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SHORT NOTE

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Occurrence of vesicular-arbuscular mycorrhizae in *Pseudotsuga menziesii* and *Tsuga heterophylla* seedlings grown in Oregon Coast Range soils

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Abstract Vesicular-arbuscular mycorrhizae (VAM) were common in seedlings of *Pseudotsuga menziesii* and *Tsuga heterophylla* grown in a greenhouse soil bioassay in soils collected from the Oregon Coast Range. Although root samples were heavily colonized by ectomycorrhizal fungi (EM), VAM colonization was observed in the cortical cells of both secondary and feeder roots. Vesicles, arbuscules, and hyphae typical of VAM occurred in 48% of 61 *P. menziesii* and 25% of 57 *T. heterophylla* seedlings. The ecological significance of VAM presence in the Pinaceae, as well as interactions among VAM, EM, and the plant host, deserve future investigation.

Key words Vesicular-arbuscular mycorrhizae · Pinaceae · *Pseudotsuga menziesii* · *Tsuga heterophylla* · Soil bioassay

Introduction

Vesicular-arbuscular mycorrhizae (VAM) develop on 60–80% of the plant species of the world, including bryophytes, pteridophytes, gymnosperms and angiosperms (Bonfante-Fasolo 1987; Trappe 1987). Pinaceae typically form ectomycorrhizae (EM), although vesicular endophytes have been reported in Pinaceae seedlings. Cázares and Trappe (1993) recently reported *Glomus*-type vesicles and hyphae, but no arbuscules, in seedlings of *Abies lasiocarpa* (Hook.) Nutt., *Pseudotsuga menziesii* (Mirb.) Franco, *Tsuga heterophylla* (Raf.) Sarg. and *T. mertensiana* (Bong.) Carr. collected from the northern Cascade Range in Washington. Other descriptions of vesicles and hyphae characteristic of "endophytic mycorrhizae" in Pinaceae occasionally have been reported in the last century (McDougall and Jacobs 1927; Henry 1933, 1934; Asai 1934; Dominik 1951; Shvartsman 1955; Golubinskaya 1967; Dowgiallo and Rambelli 1972; Malloch and Malloch 1981).

In this study, we report both vesicles and arbuscules in *P. menziesii* (Douglas fir) and *T. heterophylla* (western hemlock) seedlings grown in mixtures with *Gaultheria shallon* Pursh (salal) and *Rhododendron macrophyllum* G. Don (rhododendron) in controlled microcosms. The findings reported here are part of a broader study examining the presence of ectomycorrhizas on Ericaceae (Smith et al. 1995). The ecological significance of VAM in typically ectomycorrhizal hosts deserves future attention to determine whether this relationship enhances nutrient uptake and seeling establishment in natural ecosystems.

Materials and methods

Field procedures, seedling preparation and growth conditions

Soils were collected from three sites of young (10- to 12-year-old) Douglas fir plantations in the Oregon Coast Range. Ten soil samples were collected from each site. Soil samples were kept separate. Soils were mixed 3:1 by volume with perlite to minimize compaction. Two 7-l pots were filled with each soil sample. Two control pots for each of the three sites were prepared by use of an autoclaved mixture of soil from the 10 sample points on each site to detect mycorrhizal contaminants from the greenhouse. A total of 66 pots were set up in the greenhouse at Oregon State University. Procedures of soil collection and vegetation composition for the sites are reported in Smith et al. (1995).

About 10 weeks before soils were collected, seeds of Douglas fir and western hemlock were surface sterilized in 30% H_2O_2 for 50 min, and seeds of rhododendron and salal were surface sterilized in 15% H_2O_2 for 15 min. Seeds were rinsed with deionized water and then sown in a mixture of 1:1 vermiculite and perlite in the greenhouse. Most seeds germinated in 10 days. After 8 weeks, four seedlings, one of each species, were transferred and grown together for 8–10 months in the pots of field soil.

Seedlings were grown under sodium-vapor lamps with a 16-hr light/8-hr dark period complementing natural light and providing

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a minimum photon flux density of 280 $\mu mol \cdot s^{-1} m^{-2}$. Air temperature fluctuated between 16 and 25 °C. Seedlings were watered once or twice weekly with tap water during the first 3 months and thereafter every 7–10 days. Each pot was fertilized three times in the first 8 weeks with 400 ml of Peters fertilizer (N – P – K/473 ppm – 449 ppm – 426 ppm) amounting to 189 mg N, 180 mg P, and 170 mg K.

