1). EM colonization did not vary significantly between host species ($F$=1.19, $p$=0.32) or harvest months ($F$=1.10, $p$=0.34). Root tips with typical E-strain characteristics were present on roots at all harvest times and accounted for approximately 42% of all root tips. This morphotype was characterized by large septate, verrucose hyphae (Fig. 1 inset) with unclamped, septate outer mantle hyphae of wide diameter, and with more branched inner mantle hyphae (Fig. 1). At 3 months, an EM morphotype characterized by elongated bristle-like (or tapering) cystidia (Fig. 2) with an interlocking irregular synenchyma outer mantle pattern, typical of *Tuber* spp., colonized approximately 3% of the root tips on one seedling of *P. strobus*. A third morphotype with white, cottony outer mantle mycelium with rhizomorphs (Fig. 3) and numerous birefingent crystals (Fig. 3 inset) colonized approximately 8% of root tips. The outer and inner mantles were difficult to discern due to the abundance of emanating hyphae and crystals. Morphotype characteristics suggest either a *Suillus* or *Rhizopogon* species.

The percent colonization by DSE varied among host species ($F$=3.82, $p$=0.02). *P. contorta* was the least colonized by DSE and differed significantly from *P. strobus* ($p$=0.02), which had the highest occurrence of DSE colonization (Table 1). DSE colonization also varied significantly between all harvest months ($F$=20.57, $p<$0.0001). The highest level of DSE colonization occurred during the second harvest month, the lowest during the first, and intermediate levels in the third month (Table 1). Typical melanized septate hyphae (Fig. 4) characteristic of DSE were present in most roots, but few microsclerotia (Fig. 5) were observed.

The percent AM colonization did not vary significantly among host species ($F$=0.72, $p$=0.54); however, AM colonization did vary among harvest months ($F$=8.33, $p<$0.001), where AM colonization was significantly greater during the second harvest than at any other time (Table 1). The occurrence of AM vesicles was highly irregular. Commonly, only a few vesicles were observed per centimeter of colonized root (Figs. 6, 7, 9); on occasion, numerous vesicles occurred (Fig. 8). Although the sporadic occurrence of vesicles did not allow for any meaningful statistics, *P. strobus* did appear to be colonized with a greater number of vesicles than any other species (Table 1). None of the species showed the development of arbuscules.

**Table 1** Mean percent (±SE) of root length colonized by mycorrhizal categories

| | n | % Colonization | | | # of AM vesicles $cm^{-1}$ |
|---|---|---|---|---|---|
| | | EM | DSE | AM | |
| Harvest month | | | | | |
| 1 | 16 | 37.6 (+9.5, -9.1) | 0.68 a (+1.0, -0.6) | 0.84 a (+1.7, -0.8) | 23.0 |
| 2 | 14 | 54.0 (+10.2, -10.4) | 26.0 b (+4.7, -4.4) | 19.5 b (+6.4, -5.7) | 8.9 |
| 3 | 18 | 55.7 (+9.0, -9.2) | 6.69 c (+2.5, -2.1) | 0.63 a (+1.5, -0.6) | 23.4 |
| Host species | | | | | |
| *P. glauca x engelmannii* | 10 | 60.1 (+11.7, -12.3) | 5.81 ab (+3.2, -2.5) | 1.25 (+2.8, -1.2) | 5.8 |
| *P. banksiana* | 12 | 32.0 (+10.8, -9.9) | 12.1 ab (+3.9, -3.4) | 3.79 (+3.8, -2.5) | 8.7 |
| *P. contorta* | 12 | 55.5 (+10.9, -11.1) | 2.83 a (+2.1, -1.5) | 7.46 (+4.9, -3.7) | 10.0 |
| *P. strobus* | 14 | 48.9 (+10.3, -10.3) | 15.4 b (+3.9, -3.5) | 6.29 (+4.2, -3.2) | 26.1 |

Within each column for harvest month and host species, means with different letters are significantly different ($p$=0.05). For the number of AM vesicles $cm^{-1}$ root length, only the averages are given.
*EM* Ectomycorrhiza, *DSE* dark septate endophytes, *AM* arbuscular mycorrhiza

