Arbuscular Mycorrhizal Inoculation Following Biocide Treatment Improves *Calocedrus decurrens* Survival and Growth in Nursery and Outplanting Sites

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Abstract: Commercial production of tree seedlings often includes various biocidal soil treatments for disease control. Such treatments can be effective in eliminating or reducing disease organisms in the soil, but may also eliminate non-targeted beneficial soil organisms, such as mycorrhizal fungi, that improve seedling performance, both in the nursery as well as the outplanted environment. The arbuscular mycorrhizal fungal (AMF) relationship has been verified for some important western coniferous species such as incense cedar (*Calocedrus decurrens* [Torr.] Florin), coastal redwood (*Sequoia sempervirens* [D. Don] Endl), and western redcedar (*Thuja plicata* J. Donne ex D. Don).

This study was designed to determine the response of incense cedar after soil fumigation with and without the addition of phosphorous fertilizer and a commercial mycorrhizal inoculant containing *Glomus intraradices*. Incense cedar seedling performance was monitored in both the nursery and outplanting environments.

At the nursery, non-mycorrhizal seedlings had significantly less foliar phosphorous levels even when phosphorous fertilizers were applied. Mycorrhizal inoculation at the nursery significantly improved height and seedling survival on treated plots. Seedlings from the nursery beds were then outplanted on 2 reforestation sites. Mycorrhizal inoculation at the nursery improved survival and growth of seedlings at the outplanted site.

Keywords: bareroot seedlings, nursery culture, outplanting performance

Introduction

Under natural conditions, most plants live in close beneficial association with soil microorganisms called mycorrhizal fungi. These fungi colonize plant roots and extend the root system into the surrounding soil to form an essential link between plant and soil environment. Mycorrhizal mycelia are extensions of the plant root system and are more effective in nutrient and water absorption than plant roots by themselves. The relationship is mutually beneficial because the fungus receives essential sugars and other compounds from the plant to fuel its activities and, in return, it increases plant nutrient and water uptake, increases plant resistance to disease, and extends protection against a wide variety of environmental extremes (Harley and Smith 1983; Allen 1991). All conifer species are known to form and be dependent upon the mycorrhizal relationship in their native habitats.

Commercial production of tree seedlings often includes various biocidal soil treatments for disease control. Such treatments can be effective in eliminating or reducing disease organisms, but may also eliminate non-target beneficial soil organisms, such as mycorrhizal fungi (Menge 1982; Trappe and others 1984; Kough and others 1985). Research has shown mycorrhizal fungi are critical to the uptake of water and nutrients and seedling survival across a wide range of host and field conditions (Jackson and others 1998; Miller and others 1998; Amaranthus and Steinfeld 2003; Steinfeld and others 2003). However, nursery conditions in which water and nutrients are amply provided can decrease the need and observed benefits of the mycorrhizal

relationship. This is especially true when phosphorous is readily available (Harley 1978; Browning and Whitney 1992). Numerous practitioners, however, have observed stunting and uneven growth of conifers following biocidal treatments even after soil analysis reveals adequate levels of soil fertility. Many of these cases of uneven growth and nutrient deficiencies following biocidal treatment have documented improved growth and nutrition when inoculated with the appropriate mycorrhizal fungus (Bartschi and others 1981; Parke 1982; Parke and others 1983). In these cases, poor growth of many conifer species, despite adequate soil fertilization, may be due to the coarse root systems lacking root hairs. Mycorrhizal fungi augment the root hairs by providing increased surface area and enzyme activity to release immobile soil nutrients, such as phosphorous, zinc, copper, and others (St John 1979).

Mycorrhizal fungi can profoundly affect seedling performance in the field by mediating nutrient and water uptake and protecting against environmental extremes in the narrow window for seedling establishment (Harley and Smith 1983; Steinfeld and others 2003; Amaranthus and others 2004b). A typical forest site generally contains many mycorrhiza-forming fungal species (Amaranthus and others 1996), but populations can be dramatically reduced or eliminated following site disturbance (Perry and others 1987; Amaranthus and Trappe 1993; Page-Dumroese and others 1998). Seedlings inoculated at the nursery with the appropriate mycorrhizal fungi *before* outplanting have the ability to more quickly assimilate site resources during the critical period of seedling establishment.

