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Growth and Colonization of Western Redcedar by Vesicular-Arbuscular Mycorrhizae in Fumigated and Nonfumigated Nursery Beds

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Western redcedar (Thuja plicata Donn ex D. Don) seedlings were grown in a bareroot nursery bed that had been fumigated with methyl bromide. Seedlings grown in fumigated beds were stunted and had purple foliage. Microscopic examination showed that roots from these seedlings were poorly colonized by mycorrhizae, and only by fine vesicular-arbuscular mycorrhizae. In contrast, roots from seedlings grown in non-fumigated beds had larger shoots and green foliage and were highly colonized by both fine and coarse vesicular-arbuscular mycorrhizae. Tree Planters' Notes 42(4):14-16; 1991.

Species of cypress (Cupressaceae) and yew (Taxodiaceae) that make up significant parts of the forest landscapes of northwestern North America are dependent on vesicular-arbuscular mycorrhizae (VAM) for good growth. These fungi are mutualistic root colonizers that take carbon (C) and energy from the host plant in exchange for soil nutrients, notably phosphorus (P), and water gathered by the soil hyphae. Many other kinds of mycorrhizae exist (Harley and Smith 1983), but forest nursery crops in North America only form either ectomycorrhizae or endomycorrhizae, which are also called VAM. Most pines form ectomycorrhizae with the characteristic mantle of fungal hyphae surrounding the fine feeder roots and the *Hartig net* of hyphae surrounding cortical cells. VAM are characterized by fungal storage organs called *vesicles* and minutely branched intracellular hyphae known as *arbuscules.* VAM come in two types: the more common coarse VAM's and the fine VAM's, which are more common in stressed environments, as discussed later.

Kough et al. (1985) inoculated incense-cedar *(Libocedrus decurrens* (Torn.)), redwood *(Sequoia sempervirens* (D. Don) Endl.), giant sequoia *(Sequoiadendron giganteum* (Lindl.), and western redcedar *(Thuja plicata* Donn ex D. Don.) with three different VAM. Positive growth responses of up to 20 times the nonmycorrhizal controls occurred under conditions of limited soil phosphorus. Incense-cedar, redwood, and giant sequoia seedlings in northern California nursery beds are routinely inoculated with *Glomus* sp. (Adams et al. 1990), as experience has shown that the absence of VAM after soil fumigation leads to phosphorus deficiency and poor growth.

When western redcedars in fumigated transplant beds at the British Columbia Ministry of Forest's Surrey Nursery began to show signs of phosphorus deficiency, a deficiency of mycorrhizal colonization was suspected. Many studies have demonstrated improved P status of VAM-inoculated plants (see Harley and Smith 1983). The objective of this study was to determine whether fumigation decreased VAM colonization.

Materials and Methods

Bareroot cedar nursery beds were fumigated in May 1986 with the typical rate of 390.18 kg/ha of methyl bromide to control strawberry root weevils *(Otiorhynchus ovatus* (L.)). In July 1987, 4-month-old containerized western redcedar seedlings from the same nursery were randomly planted in fumigated and adjacent nonfumigated beds. By May 1988, plants in the fumigated beds had purple foliage and smaller shoots than plants from nonfumigated beds.

In August 1988, 10 plants each were randomly collected from one fumigated and one nonfumigated nursery bed, wrapped in plastic and shipped to the University of British Columbia for analysis. Height and root collar diameter were determined on fresh shoots, which were then oven-dried at 70 °C for 48 h and weighed. All roots under 2 mm diameter were fixed in 50% aqueous formalin/acetic acid/ ethanol (90:5:5), then later cleared and stained by a modified method of Phillips and Hayman (1970). Colonization of these fine roots by VAM was examined under the dissecting microscope at magnifications of 8 to 25 x and categorized into six classes: 0 = none; 1 = very low (1 to 5% of total fine root length colonized); 2 = low (6 to 15%); 3 = medium (16 to 30%); 4 = high (31 to 50%), and 5 = very high (>50%).

Colonization by coarse VAM was determined under the dissecting microscope at 8 to 25 x , based on the presence of stained hyphae and arbuscules in roots and large vesicles. Stained hyphae in roots with arbuscules but no large vesicles were suspected of being fine VAM. To confirm presence of fine VAM (which are too small to see without high magnification) and the absence of coarse VAM and other types of root-inhabiting fungi, a minimum of five 2-cm-long mycorrhizal root segments per plant were mounted in lactic acid on slides and examined at 400 x . We differentiated the two VAM based on the following criteria: fine VAM species have hyphae 2 to 5 µm wide and vesicles up to 5 to 10 µm long; coarse VAM species form hyphae 5 to 10µm wide and vesicles up to 100 µm long.

Growth data were checked for normality and heterogeneity and analyzed statistically using the Student's *t*-test at $P < 0.05$ (Zar 1984) for differences between means of shoot diameter, height, and dry weight. Mycorrhizal colonization data were not normally distributed and so were analyzed using the X^2 test for nonparametric data at $P < 0.05$ (Siegel 1956).

Results and Discussion

Shoot diameter and shoot dry weight were significantly lower in seedlings planted in fumigated than in nonfumigated beds (table 1). Shoot height was lower, though not significantly so, in the fumigated treatment and might have been even more so if 3 seedlings in the nonfumigated plot had not had their leaders cut to control growth.

