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Vesicular mycorrhizal colonization of seedlings of Pinaceae and Betulaceae after spore inoculation with *Glomus intraradices*

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Abstract Although Pinaceae and Betulaceae have been reported to contain *Glomus*–type root endophytes, its ecological importance and the conditions influencing this symbiosis are poorly understood. Seedlings of *Abies lasiocarpa*, *Alnus rubra*, *Pinus contorta*, *Pinus ponderosa*, *Pseudotsuga menziesii*, and *Tsuga heterophylla* were inoculated with *Glomus intraradices* to determine the vesicular-arbuscular mycorrhizae (VAM) development and responsiveness of these hosts. The role of companion VAM host plants on mycorrhizal colonization and nutrient uptake by *Pseudotsuga menziesii* was also examined by growing seedlings of *Pseudotsuga menziesii* in dual culture with VAM hosts *Thuja plicata* or *Calamagrostis rubescens*. After 8 weeks, no seedlings were colonized. At 16 weeks, 8 of 17 *Thuja plicata* seedlings grown with *Pseudotsuga menziesii* and all 18 inoculated *Thuja plicata* seedlings grown alone were colonized with vesicles and hyphae. Two of 17 inoculated *Pseudotsuga menziesii* seedlings grown in dual culture with *Thuja plicata* were colonized with abundant vesicles and hyphae. No ectomycorrhizal seedlings grown in monoculture were colonized. At 9 months, all 10 *Calamagrostis rubescens* and all 10 inoculated *Pseudotsuga menziesii* seedlings grown in dual culture were colonized by vesicles and hyphae. Two of 10 inoculated *Pseudotsuga menziesii*and 1 of 10 inoculated *Pinus ponderosa* seedlings grown in monoculture were similarly colonized. The mean phosphorus content in the needles of colonized *Pseudotsuga menziesii* seedlings grown with *Calamagrostis rubescens* was about twice as high as in noncolonized *Pseudotsuga menziesii*seedlings grown with *Calamagrostis rubescens*. Tissue nitrogen did not differ between these treatments. The results show that *Glomus intraradices* colonization of Pinaceae is most successful when a VAM host is present, although some vesicular colonization of Pinaceae occurred in the absence of a VAM host.

Key words Vesicular-arbuscular mycorrhiza · Pinaceae · Betulaceae · *Calamagrostis rubescens* · *Glomus intraradices*

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Introduction

The presence of vesicular-arbuscular mycorrhizae (VAM) in Pinaceae may be a common but overlooked phenomenon (Cázares and Trappe 1993). Descriptions of vesicles and hyphae characteristic of "endophytic mycorrhizae" in Pinaceae have been reported by McDougall and Jacobs 1927, Henry 1933, 1934, Asai 1934, Dominik 1951, Shvartsman 1955, Golubinskaya 1967, Dowgiallo and Rambelli 1972, and Malloch and Malloch 1981. 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 *Tsuga mertensiana* (Bong.) Carr. collected in the wild. Cázares and Smith (1996) described both vesicles and arbuscules in *Pseudotsuga menziesii*(Douglas-fir) and *Tsuga heterophylla*(western hemlock) seedlings grown in a greenhouse soil bioassay study. Johnson (1998) found an increased uptake of phosphorus in *Abies lasiocarpa* (subalpine fir) colonized by *Glomus*–type vesicles and hyphae compared to noncolonized *Abies lasiocarpa* in a greenhouse study. Despite recent reports of VAM in Pinaceae, their ecological significance in this typically ectomycorrhizal family remains largely unknown.

Similarly, although VAM increase growth and nutrient acquisition for some *Alnus* species, the prevalence and ecological, functional, or physiological importance of VAM on *Alnus rubra* Bong. (red alder) in Pacific Northwest forests is unclear (Molina et al. 1994). Although Rose (1980) reported VAM development on *Alnus rubra* growing in coastal Oregon and northern California forest habitats, Miller et al. (1992) found no VAM on *Alnus rubra* seedlings grown in six Oregon coastal forest soils. As *Alnus rubra* is an important commercial hardwood species in the Pacific Northwest, understanding its interaction with symbiotic fungi is important to future forest management.