Hyphal coils, arbuscules, and vesicles typical of *Glomus* species were commonly observed in the roots of all *T. pratense* seedlings grown with Pinaceae seedlings (Fig. 10). The percent root length with AM colonization was significantly greater ($p$=0.005) in Pinaceae seedlings grown in the presence of *T. pratense* (11.9% SE +4.7, −4.00) compared to those grown without *T. pratense* (8% SE +1.4, −0.7). Of the seedlings grown with *T. pratense*, seven of nine *P. strobus*, one of five *P. contorta*, three of four *P. banksiana*, and one of two *Picea glauca x engelmannii* were colonized by hyphae and/or intracellular vesicles typical of AM fungi. None of the *P. strobus* seedlings grown in the absence of *T. pratense* were colonized by AM fungi; however, two of seven *P. contorta*, one of eight *P. banksiana*, and one of six *Picea glauca x engelmannii* did become colonized in the absence of *T. pratense*.

AM vesicles and hyphae were typically observed in the longer laterals and, occasionally, in the primary and short roots. In a few instances, vesicles were observed in short roots that had epidermal and cortical cells surrounded by a Hartig net. The presence or absence of *T. pratense* did not significantly affect the EM colonization ($p$=0.55) or the DSE colonization ($p$=0.13).

There was no significant correlation identified for either DSE colonization ($r$=−0.06, $p$=0.67) or AM colonization ($r$=−0.05, $p$=0.76) with EM colonization. A significant positive correlation was found between AM and DSE colonization ($r$=0.30, $p$=0.04).

## Discussion

Soil-baiting is commonly used to determine the presence of soil fungi (Brundrett et al. 1996), and in our study, seedlings of three pine species and hybrid spruce, grown in soil collected from a disturbed forest site, became colonized by EM and AM fungi, and DSE, showing that fungal inoculum of these fungi was present in spite of continued disturbance and herbicide application. Triclopyr-based herbicides are commonly used in forestry practice to control problem woody species (Sidhu and Chakravarty 1990), and although this herbicide may reduce the growth of EM fungi in vitro (Chakravarty and Sidhu 1987), a recent study (Busse et al. 2004) has shown that Ponderosa pine, Douglas-fir, and white fir (*Abies concolor*) seedlings were heavily colonized by EM fungi in various soils treated with the herbicide. The persistence of fungal inoculum of

**Fig. 1** Ectomycorrhizal morphotype on Pinaceae seedlings grown in forest soil. Inner branched mantle hyphae of an E-strain fungus on *Pinus banksiana*. *Inset* shows a septate, verrucose extraradical hypha typical of E-strain fungi

**Fig. 2** Ectomycorrhizal morphotype on Pinaceae seedlings grown in forest soil. *Tuber* sp. morphotype with cystidia (*arrowhead*) on *Pinus strobus*. *Inset* shows outer mantle hyphae with numerous pointed cystidia

**Fig. 3** Ectomycorrhizal morphotype on Pinaceae seedlings grown in forest soil. Unknown morphotype (cf. *Suillus* or *Rhizopogon* sp.) on *Pinus banksiana* with white, cottony extraradical mycelium and rhizomorphs (*arrowhead*). *Inset* shows numerous birefringent crystals in the extraradical mycelium viewed with cross polars

**Fig. 4** Melanized, septate hyphae of a dark septate fungal endophyte in a cleared root of *Pinus strobus*

**Fig. 5** A microsclerotium (*arrowhead*) of a dark septate fungal endophyte in a cleared root of *Pinus strobus*

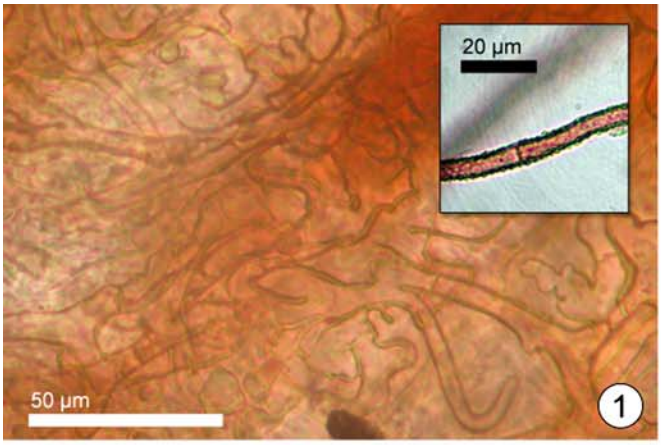
**Fig. 6** Intracellular vesicle (*v*) formation within a root cortical cell of *Picea glauca x engelmannii*