The arbuscular mycorrhizal fungal (AMF) relationship has been verified for some important western coniferous species, such as incense cedar (*Calocedrus decurrens* (Torr.) Florin), coastal redwood (*Sequoia sempervirens*), and western redcedar (*Thuja plicata*). This study was designed to determine the response of incense cedar after soil fumigation with and without the addition of phosphorous fertilizer and a commercial mycorrhizal inoculant. Incense cedar performance was monitored in both the nursery and outplanted environments.

Methods

Nursery

Uniform nursery beds were fumigated at the USDA Forest Service J Herbert Stone Nursery in Central Point, Oregon, in fall 1990. Four replicate plots of 4 treatments were installed with 1.0 m (3.3 ft) buffers separating plots. Four plots (2.0 m long by 1.25 m wide [6.6 by 4.1 ft]) were randomly assigned 1 of 4 treatments before sowing incense cedar seeds. Treatments were as follows:

1. **MYCO/no P**—Propagules of *Glomus intraradices* were added at a rate of 12,000 propagules/m^2 (1,100 propagules/ft^2), and no phosphorous fertilizer was added.
2. **MYCO/P**—Propagules of *Glomus intraradices* were added at a rate of 12,000 propagules/m^2 (1,100 propagules/ft^2), and phosphorous fertilizer was applied at a rate of 0.02 kg P_2O_5/m^2 (200 lb/ac [224 kg/ha]).
3. **No MYCO/no P**—No *Glomus intraradices* and no phosphorous were added to the plot.
4. **No MYCO/P**—No *Glomus intraradices* was added to the plot and phosphorous fertilizer was applied at a rate of 0.02 kg P_2O_5/m^2 (200 lb/ac [224 kg/ha]).

Mycorrhizal inoculum containing spores and root fragments of *Glomus intraradices* was produced on an inert clay carrier and added to the seedbed plots at the time of sowing in April 1991. Mycorrhizal propagule densities were determined using the sugar centrifugation spore extraction method and clearing and staining of the colonized root fragment techniques.

Seedlings of all treatments were grown under standard 1+0 seedling culturing practices. In winter 1991, seedlings were evaluated for stem diameter, height, seedbed density, and percent mycorrhizal colonization. Seedlings were stored in coolers at 1 °C (34 °F) until outplanting.

Outplanting Sites

Outplanting sites were 2 clearcut sites in the Illinois Valley Ranger District of the Siskiyou National Forest of southwest Oregon. Seedlings were planted on a west-facing slope (Site 1) and a south-facing slope (Site 2) in the Wood Creek drainage and at a mean elevation of 480 and 420 m (1,575 and 1,380 ft), respectively. Slope steepness ranged from 25 to 50%. Soils were fine-loamy mixed mesic Ultic Haploxeralfs, formed in colluvium derived from metavolcanic parent material of 80 to 120 cm (31 to 47 in) depth. Coarse fragments in the surface soil averaged 35%. Annual precipitation averages 210 cm (83 in), with more than 90% of it falling between mid-September and mid-May.

Outplanting sites were clearcut in winter of 1990, broadcast burned in fall of 1991, and outplanted with nursery study seedlings in spring of 1992. The fall broadcast burn intensity was severe, as all surface litter and duff layers, downed woody material less than 20 cm (8 in), leaves, and needles were completely consumed by the fire. Following the burn, bare mineral soil was exposed on 70 to 80% of the 2 clearcut sites.

Naturally reoccurring clumps of pioneering hardwoods—primarily the arbutoid or ectomycorrhizal Pacific madrone (*Arbutus menziesii* Pursh), chinkapin (*Castanopsis chrysophylla* [Dougl.] A. DC.), tanoak (*Lithocarpus densiflorus* [Hook. & Arn.] Rehd.), and California black oak (*Quercus kelloggii* Newb.), and the AMF western poison oak (*Rhus diversiloba* T. & G.)—were widespread across the 2 clearcuts.