The ecological importance of VAM or vesicular mycorrhizal (VM) colonization in typically ectomycorrhizal hosts deserves attention to determine whether this relation increases nutrient uptake and seedling establishment in natural ecosystems. The objectives of this study were (1) to determine whether different species of Pinaceae and Betulaceae can be inoculated and develop VAM from spores of *Glomus intraradices* Schenck & Smith in a controlled environment, (2) to determine the influence of typically VAM host species on colonization of neighboring Pinaceae hosts, (3) to assess the period of seedling development when host species become colonized by *Glomus intraradices*, and (4) to determine the effect of VAM colonization on *Pseudotsuga menziesii* tissue nutrients such as phosphorus and nitrogen.

Materials and methods

Inoculation

In two experiments, a mixture of 0.4 g of Nutri-link inoculum (NPI, Salt Lake City) in a dried-clay carrier containing about 500 *Glomus intraradices* spores/g was placed in a central column (about 150 mm long, 5 mm diameter) in the growing medium beneath surface-sterilized seeds. A glass rod was inserted into the moistened growing medium to create the hollow column for adding the inoculum. An equal amount of dried-clay carrier containing no spores was placed similarly beneath noninoculated controls.

Seed germination and growing conditions

In experiment 1, seeds of *Pinus contorta* Dougl. (lodgepole pine), *Pinus ponderosa* Laws. (ponderosa pine), and *Pseudotsuga menziesii* were surface sterilized in 30% hydrogen peroxide for 45 min, *Abies lasiocarpa* and *Tsuga heterophylla* for 20 min, and *Alnus rubra* and *Thuja plicata* Donn. (western red cedar) for 10 min, then spread to dry on paper towels and cold stratified at 4 °C for 1 week. Seeds were then placed in Supercells (160 ml capacity) (Stuewe & Sons, Corvallis) filled to 2.5 cm from the top with pasteurized Willamette sandy loam (pH 6.9) containing 0.06% total nitrogen, 5 mg kg^{-1} phosphorus, 55 mg kg^{-1} potassium, 4.8 mequiv magnesium, and 10.9 mequiv calcium per 100 g of soil. Seeds were planted in each container and covered with a thin layer of white quartz sand (8 grade). Most seeds germinated between 15 and 18 days. *Tsuga heterophylla* germinated in 28–31 days. Seedlings were watered twice weekly with deionized water. Four-week-old seedlings were fertilized weekly with 10 ml Long Ashton nutrient solution (Hewitt 1966) modified to 1/4 strength phosphorus (11 ppm) to promote VAM colonization. Seedlings were harvested at 8 and 16 weeks after germination.

In experiment 2, seeds of *Alnus rubra*, *Pseudotsuga menziesii*, *Tsuga heterophylla*, and *Pinus ponderosa* were aerated in Captan (1 tablespoon/gallon) (Zeneca, Wilmington) for 15 h, then stored in plastic bags at 4 °C for 10 days. *Calamagrostis rubescens* Buckl. (pinegrass) seed was aerated in Captan for 30 min and sown concomitantly with the other seeds with equal amount by volume of *Calamagrostis rubescens* seed (about 50 seeds) in each container. Seeds were placed in Supercells filled 2.5 cm from the top with vermiculite and perlite in equal parts by volume. Seeds germinated in 12–14 days. Seedlings were watered as described in experiment 1 and fertilized 5 times with 20 ml Long Ashton nutrient solution, modified as described in experiment one, at about 6-week intervals, beginning when plants were 6 weeks old. Seedlings were harvested 9 months after germination.

Seedlings in both experiments were grown in a growth room under artificial Gro-lux light (Sylvania, Hillsboro) at an average of 96.5 $\mu mol/m^{-2}$ sec^{-1} with a 16-h photoperiod. Air temperature was 20–22 °C throughout the whole experiment.