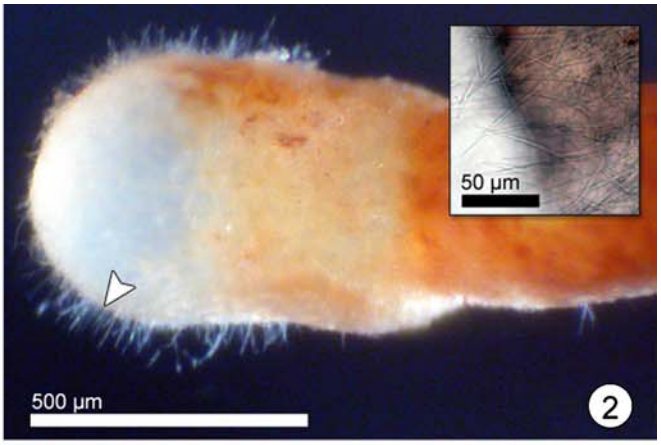
**Fig. 7** Cleared and stained root of *Pinus strobus* with vesicles (*v*) and hyphae (*arrow*)

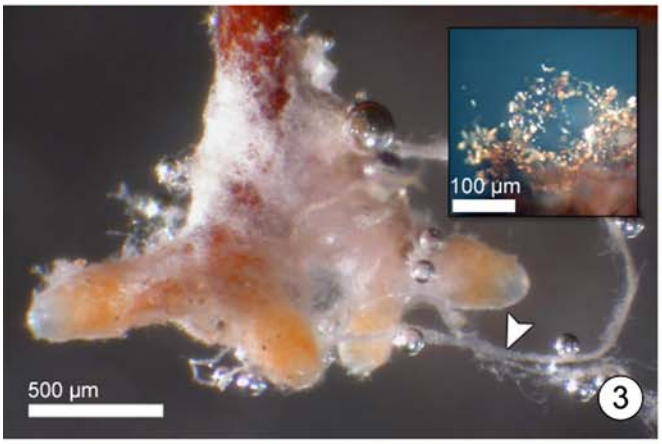
**Fig. 8** Numerous vesicles (*v*) in a cleared root of *Pinus strobus*

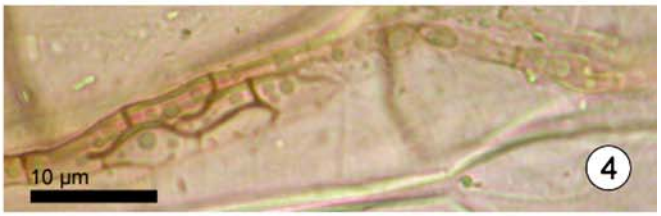
**Fig. 9** Internal hyphae (*arrow*) and vesicles (*v*) in a root of *Picea glauca x engelmannii* (hybrid spruce)

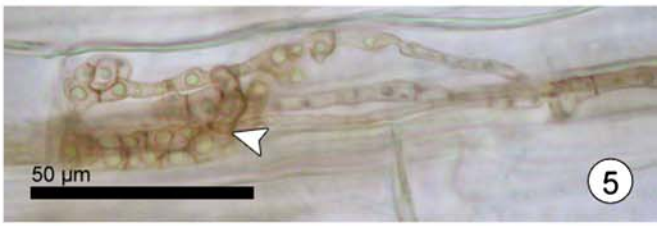
**Fig. 10** Heavily colonized root of *Trifolium pratense* showing an appressorium (*arrowhead*), internal hyphae and arbuscules (*A*), and vesicles (*V*) typical of arbuscular mycorrhizas