Outplanting Procedure—In April 1992, 4 planting blocks of 10 by 10 m (33 by 33 ft) were established at each of the 2 clearcut test sites. Seedlings were sorted on the landing before outplanting to assure seedlings of similar size would be outplanted for each treatment. Each block was located entirely on the same aspect and slope. Sixteen incense cedar seedlings from each nursery treatment were arrayed in a 4 by 4 pattern with 0.5 m (1.6 ft) spacing between seedlings and 1.0 m (3.3 ft) spacing between treatments.

Plastic netting was placed around seedlings following outplanting to reduce browsing by deer. The stem diameter, 1 cm (0.4 in) above the soil surface, was recorded for each seedling at outplanting time. Seedling survival, stem diameter, and leader growth were measured for all surviving seedlings 14 months following outplanting.

Mycorrhizal Colonization—On each site, 2 seedlings per treatment and per replication were randomly selected for mycorrhizal colonization percentage at the time of lifting and 14 months after outplanting. Root systems were extracted from soil, taken to the laboratory, and gently washed free of soil and extraneous material. Arbuscular mycorrhizal colonization was determined by cutting fine root samples into segments that would fit handily in small capsules used for clearing and staining. Roots were cleared in 10% KOH solution, steamed 72 hours, rinsed with tap water, transferred to 1% HCL solution for 30 minutes, then rinsed again with tap water. Cleared samples were transferred into a staining solution of 0.5% trypan-blue in lactoglycerol, steamed for 60 minutes, rinsed with tap water, and stored in refrigerated cold water until microscopic examination. Cleared and stained root segments from each capsule were examined and tallied for the presence of arbuscular spores, vesicules, and arbuscules of mycorrhizal fungi using a dissecting microscope and sub-sample with the compound microscope. Counts were tallied on a graduated Petri dish.

Statistical Analyses

A statistical randomized block design and the analysis was performed utilizing ANOVA and Tukey's multiple range testing. Comparisons of nursery seedling stem diameter, height, seedbed density, foliar phosphorous content, and mycorrhizal colonization data were performed. Similarly, comparison of seedling stem diameter, height, survival, and mycorrhizal colonization data were compared by treatment for each of the 2 clearcut test sites.

Residuals from the data on stem diameter, height, and mycorrhizal colonization were plotted to determine if a log-normal transformation was necessary to compensate for log-normally distributed values. This indeed was the case, so the data were accordingly transformed to produce a relatively normal distribution (Steel and Torrie 1960).

Results

Nursery

Seedling heights, stem diameters, seedbed densities, mycorrhizal colonization, and foliar phosphorus levels after lifting in winter 2001 are shown in Figures 1 through 5. MYCO/no P seedlings had significantly greater mycorrhizal colonization compared to all other treatments ($P < 0.05$). MYCO/P seedlings had significantly greater mycorrhizal colonization and height growth compared to No MYCO/P and No MYCO/No P treatments. MYCO/No P and MYCO/P seedlings had significantly greater foliar phosphorous levels compared to No MYCO/P and No MYCO/No P treatments.

Outplanting Sites

Figures 6 through 9 show seedling heights, stem diameters, survival, and mycorrhizal colonization after 14 months on the outplanting sites. MYCO/no P and MYCO/P seedlings had significantly greater mycorrhizal colonization, stem diameter, and height compared to No MYCO/P and No MYCO/no P treatments at both clearcut sites ($P < 0.05$).