Experimental design

The *Pseudotsuga menziesii* component of experiment 1 was a 2 × 2 factorial design consisting of four treatments: noninoculated plants grown in monoculture, spore-inoculated plants grown in monoculture, noninoculated plants grown in dual culture with *Thuja plicata* and spore-inoculated plants grown in dual culture with *Thuja plicata*. Eighteen replicates were planted for each treatment and harvest time; treatments were harvested at 8 and 16 weeks. Additionally, 18 replicates of inoculated and noninoculated *T. plicata* were grown in monoculture to determine inoculum viability. An inoculation treatment and a noninoculation treatment were applied to all other species in a complete randomized design with 8 replicates in each treatment.

The *Pseudotsuga menziesii* component of experiment 2 was a 2 × 2 factorial design again of four treatments: noninoculated plants grown in monoculture, spore-inoculated plants grown in monoculture, noninoculated plants grown in dual culture with *Calamagrostis rubescens*, and spore-inoculated plants grown in dual culture with *Calamagrostis rubescens*. Ten replicates were planted for each treatment. Additionally, 10 replicates of inoculated and noninoculated *Calamagrostis rubescens* were grown alone to determine inoculum viability. Inoculation and noninoculation treatments were applied to all other species in a complete randomized design with 10 replicates of each treatment. Because space was limited in the growth room, it was not possible to test all species in dual culture with a VAM host.

Clearing and staining

Entire root systems were cleared then stained in a solution of 0.05% trypan-blue in lactoglycerol according to methods described by Cázares and Trappe (1993) based on a modification of Phillips and Hayman (1970).

Assessing VAM colonization

Vesicular-arbuscular mycorrhiza colonization was determined by stereo- and compound microscopy. In experiment 1, each root system was assessed for the presence or absence of vesicles and arbuscules. In experiment 2, each 5-mm section of seedling root systems was systematically examined according to the method described by Johnson (1998) with the exception that the presence of vesicles or arbuscules, but not hyphae, was quantified. This method of assessing percentage of colonization represents the percentage of segments in a root colonized by the fungus, but does not provide a complete estimate of the percentage of root length col-

onized because not all segments were entirely colonized. It does, however, reveal the distribution of colonization throughout the root system (Read et al. 1976). *Glomus* hyphae were present in some segments where vesicles were absent and difficult to detect in lignified roots. The presence of vesicles or arbuscules rather than hyphae was used to determine colonization so as to avoid overestimating the presence of *Glomus* endophytes (Cázares and Trappe 1993).

Presence of vesicles or arbuscules in *Calamagrostis rubescens* was visually estimated by a modification of the nonsystematic method developed by Kormanik and McGraw (1982). The percentage of roots colonized was converted into abundance classes as follows: 1:1–25% colonization; 2:26–50%; 3:51–75%; 4:76–100%.

Biomass and leaf tissue analyses

Roots and shoots were separated, dried for 48 h at 70 °C, and weighed to 0.01 g. *Pseudotsuga menziesii* needles from all seedlings in the four treatments and *Calamagrostis rubescens* blades from the two treatments with *Pseudotsuga menziesii* were removed for analysis of percentage phosphorus and nitrogen with the microkjeldahl digest method (Bremner and Mulvaney 1982).

Statistical analysis

Visual examination of the data for root and shoot biomass and foliar concentration of phosphorus and nitrogen revealed that distributions for percentage phosphorus in *Pseudotsuga menziesii* and root biomass in *Calamagrostis rubescens* were not normally distributed, and variability was not constant. A square root transformation for percentage phosphorus in *Pseudotsuga menziesii* and a natural log transformation for root biomass of *Calamagrostis rubescens* stabilized the variance and produced more symmetric distributions for the analysis. Root and shoot biomass and foliar concentration of nitrogen and phosphorus measured on *Pseudotsuga menziesii*, and also measured on *Calamagrostis rubescens*, were compared among treatments by use of analysis of variance (ANOVA). When overall differences were detected, between-treatment differences were determined with Fisher's protected least significant difference test at $\alpha = 0.05$.