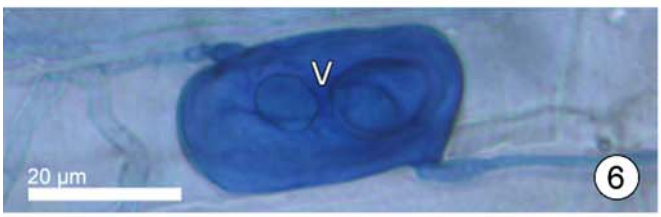

20 µm
50 µm
1

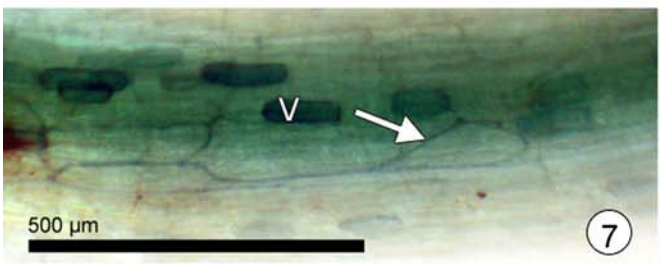

50 µm
500 µm
2

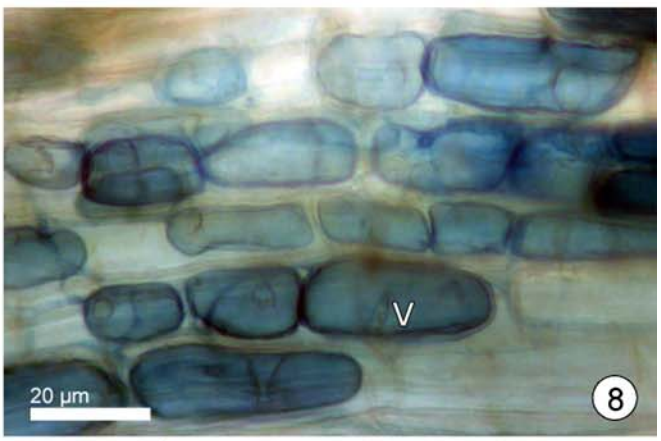

100 µm
500 µm
3

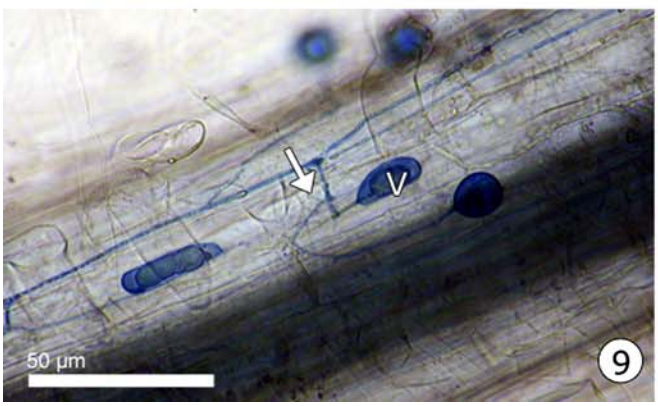

10 µm
4

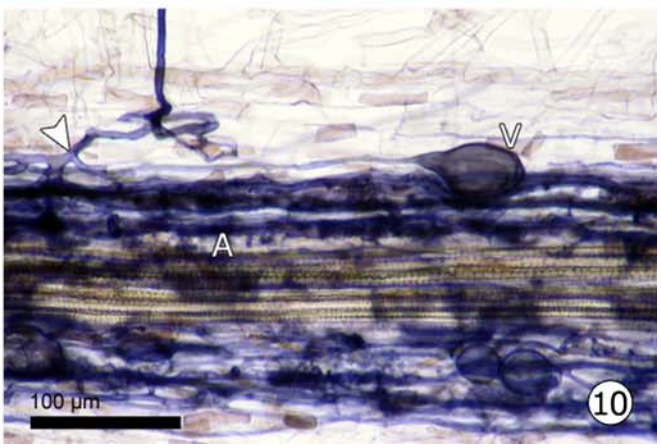

50 µm
5


V
20 µm
6


V
500 µm
7


V
20 µm
8


V
50 µm
9


V
A
100 µm
10

the three categories of root-associated fungi examined in the present study, supports findings by Horton et al. (1998) who assessed soil fungi after a wildfire.