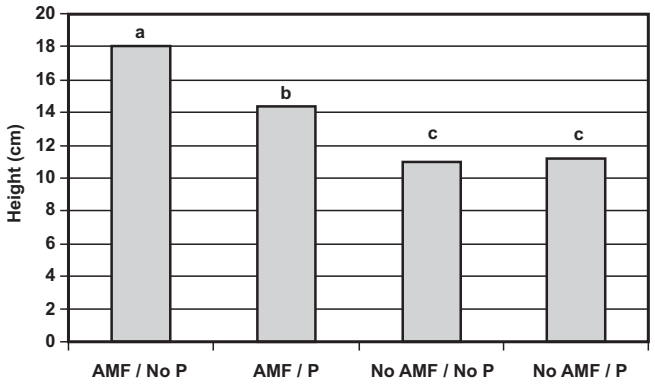

Figure 1—Height (cm) of *Calocedrus decurrens* seedlings grown at J Herbert Stone Nursery. Alpha symbols denote statistically significant results ($P < 0.05$).

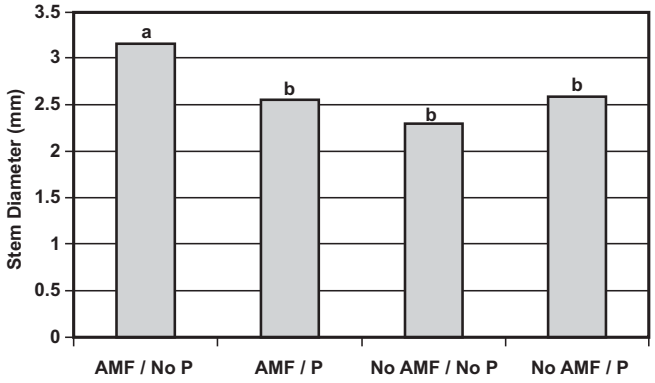

Figure 2—Stem diameter (mm) of *Calocedrus decurrens* seedlings grown at J Herbert Stone Nursery. Alpha symbols denote statistically significant results ($P < 0.05$).

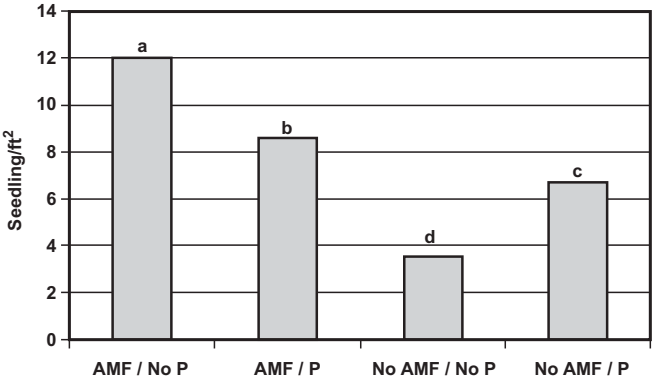

Figure 3—Seed bed density of *Calocedrus decurrens* seedlings grown at J Herbert Stone Nursery. Alpha symbols denote statistically significant results ($P < 0.05$).

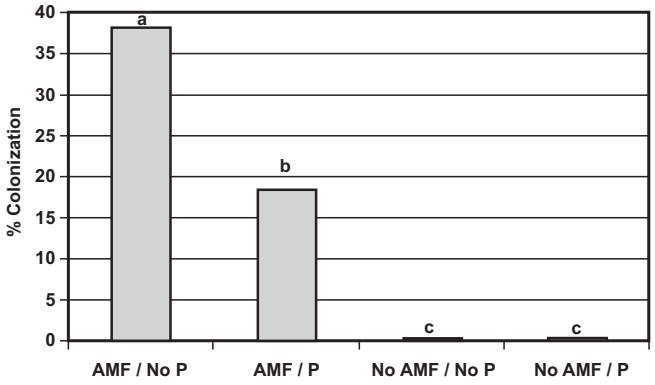

Figure 4—Percent mycorrhizal colonization of *Calocedrus decurrens* seedlings grown at J Herbert Stone Nursery. Alpha symbols denote statistically significant results ($P < 0.05$).