Chi-square tests were used to compare colonization percentage of *Calamagrostis rubescens* by VAM between cultures of *Calamagrostis rubescens* only, with spores, with *Pseudotsuga menziesii* and spores, and with *Pseudotsuga menziesii*.

Results

Colonization results

In experiment 1, no seedlings harvested at 8 weeks were colonized. At 16 weeks, 8 of 17 inoculated *Thuja plicata* seedlings grown with *Pseudotsuga menziesii* and all 18 inoculated *Thuja plicata* seedlings grown alone were colonized with vesicles and hyphae. Two of 17 inoculated *Pseudotsuga menziesii* seedlings grown in dual culture with *Thuja plicata* were colonized with vesicles and hyphae. No ectomycorrhizal species grown in monoculture were colonized.

In experiment 2, vesicles and hyphae were abundant throughout the root systems of all *Calamagrostis rubescens* seedlings as well as in all 10 inoculated *Pseudotsuga menziesii* seedlings grown in dual culture with *Calamagrostis rubescens*. Vesicular mycorrhiza colonization generally did not occur in seedlings grown in the absence of a VAM host. Vesicles and hyphae were present, however, in 2 of 10 *Pseudotsuga menziesii* and 1 of 10 *Pinus ponderosa* seedlings grown alone. Colonization occurred in the upper unbranched portion of the main root; three vesicles also were detected in a primary root of the *Pinus ponderosa* seedling, about 30 mm from the root collar. No VAM colonization was detected in *Tsuga heterophylla* or *Alnus rubra* seedlings.

Ninety percent of the *Pseudotsuga menziesii* seedlings grown with *Calamagrostis rubescens* were >25% colonized, and 30% were >50% colonized. The highest colonization percentage was 65% and the lowest 18%. Percentage colonization was greater in primary roots than in laterals. Ninety percent of the seedlings had >25% primary root colonization, whereas only 30% of the seedlings had >25% lateral root colonization. Although the upper unbranched portions of the 10 root systems were 100% colonized, vesicular colonization typically was distributed throughout the root systems. Forty percent of the seedlings, however, had no lateral root colonization.

Most (13/20) of the *Calamagrostis rubescens* seedlings were in the second colonization abundance class (26–50% colonization), 20 and 15% were in the first and third colonization abundance classes, respectively. There was no difference in colonization of *Calamagrostis rubescens* between mono- and dual-culture treatments.

No vesicles, arbuscules, or hyphae were detected in the noninoculated treatments of either experiment. No arbuscules were observed in colonized seedlings. Ectomycorrhizal colonization was not detected.

Foliar nutrient and biomass results

In experiment 2, phosphorus concentration in *Pseudotsuga menziesii* differed significantly among treatments ($P = 0.0001$) (Table 1). Phosphorus concentration was significantly greater in plants grown alone than in plants grown with *Calamagrostis rubescens*. Mean phosphorus concentration is estimated to be two times greater in the inoculated *Pseudotsuga menziesii* seedlings when grown in dual culture with *Calamagrostis rubescens* than in the noninoculated *Pseudotsuga menziesii* seedlings (95% confidence interval for the difference between means on back-transformed data: 1.6–4.5 times, $P \leq 0.05$). Phosphorus concentration in *Calamagrostis rubescens* grown with *Pseudotsuga menziesii* did not differ between the inoculated and noninoculated treatments (Table 2).

Nitrogen concentration in *Pseudotsuga menziesii* differed significantly among treatments ($P = 0.0001$) (Table 1). Nitrogen concentration was significantly greater in plants grown alone than in plants grown with *Calamagrostis rubescens*. Nitrogen concentration, however, did not differ between inoculated and noninoculated seedlings grown with *Calamagrostis rubescens*. Nitro-

Table 1 Mean percentages of foliar phosphorus (back transformed) (% *P*) and nitrogen (% *N*), nitrogen to phosphorus ratios (*N/P*) mean dry weights (g) of roots and shoots, and root to shoot ratios of *Pseudotsuga menziesii* seedlings grown with and without *Calamagrostis rubescens* and inoculated with or without *Glomus intraradices* spores. Confidence intervals are shown in parentheses. Values followed by different letters are significantly different at $\alpha = 0.05$

