Use of VA Mycorrhizal Innoculum to Improve Growth of Forest Tree Seedlings in Fumigated Soil[1]

Tim Wood, Libby Nance, Steve Jedrzejek, and Greg Johnson[2]

Wood, Tim; Nance, Libby; Jedrzejek, Steve; Johnson, Greg; 1989. Use of VA Mycorrhizal Innoculum to Improve Growth of Forest Tree Seedlings in Fumigated Soil. In: Landis, T.D., technical coordinator. Proceedings, Intermountain Forest Nursery Association; 1989 August 14-18; Bismarck, ND. General Technical Report RM-184. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station: 58-59. Available at: http:www.fcnanet.org/proceedings/1989/wood.pdf

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Inoculation of fumigated nursery soils with VA mycorrhizal fungi improved the growth and quality of Russian olive, Sierra redwood, incense cedar and western red cedar seedlings in two West Coast forest nurseries. Experiments were used to identify superior fungal strains for use on acid, low-phosphorus soils. Commercial VA mycorrhizal inoculants, based on these strains, are under development.

INTRODUCTION

Forest nursery soils are often fumigated with methyl bromide to promote production of disease-free seedlings. Methyl bromide is a general biocide, and in addition to killing soil-borne pathogens, it eliminates beneficial vesicular-arbuscular (VA) mycorrhizal fungi. VA mycorrhizal fungi form symbiotic associations with the roots of some forest tree species (most notably redwoods, cedars, junipers and many broadleaf species), and they aid those plants in uptake of nutrients. Elimination of these fungi via fumigation can lead to plant nutrient deficiencies, stunting and crop loss, particularly on low-phosphorus and/or phosphorus-fixing soils.

The studies reported here compare the efficacies of several VA mycorrhizal fungi in promoting the growth and quality of tree seedlings on fumigated soils in two West Coast forest nurseries.

METHODS AND MATERIALS

In the spring of 1988, field trials were established at the California Department of Forestry nursery at Magalia, CA, and at the D.L. Phipps State Forest Nursery at Elkton, OR.[3] The soils at Magalia were acid clay loams (pH 5.0 - 5.3). They showed significant phosphorus (P) fixation capacities, and contained low levels of water extractable P (0.2 µg P/g soil as measured in 1:10 soil:water extracts). Elkton soils were acid sandy loams (pH 4.7 - 4.8). They showed moderate P fixation capacities, and contained variable levels of water soluble P (1.2 - 5.0 µg P/g soil).

Cultural practices and experimental procedures used at the two nurseries were similar. Seven months prior to the field tests, soils were fumigated with methyl bromide - chloropicrin mixtures at 393 kg/ha. In March, 1988, prior to seeding, ground was power harrowed and beds (1.2 m wide) were shaped. Beds were then measured off to delineate meter-long inoculation plots separated by 3-4 m non-inoculated buffers. Plots were laid out in complete randomized blocks with 4-5 repetitions per tree species x inoculation treatment.

Three strains of VA mycorrhizal fungi were chosen for the field tests at each nursery. Selections were based on superior performance in greenhouse trials involving acid soils. Inoculants consisted of colonized root fragments and fungal spores in a moist sand carrier. Each treatment plot was inoculated by incorporating 750 g of this material uniformly into the upper 15-20 cm of the soil. Non-inoculated control plots received 750 g of sand carrier only.

Following inoculation, beds were seeded using an eight-row seed drill. Russian olive (<u>Elaeagnus angustifolia</u>), Sierra redwood (<u>Sequoia gigantea</u>) and incense cedar (<u>Calocedrus decurrens</u>) were sown at Magalia. Sierra redwood, incense cedar and western red cedar (<u>Thuja plicata</u>) were sown at the Elkton nursery. Seedlings were grown using cultural practices standard to the two nurseries.

In April, 1989, the seedlings were harvested. Thirty plants from each plot were selected at random and were measured to determine height and caliper. In addition, 50 seedlings of each species were subjected to dimension analysis. Equations for estimating seedling dry weight and seedling root volume from caliper measurements were developed. For all species, regression equations took the form:

dry weight or root volume = m (caliper2.5)

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1. Paper presented at the Intermountain Nursery Association Conference, Bismark ND, August 14-18, 1989.

2. Tim Wood, Libby Nance, Steve Jedrezejek and Greg Johnson are Senior Scientist, Research Associate, Scientist and General Manager respec tively at NPI, 417 Wakara Way, Salt Lake City, UT 84108.

3. The authors would like to acknowledge the assistance of Bill Krelle and David Pilz who over saw site preparation and maintenance of the trials at the CDF and D.L Phipps nurseries respectively.

In all instances, correlation coefficients for these regression equations ranged from 0.80 to 0.98. Seedling dry weights and root volumes were then estimated for the 30 seedlings in each field plot using caliper data. Finally, for each tree species x inoculation treatment, means and standard deviations for seedling height, caliper, estimated dry weight and estimated root volume were calculated. Analyses of variance were run, and significant differences between inoculation treatments were determined using Tukey's *w* procedure, $p \leq 0.05$.