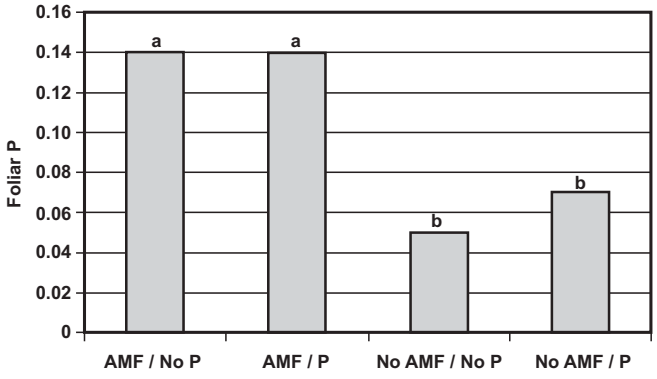

Figure 5—Percent foliar phosphorous (P) level of *Calocedrus decurrens* seedlings grown at J Herbert Stone Nursery. Alpha symbols denote statistically significant results ($P < 0.05$).

MYCO/no P seedlings had significantly greater survival percentage compared to No MYCO/P and No MYCO/No P treatments at both sites. MYCO/P seedlings did not survive significantly better than No MYCO/P seedlings at Clearcut Site 1 and No MYCO/no P seedlings at Clearcut Site 2. MYCO/No P had significantly greater height, survival, and mycorrhizal colonization than MYCO/P seedlings at Clearcut Site 1.

Discussion

In this study, both incense cedar growth and survival was influenced in both nursery and outplanting environments following AMF inoculation in fumigated nursery beds. Response was modified only slightly by the addition of phosphorous fertilizer at the nursery. Phosphorous addition in the MYCO/P treatment did significantly reduce the level of mycorrhizal colonization compared to the No MYCO/P treatment. However, even mycorrhizal colonization at 18% in the MYCO/P treatment was sufficient for the seedlings to significantly improve their growth performance

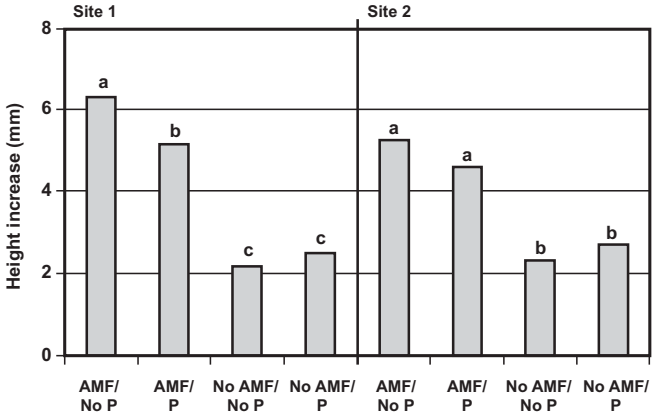

Figure 6—Height growth (mm) 14 months following outplanting. Alpha symbols denote statistically significant results ($P < 0.05$).

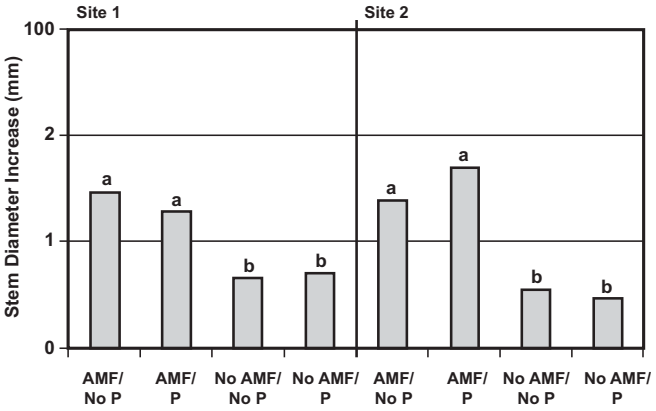

Figure 7—Stem diameter growth (mm) 14 months following outplanting. Alpha symbols denote statistically significant results ($P < 0.05$).