	P. menziesii $n=10$	*P. menziesii* + spores $n=9$	*P. menziesii* + *C. rubescens* $n=10$	*P. menziesii* + *C. rubescens* + spores $n=10$
%P	0.114 a (0.098, 0.132)	0.073 b (0.051, 0.099)	0.006 c (0.003, 0.011)	0.018 d (0.010, 0.078)
%N	1.744 a (1.603, 1.885)	1.408 b (1.162, 1.654)	0.724 c (0.624, 0.814)	0.745 c (0.641, 0.849)
N/P	15.272	19.314	118.689	41.389
Root dry weight	0.111 a (0.093, 0.129)	0.105 a (0.084, 0.126)	0.053 b (0.044, 0.062)	0.050 b (0.041, 0.059)
Shoot dry weight	0.133 a (0.105, 0.161)	0.131 a (0.110, 0.152)	0.078 b (0.067, 0.090)	0.072 b (0.049, 0.095)
Root/shoot	0.835	0.802	0.680	0.694

Table 2 Mean percentages of foliar phosphorus (% *P*) and nitrogen (% *N*), nitrogen to phosphorus ratios (*N/P*), mean dry weights (g) of roots (back transformed) and shoots, and root to shoot ratios of *Calamagrostis rubescens* grown with *Pseudotsuga menziesii* and inoculated or not with *Glomus intraradices* spores. Confidence intervals are shown in parentheses. Values followed by different letters are significantly different at $\alpha = 0.05$ (*ND* not determined)

	C. rubescens $n=10$	*C. rubescens* + spores $n=10$	*C. rubescens* + *P. menziesii* $n=10$	*C. rubescens* + *P. menziesii* + spores $n=10$
%P	ND	ND	0.089 a (0.075, 0.103)	0.088 a (0.070. 0.106)
%N	ND	ND	1.433 a (1.274, 1.592)	1.364 a (1.140, 1.588)
N/P	ND	ND	16.101	15.500
Root dry weight	0.207 a (0.151, 0.284)	0.172 a (0.145, 0.203)	0.178 a (0.118, 0.270)	0.117 b (0.089, 0.155)
Shoot dry weight	0.334 a (0.293, 0.376)	0.296 a (0.257, 0.335)	0.321 a (0.266, 0.376)	0.284 a (0.233, 0.335)
Root/shoot	0.620	0.581	0.557	0.413

gen concentration in *Calamagrostis rubescens* grown with *Pseudotsuga menziesii* did not differ between the inoculated and noninoculated treatments (Table 2).

Shoot and root biomass of *Pseudotsuga menziesii* differed significantly among treatments ($P = 0.0001$) (Table 1). Root and shoot biomass was significantly greater for plants grown alone than for plants grown with *Calamagrostis rubescens*. Shoot and root biomass of *Pseudotsuga menziesii*, however, did not differ between inoculated and noninoculated plants grown alone or grown in dual culture with *Calamagrostis rubescens*. Root biomass of *Calamagrostis rubescens* differed significantly among treatments ($P = 0.03$) (Table 2). The median root biomass of noninoculated plants grown in dual culture with *Pseudotsuga menziesii* was estimated to be 1.5 times higher than the median root biomass for inoculated plants grown in dual culture with *Pseudotsuga menziesii* (95% confidence interval is 1.4–1.6 times, $P \leq 0.05$). Shoot biomass of *Calamagrostis rubescens* did not differ between inoculated and noninoculated treatments grown in dual culture with *Pseudotsuga menziesii* or between inoculated and noninoculated treatments of *Calamagrostis rubescens* grown alone.