The percentage of short roots colonized by EM fungi varied from approximately 30 to 90%, depending on conifer species and date of harvest. Common sources of EM fungal inoculum in forest soils include spores, hyphae, and sclerotia (Brundrett 1991). The dominance of E-strain morphotypes on root tips suggests that spores were likely an important inoculum source. This group of fungi is known to disperse very effectively from spores (Jones et al. 2003) and is known to be abundant in disturbed forest sites (Yu et al. 2001a). A *Tuber* morphotype was identified, but only on a small number of short roots. Viable hyphae remaining on or in root pieces might also have been a source of E-strain fungi as well as the *Tuber* and *Suillus* and (or) *Rhizopogon* white rhizomorphic morphotypes.

In a similar study, which assessed AM colonization using soil from Douglas-fir plantations, Cázares and Smith (1996) reported that seedling roots of Douglas-fir and western hemlock were heavily colonized by EM fungi. A detailed analysis of the extent of colonization and the morphotypes recognized are provided in Smith et al. (1995).

Residual root fragments of understory plants in the soil used in this study were likely the main source of AM propagules. It is unlikely that a hyphal network was retained due to soil mixing before seeding, as it is well established that soil disturbance destroys this network (Jasper et al. 1989; Evans and Miller 1990; Bellgard 1993). Spores may also have indirectly contributed to AM colonization of the Pinaceae seedlings by germinating, initially colonizing the *T. pratense* in the pots and subsequently colonizing the conifer seedlings via inoculum in the form of extraradical hyphae. It is unlikely that germinating spores directly resulted in colonization of the Pinaceae seedlings; Giovannetti et al. (1994) demonstrated that non-AM hosts lack the necessary compounds in their root exudates to stimulate hyphal growth, appressorium formation, and the differentiation of hyphae that is typically induced by root exudates of AM hosts. Only hyphae and vesicles were identified in conifer roots that became colonized, a common feature reported previously for Pinaceae seedlings (Cázares and Trappe 1993; and papers cited therein; Smith et al. 1998). Smith et al. (1998) found an increase in percent foliar phosphorus (P) in *Pseudotsuga menziesii* seedlings when grown in the presence of an AM host colonized with *G. intraradices*, although only hyphae and vesicles were present in roots, suggesting that arbuscules may not be necessary for P uptake, as proposed earlier by Smith and Smith (1996). This is supported by van Aarle et al. (2005) who found acid phosphatase activity present in hyphal coils of *Paris*-type AMs indicating that they may be important in P uptake.

To date, AMs described in roots of members of the Pinaceae are of the *Glomus* type with the formation of hyphae and vesicles being the dominant intracellular structures. Vesicles, which typically store lipids used to further fungal growth or spore development, may also function as propagules; Biermann and Linderman (1983) showed that vesicles of *Glomus* spp. isolated from roots could act as viable inoculum. The formation of vesicles in pine and hybrid spruce roots may serve as a bank of propagules for AM species when inoculum levels in the soil decrease dramatically due to disturbance or fire.

There is little information available concerning the type of dark septate fungal inoculum present in soils. Currah et al. (1993) suggested that sloughed root cells containing microsclerotia may be a source of inoculum. The demonstration that DSE microsclerotia contain deposits of polysaccharides, polyphosphate, and proteins (Yu et al. 2001b) strengthens the suggestion that they may act as propagules. In addition, it is likely that the highly melanized hyphae may also persist in soils to act as inocula (Jumpponen and Trappe 1998). The widespread distribution of DSE and the number of plant species in which they have been reported (Jumpponen and Trappe 1998) has led Mandyam and Jumpponen (2005) to suggest that these fungi may be as abundant as mycorrhizal fungi. It is still unclear how persistent DSEs are in soils after disturbance. In our study, few microsclerotia were observed in Pinaceae seedling roots, and this may reflect either a lack of time for these to form or an unfavorable nutrient status in the seedlings for their formation. To our knowledge, the factors controlling microsclerotium formation in DSEs are unknown.