Mycorrhizal colonization of plants in the nonfumigated bed was significantly higher than in the fumigated bed ($X^2 = 81.6$). Only fine VAM were present in roots from the fumigated bed, whereas both fine and coarse VAM were present in 6 of the 10 plants from the nonfumigated bed. From this study, we do not know if the decreased plant growth resulted from changes in VAM colonization due to fumigation or from the lack of other beneficial soil organisms that were also killed. Residual soil toxicity from fumigation is not considered a problem in this case as a full year had passed between fumigation and planting. However, replacement of VAM fungi in fumigated bareroot nurseries has corrected similar poor growth of incense-cedar, giant sequoia, and redwood (Adams et al. 1990), and we believe that the same would be true with bareroot western redcedar.

Twenty-seven months after fumigation and 13 months after transplanting, almost all of the plants from the fumigated bed were mycorrhizal, though not always to the same extent as the seedlings from the nonfumigated bed (table 1). Based on other studies of containerized nurseries in British Columbia, we are confident that the plants were virtually nonmycorrhizal at transplanting even though we did not examine these plants. It appears that plants going into the nonfumigated bed were colonized shortly after transplanting, whereas those going into the fumigated bed were either not colonized until mycorrhizal propagules were reintroduced into the soil or colonized very slowly by a small number of surviving propagules. Of particular interest, however, is the apparent difference in colonization behavior of the fine and coarse VAM. If both VAM had been eradicated by the fumigation, then we can deduce, based on these data, that the fine VAM is a better recolonizer than the coarse VAM. On the other hand, it is possible that some propagules of the fine VAM survived fumigation.

We have found that fine VAM often dominate western redcedar roots under stressful or disturbed conditions (Berch et al. in prep.). Containerized seedlings from the MacMillan Bloedel Nursery at Nanaimo raised in peat-vermiculite mix were essentially nonmycorrhizal, but for the occasional plant

Table 1—*Mean shoot growth and root colonization of western redcedar in fumigated and nonfumigated beds at Surrey Nursery* (N = 10)

	Diameter (mm)	Height (cm)	Dry weight (g)	Mean root colonization class*	Type of endophyte
Fumigated	9.43 (± 2.10) a	62.7 (± 10.1) a	22.7 (± 10.4) a	3.3 a	F
Nonfumigated	12.42 (± 2.57) b	67.6 (± 6.60) a	43.1 (± 16.9) b	5.0 b	F or F + C

Numbers within columns with the same letter in common differ significantly ($P < .05$). Root colonization is expressed as mean colonization class, with 0 = none, 1 = 1 to 5%, 2 = 6 to 15%, 3 = 16 to 30%, 4 = 31 to 50%, 5 = 50%. Type of endophytes: F = fine only; F + C = fine and coarse. Values are ± 1 standard deviation.

* χ^2 Test of heterogeneity = 81.6 (critical value, $P < 0.05$ = 18.31).

with low levels of the fine VAM. After outplanting on sites pretreated with different slash-burn intensities, the majority of these young plants on the severely burned site were colonized by fine VAM alone or by both VAM. On the lower intensity burn site, the coarse VAM was dominant, which suggests that the fine VAM is better adapted to stressful conditions. Further substantiation of this comes from a pot bioassay in which soils were dried, ground, and, used as inocula to determine propagule density; in this test only fine VAM colonized the test plants (Mike Curran, Ministry of Forests, Nelson, BC, personal communication). This implies that fine endophyte propagules survive the rigors of drying and grinding better than coarse endophytes. Parke et al. (1983) also reported that western redcedar formed only fine VAM after inoculation with fresh, sieved forest litter or mineral soil.

Kough et al. (1985) reported that growth response to VAM inoculation in pots of incense-cedar, redwood, giant sequoia, and western redcedar generally decreased with seedling age up to about 11 months, which may reflect the depressing effects of small rooting volume. Our cedars were approximately 4 months old when transplanted and 17 months old when harvested, yet they still showed growth differences that may be due to colonization. This may reflect the difference in growth potential of plants in pots and plants in a bareroot nursery.

Recommendations

This study suggests that VAM fungi are important to the growth of bareroot western redcedar. Ideally, our observations should be verified in a study designed specifically for that purpose in which nonmycorrhizal redcedar seedlings would be planted into prefumigated soils and some would be inoculated with pure VAM inoculum free of other microorganisms that might affect plant growth. It would also be possible to examine the differences between fine and coarse vesicular-arbuscular mycorrhizae in terms of propagule survival of treatments such as soil fumigation and slash burning. This could be achieved in closed chambers, where airborne propagules are eliminated, if cedars were germinated in treated and untreated soils and allowed to form mycorrhizae with the VAM that survived treatment. Given the improved growth of redwoods at the Ben Lomond State Forest Nursery after inoculation with a VAM (Adams et al. 1990) and the fact that western redcedar in pots grows best when mycorrhizal (Kough et al. 1985, Parke et al. 1983), we can hypothesize that it grows best in bareroot nurseries when mycorrhizal. Soil transfer or application of commercial inoculum will result in the recolonization of sterilized seed beds. Because of their ability to improve nutrient status of seedlings, including western redcedar (Kough et al. 1985), it would be interesting to determine whether VAM could substitute wholly or in part for P fertilizer application in bareroot nurseries.

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