Discussion

Spore inoculum of *Glomus intraradices* produced vesicles and hyphae in Pinaceae seedlings in mono- and dual culture with a VAM host plant. The general absence of colonization in monoculture treatments suggests that colonization of *Pseudotsuga menziesii* in the dual-culture treatments occurred via hyphae growing from the roots of colonized VAM hosts (*Thuja plicata* in experiment 1 and *Calamagrostis rubescens* in experiment 2). Root exudates of some host plants induce considerable hyphal proliferation in VAM fungi (Graham 1982; Bécard and Piché 1989; Giannazzi-Pearson et al. 1989; Nair et al. 1991; Giovanetti et al. 1993). Root-to-

root contact rather than spores has been suggested as the major source of VAM inoculation in grassland and deciduous woodland communities (Read et al. 1976). We cannot discount the possibility, however, that *Glomus* spore germination was stimulated by *Thuja plicata* or *Calamagrostis rubescens* exudates leading to subsequent colonization of *Pseudotsuga menziesii* roots by hyphae derived from spores. The presence of hyphae and vesicles in two *Pseudotsuga menziesii* and one *Pinus ponderosa* seedling grown in monoculture suggests that Pinaceae root exudates stimulate *Glomus* hyphae to penetrate to some extent. Johnson (1998) recorded similar colonization results in *Abies lasiocarpa* grown in monoculture and dual culture with *Calamagrostis rubescens*.

This study reveals variation in the colonization potential of *Glomus intraradices* depending on whether it is already linked to a compatible host. *Glomus intraradices* colonization of Pinaceae was most successful when a VAM host plant was present, although some colonization of Pinaceae occurred in the absence of a VAM host. Massicotte et al. (1994) determined that the presence of a companion plant affects the specificity of some ectomycorrhizal fungi, resulting in colonization of alternate host plants grown in dual culture with a companion plant but not when grown in monoculture. Species not colonized by VAM in this study and tested only in monoculture (*Pinus contorta*, *Alnus rubra*, *Abies lasiocarpa*, and *Tsuga heterophylla*) may have become colonized by VAM fungi if grown with VAM host plants. Indeed, Johnson (1998) recorded VM colonization in *Abies lasiocarpa* grown with *Calamagrostis rubescens*. *Alnus spp.* grown in monoculture, however, have been successfully inoculated with VAM fungi, thereby suggesting that factors other than the presence of companion plants contribute to VAM colonization of *Alnus* and perhaps other ectomycorrhizal species (Lee 1988; Chartarpaul et al. 1989; Russo 1989; Fraga-Beddiar and Le Tacon 1990).

Results of experiment 1 of this study and the study by Johnson (1998) indicate that tree seedlings become mycorrhizal at an early stage in their development. In experiment 1, VM colonization of *Thuja plicata* and *Pseudotsuga menziesii* occurred between 8 and 16 weeks. Johnson (1998) found abundant VM colonization in *Calamagrostis rubescens* and *Abies lasiocarpa* seedlings harvested at 14 weeks. Based on the report by Read et al. (1976) that grassland species can become colonized at the cotyledon stage, we speculate that the *Calamagrostis rubescens* in the study by Johnson (1998) became colonized earlier than 8 weeks, resulting in a greater percentage of colonized *Abies lasiocarpa* seedlings (100%) harvested at 14 weeks than *Pseudotsuga menziesii* seedlings (12%) grown with *Thuja plicata* and harvested at 16 weeks in experiment 1 of this study.

Differences in foliar nutrient concentrations and biomass between mono- and dual-culture treatments in experiment 2 of this study can be attributed to greater competition for available nutrients in the dual culture than monoculture microcosms. These striking differences between mono- and dual-culture treatments were not mirrored in the foliar nutrient concentrations in the study by Johnson (1998), probably because competition was a weaker factor at the 14-week harvest age in the study by Johnson (1998) than at the 9-month harvest age in this study. *Glomus intraradices* colonization of *Pseudotsuga menziesii* seedlings grown in dual culture and harvested at 9 months resulted in an estimated twofold increase in mean phosphorus levels of fir needles over that of the noninoculated *Pseudotsuga menziesii* grown in dual culture. Johnson (1998) recorded that *Glomus intraradices* colonization of *Abies lasiocarpa* seedlings grown in dual culture and harvested at 14 weeks led to a 10.5-fold increase in mean phosphorus levels of *Abies lasiocarpa* needles over that of noninoculated *Abies lasiocarpa* grown in dual culture.