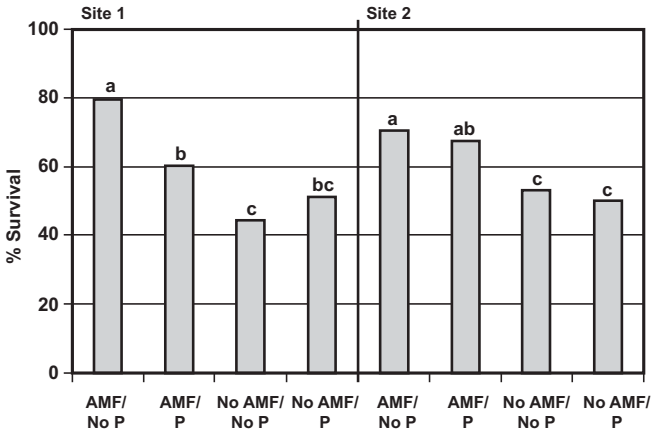

Figure 8—Survival percentage 14 months following outplanting. Alpha symbols denote statistically significant results ($P < 0.05$).

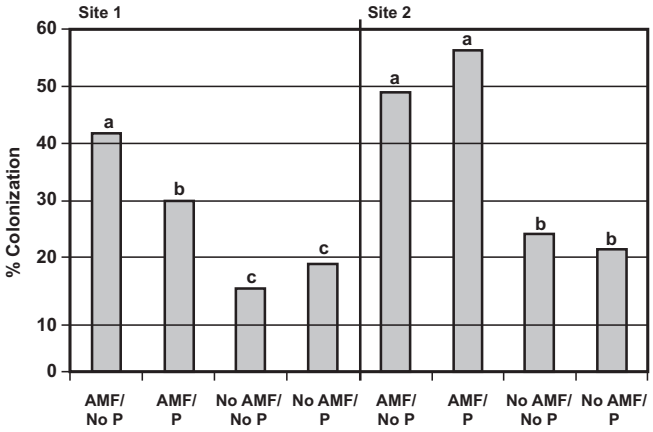

Figure 9—Mycorrhizal colonization percent 14 months following outplanting. Alpha symbols denote statistically significant results ($P < 0.05$).

and foliar phosphorous contents compared to the non-inoculated controls.

Young incense cedar seedlings inoculated and colonized with AMF clearly produce more uniform seedlings with improved height and bed density compared to No MYCO/P and No MYCO/No P seedlings. No MYCO/P and No MYCO/No P seedlings grew at lower densities and should have, as a result, greater stem diameter and height. In this study, the opposite was true—non-inoculated seedlings, which grew at low densities, had significantly less stem diameter and height.

Following fumigation, the addition of 0.02 kg P_2O_5/m^2 (200 lb/ac [224 kg/ha]) fertilizer should have provided enough phosphorous to the soil to saturate P-binding sites so that this essential nutrient would have been readily available to the roots. The bronzing effect and low foliar P level in the No MYCO/P treatment indicates that the higher level of phosphate was apparently inadequate for sufficient P uptake when incense cedar is non-mycorrhizal. The pre-existing soil phosphorous levels were adequate for MYCO/No P to have sufficient foliar P levels for adequate growth, even without the addition of P fertilizer.

At the seedling stage of plant growth, phosphorous uptake is presumably limited by the relatively small volume of soil occupied by root systems. AMF hyphae occupy a greater soil volume and produce specific enzymes for P extraction. In this study, the presence of AMF significantly improved seedling P nutrition at the nursery.

While phosphorous is generally very mobile in plant tissue, the only phosphorous reserve in young seedlings comes from the seeds themselves. As seed reserves become exhausted, the mycorrhizal association for P uptake is critical. Young seedlings, therefore, may be more responsive to mycorrhizal colonization than older plants. Although both young and old plants require and benefit from the mycorrhizal association, the survival and growth response may be more dramatic for younger plants because of their undeveloped root systems.

High levels of soil phosphorous have been shown to reduce or eliminate mycorrhizal colonization of conifer species (Harley and Smith 1983). Kough and others (1985) found, in a greenhouse study, that AMF-inoculated western redcedar, incense cedar, coastal redwood, and giant sequoia (*Sequoiadendron giganteum*) seedlings produced 100 to 2,000% more biomass than non-inoculated seedlings at low P (11 ppm), and from equality to a 500% increase at higher P (43 ppm). In their study, AMF inoculation enhanced seedling uniformity and size in all the tree species. Our results with incense cedar support their findings in the more operational environments of a production nursery and reforestation sites.