A poor correlation between the level of EM colonization and the level of colonization by both AM and DSE fungi may be due to the different niches these fungi occupied within the root system. EM typically formed on short roots while, with a few exceptions, AM and DSE colonized the longer lateral roots and sometimes the primary root. A similar distribution of EM and DSE was reported for *P. contorta* roots colonized by *Phialocephala fortinii* (O'Dell et al. 1993). The positive correlation between the percent colonization of AM and DSE may indicate similar pathways of entrance into roots of host species. Both AM and DSE fungi had low levels of colonization after the first month during which time fungal and seedling root growth are likely initiating. Both DSE and AM colonization peaked after the second month suggesting an increase in the rate of fungal development and colonization relative to seedling root extension. The decline of AM and DSE colonization during the third month suggests an increase in the seedling root length growth over AM and DSE colonization rates. This may be due to a lack of resources for the fungi as a result of changing soil or host plant interactions after 2 months of growth, possibly a result of poor growing

conditions. Interestingly, the occasional co-occurrence of AM and EM within the same root fragment was noted. The co-occurrence of EM and AM in *Eucalyptus dumosa* has been described to occur in successional stages: AM colonization occurring at an early stage of growth within the cortical cells basipetal to the root apex, and EM fungi forming a Hartig net and mantle around the root apex at a later stage, creating different spatial and temporal niches (Chilvers et al. 1987). In our study, roots colonized by both EM and AM fungi showed a similar differentiation; AM structures occurred most commonly in the cortical cells of longer lateral roots, whereas EMs were mostly confined to the short roots. When they did co-occur, AMs most likely colonized the root first followed by the formation of an EM mantle and Hartig net.

**Acknowledgements** We thank the Natural Sciences and Engineering Research Council of Canada for financial support in the form of a Discovery grant to RLP and an Undergraduate Summer Fellowship to MP. We thank Forrest Phillips for help with the plate and Linda Tackaberry for comments on the manuscript. We thank Dr. Scott Green, UNBC, for the hybrid spruce seeds.

## References

Ahlich K, Sieber TN (1996) The profusion of dark septate endophytic fungi in non-ectomycorrhizal fine roots of forest trees and shrubs. New Phytol 132:259–270

Agerer R (1987–2006) Colour atlas of ectomycorrhizae, 1st–13th edition. Einhorn, Schwäbisch Gmünd

Bellei MM, Garbaye J, Gil M (1992) Mycorrhizal succession in young *Eucalyptus viminalis* plantations in Santa Catarina (south Brazil). For Ecol Manage 54:205–213

Bellgard SE (1993) Soil disturbance and infection of *Trifolium repens* roots by vesicular–arbuscular mycorrhizal fungi. Mycorrhiza 3: 25–29

Biermann B, Linderman RG (1983) Use of vesicular-arbuscular mycorrhizal roots, intraradical vesicles and extraradical vesicles as inoculum. New Phytol 95:97–105

Brundrett MC (1991) Mycorrhizas in natural ecosystems. Adv Ecol Res 21:171–197

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research (ACIAR) Monograph 32, 374 pp

Busse MD, Fiddler GO, Ratcliff AW (2004) Ectomycorrhizal formation in herbicide-treated soils of differing clay and organic matter content. Water Air Soil Pollut 152:23–34

Cázares E, Smith JE (1996) Occurrence of vesicular–arbuscular mycorrhizae in *Pseudotsuga menziesii* and *Tsuga heterophylla* seedlings grown in Oregon Coast Range soils. Mycorrhiza 6:65–67

Cázares E, Trappe JM (1993) Vesicular endophytes in roots of the Pinaceae. Mycorrhiza 2:153–156

Chakravarty P, Sidhu SS (1987) Effect of glyphosate, hexazinone and triclopyr on in vitro growth of five species of ectomycorrhizal fungi. Eur J For Pathol 17:204–210