Clearing and staining

Pinaceae seedlings from each pot were examined. A representative sample consisting of 3–5 entire root lengths were randomly selected from each root system that previously had been washed free of soil in running tap water. Roots were cleared and then stained in a solution of 0.05% trypan-blue in lactoglycerol according to methods described by Cázares and Trappe (1993) and modified from Phillips and Hayman (1970).

Assessing VAM colonization

VAM colonization was determined by use of stereo- and compound microscopy and a modification of a nonsystematic method developed by Kormanik and McGraw (1982). The percentage of root length colonized from the random sample was quantified as follows: 1–25%, 26–100%, and complete absence of vesicles/arbuscules. In a few cases, *Glomus*-type hyphae were apparently present but vesicles and arbuscules were absent, so to avoid overestimating these endophytes, we chose presence of either or both vesicles and arbuscules and the colonization criterion. Chi square tests were used to statistically compare presence or absence of VAM on Pinaceae between species and between sites.

Results

A total of 118 seedlings (61 Douglas fir, 57 western hemlock) was examined for the presence of VAM (Table 1). Colonization differed between species ($P=0.03$) but not between soils from the three sites. Forty-eight percent of the Douglas fir (95% confidence interval is 35–60%) and 25% of the western hemlock (95% confidence interval is 13–36%) seedlings were colonized.

Vesicles, arbuscules and nonseptate hyphae occurred in the cortical cells of both secondary and ectomycorrhizal feeder roots; vesicles were more common than arbuscules. VAM fungi often were the only fungi seen in the cortical cells, but EM and dark septate endophytes frequently were present in the same root. Vesicles and hyphae were seen in the cortical cells beneath the EM mantle. With the exception of one Douglas fir seedling, VAM colonization for all seedlings was recorded in the 1–25% category range.

Table 1 Percentage of Douglas fir and western hemlock seedlings with *Glomus*-type vesicles and/or arbuscules in their root systems

Taxon	Vesicle/arbuscule status[a]			
	1–25%	26–100%	0%	*n*
Douglas fir	46	2	52	61
Western hemlock	25	0	75	57

[a] Percentage of root sample length colonized by *Glomus* vesicles or arbuscules

Discussion

The occurrence of both EM and VAM in individual tree genera is well known. Harley and Smith (1983) reported that species of *Acacia, Casuarina, Cupressus, Juniperus, Populus, Salix, Tilia* and *Ulmus* could form either EM or VAM. Molina et al. (1992) list 42 plant genera known for their ability to form both EM (or ericoid) and VAM. Succession from VAM to EM in the same root system has been described for *Helianthemum* (Read et al. 1977), *Eucalyptus* (Lapeyrie and Chilvers, 1985; Chilvers et al. 1987) and *Alnus* (Molina et al. 1994). We have no evidence that VAM/EM succession occurred in our Douglas fir and western hemlock seedlings, but we suppose that VAM fungi penetrated the cortical cells before the EM fungi mantled the root surface. A sequential harvest scheme is needed to address this question.

The ecological importance of VAM presence in the Pinaceae as well as interactions between VAM, EM and the plant host are not clear. Lapeyrie and Chilvers (1985) suspect that predominantly EM tree species may be capable of brief VAM episodes in the seedling stage and the VAM may be important to the early establishment of plants in low nutrient or calcareous soils. Chilvers et al. (1987) considered VAM fungi well adapted to rapid primary colonization and perpetuation within individual roots but inferior to EM fungi for secondary colonization because of slow hyphal spread via root branches.

Nearly twice as many Douglas fir as western hemlock seedlings were colonized by VAM in our study. Kropp and Trappe (1982) suggest that hosts, such as hemlock, regenerating in the understory, adapt to the mycorrhizal system established by the overstory hosts. Because Douglas fir characteristically forms pure stands after a disturbance, we hypothesize that VAM fungi colonize typically EM hosts that establish early in areas where EM propagules are sparse or absent more readily than EM hosts that establish later in plant community succession.

Douglas fir and western hemlock genreally have been considered obligate EM hosts. EM root tips are relatively easy to assess without differential staining and by direct observation with a stereomicroscope. The presence of VAM and dark septate endophytes would be overlooked with this procedure. The presence of VAM in Douglas fir and western hemlock generates many questions about their presence and function in different habitats, associations with plants at certain ages, occurrence with other species of Pinaceae, and the ecological importance of different mycorrhizal types in forest ecosystems.

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