Results of this study and the study by Johnson (1998) indicate an uptake of phosphorus in Pinaceae seedlings colonized by *Glomus* vesicles and hyphae only. The increased %P in the *Glomus*–colonized compared to noninoculated *Pseudotsuga menziesii* seedlings grown in dual culture contrasts with the %P decrease in *Glomus*–inoculated compared to noninoculated *Pseudotsuga menziesii* seedlings grown in monoculture. This suggests that colonization, and not simply the presence of the fungus, contributes to increased P uptake (Table 1). It appears that acid phosphatases released by the fungus into the substrate are not simply liberating P, which is then taken up in the absence of colonization.

There are many reports suggesting that arbuscules must be present to define a functional symbiosis (Hirrel et al. 1978; Parke and Linderman 1980; Malajczuk et al. 1981; Marx et al. 1982; Stasz and Sakai 1984; Giovannetti and Lioi 1990; Giovannetti et al. 1994). Arbuscules have shown strong association with phosphorus transport based on ATPase activity (Marx et al. 1982). However, Gianinazzi-Pearson et al. (1991) detected ATPase activity in the fungal plasma membranes of external and intercellular hyphae formed by *Glomus intraradices* in *Allium cepa* L. Smith and Smith (1990) also discussed the importance of ATPase activity and distribution in 'atypical' mycorrhizas lacking arbuscules or intercellular phases, and reports by Smith and Dickson (1991) and Smith et al. (1994) showed that fluxes of P across the hyphal interface are too large to be ignored. The reader is referred to Smith and Smith (1996) for a recent review of function and structure in VAM symbiosis.

In this study and the study by Johnson (1998), VM colonization did not to influence nitrogen uptake; foliar nitrogen concentrations did not differ significantly between the colonized and noncolonized treatments grown in dual culture with *Calamagrostis rubescens*.

Nutrient ratios within foliage are important indicators of nutrient interactions and balance that influence conifer tree growth (Ingestad 1979; Comerford and Fisher 1984; Perry 1994). A foliar nitrogen to phospho-

rus ratio greater than 14 or 15 indicates a sufficiency of nitrogen with respect to phosphorus (Comerford and Fisher 1984). Nitrogen to phosphorus ratios measured in the dual-culture treatments suggest that phosphorus was severely limiting in the *Pseudotsuga menziesii* seedlings. The nitrogen to phosphorus ratio for the colonized *Pseudotsuga menziesii* seedlings, although not optimal, was dramatically lower than in the noncolonized seedlings grown in dual culture with *Calamagrostis rubescens*. Interestingly, root biomass of *Calamagrostis rubescens* roots was significantly lower in the pots where *Pseudotsuga menziesii* was colonized than in the noncolonized treatment. Shoot biomass, nitrogen and phosphorus concentrations of the *Calamagrostis rubescens*, however, did not differ. The increased ability of the colonized *Pseudotsuga menziesii* seedlings to take up phosphorus as compared with the noncolonized seedlings may have adversely influenced *Calamagrostis rubescens* root growth.

Events triggering *Glomus* spore germination in temperate coniferous systems are poorly known. Cázares and Trappe (1993) observed greater VM colonization levels in Pinaceae seedlings collected from openings than beneath canopies and hypothesized that *Glomus*–type colonization of Pinaceae was influenced by a greater abundance of VAM fungal propagules and hosts in the openings. Phosphorus uptake by VAM may be important to typically ectomycorrhizal seedlings establishing in natural environments where ectomycorrhizal propagules are sparse or absent. Lapeyrie and Chilvers (1985) suspect that predominantly ectomycorrhizal *Eucalyptus* species are capable of brief VAM episodes in the seedling stage and that 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 ectomycorrhizal fungi for secondary colonization because of slow hyphal spread via root branches. Future studies are needed to determine the relative benefits to establishment and early growth conferred by VAM, VM, and ectomycorrhizas in typically ectomycorrhizal hosts.

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