RESULTS

Inoculation of fumigated nursery soils with VA mycorrhizal fungi generally improved the growth of the tree seedlings tested. Importantly, there were significant differences in efficacy between fungal strains. Sample results are given in Tables 1 and 2.

Table 1: 1988 VAMF Strain selection trial with one-year Russian olive (E. angustifolia) seedlings. California Department of Forestry Nursery Magalia, Ca.

VAMF Strain No.	Seedling Height (cm)	Seedling Caliper (mm)
25	26.9 ± 6.7 [b]	2.8 ± 0.6 [b]
60	22.1 ± 5.0 [b]	2.6 ± 0.3 [b]
31	9.6 ± 4.8 [a]	1.4 ± 0.4 [a]
Control	9.0 ± 3.8 [a]	1.4 ± 0.5 [a]

VAMF Strain No.	Est. Seedling Dry neight(g)	Est. Seedling Root volume (ml)
25	3.0 ± 1.3 [b]	5.2 ± 2.3 [b]
60	2.1 ± 0.5 [ab]	3.6 ± 0.9 [ab]
31	0.6 ± 0.4 [a]	1.0 ± 0.7 [a]
Control	0.8 ± 0.9 [a]	1.3 ± 1.5 [a]

Mean separation within columns by Tukey's *W*-procedure ($p \leq 0.05$)

At the Magalia nursery, Strain 25, a Glomus intraradices isolate adapted to acid soils, proved to be superior. When applied to beds of Russian olive, it increased seedlings height growth threefold, caliper growth two-fold, and estimated seedling dry weight and root volume more than three-fold (Table 1). Strain 60 also improved the growth of Russian olive. Seedlings inoculated with Strain 31 showed no improvements over non-inoculated controls. Similar responses to inoculation were observed with Sierra redwood and incense cedar at the Magalia nursery, and in each case, Isolate 25 was the superior inoculant.

VA mycorrhiza inoculation also improved seedling growth at the Elkton nursery, and again Isolate 25 was superior. In comparison to noninoculated controls, incense cedar seedlings showed two-fold increases in height and caliper, and five-fold increases in estimated seedling dry weight and root volume when inoculated with this strain (Table 2). Isolates 31 and 54 also improved seedlings growth, but increases were only about half as large as those found with Strain 25. Similar responses to inoculation were found with Sierra redwood and with western red cedar at Elkton, and again, in each case, Isolate 25 produced superior seedling growth.

Table 2: 1988 VAMF Strain selection trial with one-year incense cedar (C. decurrens) seedlings. D.L Phipps State Forest Nursery Elkton, Or.

VAMF Strain No.	Seedling Height (cm)	Seedling Caliper
25	31.7 ± 0.9 [c]	4.5 ± 0.6 [c]
54	24.5 ± 2.5 [b]	3.4 ± 0.7 [b]
31	21.2 ± 4.0 [b]	3.2 ± 1.0 [b]
Control	14.6 ± 3.8 [a]	2.2 ± 0.5 [a]

VAMF Strain No.	Met. Seedling Dry weight (g)	Est. Seedling Root volume (ml)
25	3.9 ± 1.5 [c]	2.0 ± 0.7 [c]
54	2.2 ± 1.1 [b]	1.1 ± 0.6 [b]
31	2.0 ± 1.6 [ab]	1.0 ± 0.8 [ab]
Control	0.7 ± 0.3 [a]	0.4 ± 0.2 [a]

Mean separation within colums by Tukey's W-procedure ($p \leq 0.05$)

DISCUSSION AND CONCLUSIONS

Inoculation of fumigated nursery soils with VA mycorrhizal fungi significantly improved the growth of Russian olive, Sierra redwood, incense cedar and western red cedar in two West Coast forest nurseries. Several strains of fungi were compared, and in both nurseries and with all tree species tested, one isolate, a Glomus intraradices strain, was most efficacious. This fungus gave two-to-five-fold increases in seedling height, caliper, dry weight and root volume over non-inoculated control plants. Of the other isolates tested, some gave intermediate responses, and some yielded no improvements in seedling growth.

These results underscore the importance of strain selection in the development and use of mycorrhizal inoculants. The VA mycorrhizal fungi tested in this study showed little if any host specificity in the sense that a single isolate gave the superior growth responses across all three species tested. However, in testing more than 100 strains in greenhouse experiments, we have found these fungi to be specific to soil type, and in particular to soil pH. Some strains work well in neutral-to-basic soils (pH 6.5 and above), while others, like Strain 25 in this study, give superior plant growth on acid soils below pH 5.5. In all cases, inoculation with VA mycorrhizal fungi is most efficacious when soils have low levels of available phosphorus and/or significant phosphorus fixation capacities. Commercial VA mycorrhizal inoculants comprising the superior fungi identified in these studies, are now being developed for use in forest nurseries.