The increased survival and size of seedlings colonized by AMF mycorrhizae in our study has been reported on other host plants (Cooper 1981; Biermann and Linderman 1983; Kough and others 1985). The economic benefit after fumigation is clear—increased size and higher seedbed densities in the mycorrhizal-treated beds means more seedlings acceptable for outplanting.

At outplanting sites 1 and 2, results paralleled those at the nursery. MYCO/no P and MYCO/P seedlings grew better compared to No MYCO/P and No MYCO/No P seedlings. At the clearcut Site 1, however, the higher mycorrhizal colonization of MYCO/no P cedar seedlings at outplanting apparently improved their survival and height growth when compared to MYCO/P seedlings. Numerous other studies have shown the effectiveness of AMF in promoting plant nutrition and establishment on tree hosts (Graham and others 1982; Furlan and others 1983; Amaranthus and Trappe 1993; Pattinson 2001a).

Still other studies have examined the use of AMF inoculum to encourage the re-establishment of postfire native vegetation (Bellgard and others 1994; Rashid and others 1997; Pattinson and others 2001a,b). This study further supports the use of AMF inoculum on disturbed sites to encourage plant establishment and early conifer growth.

Timber harvest and site preparation are the 2 most common and widespread deliberate forest activities in the Pacific Northwestern United States. They significantly alter both the above and below ground environments. The outplanting test sites chosen for this study were severely disturbed by clearcutting and the intense fire that resulted from the fall prescribed burn, which likely reduced indigenous AMF populations. Other studies have shown reductions in AMF activity following vegetation removal and intense fire.

Fourteen months after outplanting, the No MYCO/P and No MYCO/No P groups still had significantly lower mycorrhizal colonization and less growth than MYCO/P and MYCO/No P treatments. The 2 clearcut sites were burned according to management prescription, and the intensity of the fire likely reduced the mycorrhizal colonization potential of the sites. Recent studies have examined the impact of wildfire and post-fire reestablishment (Vilarino and Arines 1991; Amaranthus and Trappe 1993; Bellgard and others 1994; Amaranthus and others 2004a). This study's data indicate the 2 clearcut and burned outplanting sites had lost their ability to rapidly form mycorrhizae for outplanted seedlings. Where the mycorrhizal forming potential of a site has been reduced, mycorrhizal inoculation following fumigation may allow seedlings to more rapidly acquire site resources in the outplanted environment.

Many foresters have observed a significant lag in the growth of cedar seedlings following outplanting. In this

study, mycorrhizal-inoculated incense cedar seedlings grew more rapidly in the field than non-inoculated nursery seedlings, and thus may be a vital tool to encourage rapid growth of AMF host seedlings.

Summary

Mycorrhizal inoculation with *Glomus intraradices* following fumigation of nursery soils greatly enhanced *Calocedrus decurrens* performance at both the nursery and outplanting sites. The response was modified only slightly by the addition of phosphorous fertilizer at the nursery. Phosphorous addition in the MYCO/P treatment significantly reduced the level of mycorrhizal colonization compared to the No MYCO/No P treatment. However, even with the additional phosphorus treatment at the nursery, the seedlings averaged 18% mycorrhizal colonization root system performance and foliar phosphorous contents when compared to the non-inoculated controls.

Young incense cedar seedlings inoculated and colonized with AMF clearly produced more seedlings with improved height and stem diameter compared to No MYCO/P and No MYCO/No P seedlings. After 14 months planted in the clearcut sites, incense cedar seedlings not inoculated at the nursery still had significantly less mycorrhzal colonization compared to nursery inoculated seedlings.

Increased nursery survival and seedling size are tangible economic returns for the production nursery. Increased field survival and growth are important goals for foresters on difficult sites. Nursery practices, such as using fumigants, may produce non-mycorrhizal seedlings that perform poorly upon outplanting, especially on sites where the period for seedling establishment is limited and native mycorrhizal colonization potential is low.

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