Chen YL, Brundrett MC, Dell B (2000) Effects of ectomycorrhizas and vesicular-arbuscular mycorrhizas, alone or in competition, on root colonization and growth of *Eucalyptus globulus* and *E. urophylla*. New Phytol 146:545–556

Chilvers GA, Lapeyrie FF, Horan DP (1987) Ectomycorrhizal vs endomycorrhizal fungi within the same root system. New Phytol 107:441–448

Currah RS, Tsuneda A, Murakami S (1993) Morphology and ecology of *Phialocephala fortinii* in roots of *Rhododendron brachycarpum*. Can J Bot 71:1639–1644

Evans DG, Miller MH (1990) The role of the external mycelial network in the effect of soil disturbance upon vesicular–arbuscular mycorrhizal colonization of maize. New Phytol 114:65–71

Giovannetti M, Sbrana C, Logi C (1994) Early processes involved in host recognition by arbuscular mycorrhizal fungi. New Phytol 127:703–709

Horton TR, Cázares E, Bruns TD (1998) Ectomycorrhiza, 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

Ingleby K, Mason PA, Last FT, Fleming LV (1990) *Identification of ectomycorrhizas*. London, UK: Institute of Terrestrial Ecology Research Publication No. 5, HMSO

Jasper DA, Abbott LK, Robson AD (1989) Soil disturbance reduces the infectivity of external hyphae of vesicular-arbuscular mycorrhizal fungi. New Phytol 112:93–99

Johnson KA (1998) Factors affecting soil nutrients and biology in four Western United States subalpine forest sites. PhD thesis, Oregon State University

Jones MD, Durall DM, Cairney JWG (2003) Ectomycorrhizal fungal communities in young forest stands regenerating after clearcut logging. New Phytol 157:399–422

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

Lapeyrie FF, Chilvers GA (1985) An endomycorrhiza–ectomycorrhiza succession associated with enhanced growth of *Eucalyptus dumosa* seedlings planted in calcareous soil. New Phytol 100:93–104

Lodge D, Wentworth J (1990) Negative association among VA-mycorrhizal fungi and some ectomycorrhizal fungi occupying the same root system. Oikos 57:347–356

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

Molina R, Massicotte HB, Trappe JM (1992) Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. In: Allen MF (ed) Mycorrhizal functioning: an integrative plant–fungus process. Chapman & Hall, New York, pp 357–423

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

Peterson RL, Massicotte HB, Melville LH (2004) *Mycorrhizas: Anatomy and Cell Biology.* Ottawa, NRC Research Press

SAS Institute Inc. (2002–2003) SAS 9.1 for Windows. Statistical Software. Cary, NC, USA

Sidhu SS, Chakravarty P (1990) Effect of selected forestry herbicides on ectomycorrhizal development and seedling growth of lodgepole pine and white spruce under controlled and field environment. Eur J For Pathol 20:77–94

Smith JE, Johnson KA, Cázares E (1998) Vesicular mycorrhizal colonization of seedlings of Pineaceae and Betulaceae after spore inoculation with *Glomus intraradices*. Mycorrhiza 7:279–285

Smith JE, Molina R, Perry DA (1995) Occurrence of ectomycorrhizas on ericaceous and coniferous seedlings grown in soils from the Oregon Coast Range. New Phytol 129:73–81

Smith FA, Smith SE (1996) Mutualism and parasitism: diversity in function and structure in the 'arbuscular' (VA) mycorrhizal symbiosis. Adv Bot Res 22:1–43

Smith SE, Read DJ (1997) *Mycorrhizal symbiosis*. London, UK: Academic

Soil Survey of Wellington County. (1963) Report N$^{o}$ 35 of the Ontario Soil Survey. Ottawa, Ontario: Department of Agriculture

van Aarle IM, Cavagnaro TR, Smith SE, Smith FA, Dickson S (2005) Metabolic activity of *Glomus intraradices* in *Arum*- and *Paris*-type arbuscular mycorrhizal colonization. New Phytol 166:611–618

Yu TEJ–C, Egger KN, Peterson RL (2001a) Ectendomycorrhizal associations—characteristics and functions. Mycorrhiza 11: 167–177

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