Nursery Soil Fumigation and Outplant Performance

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Abstract

Most bareroot reforestation nurseries in the Pacific Northwest use soil fumigation to maximize size and quality of seedlings and minimize disease. Root disease is hard to quantify at an operational level, and fumigation effects on seedling field performance are uncertain. We conducted three nursery trials that included standard methyl bromide + chloropicrin (MBC) and nonfumigated (control) treatments and evaluated subsequent performance either in the woods or in large containers. Despite differences in nursery culling rates, survival did not differ in the two studies in the woods. Initial size differences at planting persisted, however, with seedling stem volumes 37 and 45 percent greater for MBC compared with control seedlings for the 2009 and 2010 outplanting studies, respectively. In the third trial, potted seedlings were placed in two moisture regimes (high and low) with high (control) or low (MBC) levels of root pathogens. Survival did not differ after one growing season. Both moisture and pathogens influenced seedling morphology during the study. High pathogen treatments continued to have significantly higher levels of root pathogens at the end of the first growing season regardless of moisture regime and likely played a role in reducing first-season shoot and root volumes. This paper was presented at the Joint annual meeting of the Western Forestry and Conservation Nursery Association and the Pacific Northwest Reforestation Council (Corvallis, OR, October 11–12, 2017).

Introduction

Bareroot reforestation nurseries in the Pacific Northwest have relied for decades on the use of soil fumigants. Many growers use a combination of methyl bromide and chloropicrin to address soilborne insects, weeds, and pathogens. In addition to reducing the amount of pesticides applied during a crop rotation, soil fumigation prior to sowing or transplanting maximizes size, quality, and health of seedlings at harvest. Although morphology and physiology are relatively easy to assess at time of grading, pathology, particularly the level of root pathogens on a crop, is not. A challenge for nursery managers and foresters is to identify, beyond culling for failure to meet minimum size specifications and other observable defects, what impact root pathogens may or may not have on the performance of outplanted seedlings.

Added to this uncertainty is the increasing regulatory pressure on not only methyl bromide, identified as an ozone-depleting compound, but all commercially available soil fumigants (Enebak 2007, Masters 2005, EPA 2017). Buffer zone requirements have increased fumigation costs and, in some cases, restricted the use of fumigation entirely in increasingly suburban situations (Weiland et al. 2013). Many nurseries, to reduce buffer-zone limits, pay an extra expense for the contract fumigator to split applications to the same field on different dates.

Given the unknown fate of nursery soil fumigation, we wanted to investigate how the presence or absence of nursery soil fumigation impacts seedling outplant performance. Many studies have examined the relation that seedling size has on seedling performance in the woods. Stem diameter and its corollary root volume, in particular, are important in maximizing early survival and growth (for example, Rose et al. 1991). Relatively few studies, however, have looked at a gradient of root pathogens and subsequent outplanting success.

In a review of relevant literature, Dumroese and James (2005) found mixed evidence as to the impact of root disease on seedling performance. Several researchers (Axelrood et al. 1998, Dumroese et al. 1993, Smith 1967) found that nursery root diseases continued to be present in the woods but steeply declined during the initial years following outplanting and were found only on nursery-initiated roots.

Specifically, *Fusarium oxysporum*, a major nursery pathogen on many conifers, seemed to compete poorly with, and even be antagonized by, forest soil microorganisms.

Hansen et al. (1980) found that Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco) seedlings with severe root-disease symptoms had the highest mortality and poorest growth 18 months after planting, but seedlings with "inconspicuous symptoms" fared as well as healthy, control seedlings. Similarly, Dumroese et al. (2000) compared *Cylindrocarpon*-infected and noninfected western white pine (*Pinus monticola* ex D. Don), where both groups met packable specifications of firm root plug and minimum height and root collar diameter and found no differences in outplanting survival and growth.

Dumroese and James (2005) suggest that, unless obvious deficiencies are noted, seedlings meeting nursery standards for quality (morphological and physiological) should perform well on most outplanting sites even if root disease symptoms are present. Although root pathogens from the nursery may persist after outplanting, they compete poorly in the rhizosphere when new roots penetrate into forest soil. Nonetheless, seedlings with root disease symptoms should be given critical attention at grading and may need to be subjected to additional testing, such as a root growth potential assay (Dennis and Trotter 1998). Ideally, nurseries will use effective integrated pest management techniques to mitigate root pathogens and avoid potential pathogen-induced losses after outplanting. The series of studies described in this article examined the integrated pest management tool of nursery soil fumigation and its influence on seedling quality, root disease symptoms, and subsequent outplanting performance.

Materials and Methods

In two studies, initiated in 2009 and 2010, we evaluated seedling outplant performance from the nursery to the woods. In a third study initiated in 2017, we examined seedling performance after transplanting into large containers. For all three studies, we used 1+1 Douglas-fir seedlings grown in replicated nursery fumigation trials at Webster Nursery (Latitude 46.949, Longitude -122.952, slightly south of Olympia, WA). For all three trials, we compared seedlings from methyl bromide + chloropicrin (MBC; operational standard) versus no-fumigation (control) nursery treatments. For more information regarding the nursery fumigation trials, see Khadduri (2010), Khadduri et al. (2017), and Weiland et al. (2011).

Study 1: 2009 Nursery to Woods Evaluation

In late January 2009, we lifted bareroot 1+1 Douglas-fir seedlings grown with or without nursery fumigation (methyl bromide:chloropicrin 67:33 350 lb/ac [392 kg/ha] tarped with high-density polyethylene [HDPE] plastic), culled out those that did not meet minimum specifications, and stored the rest at 34 °F (1.5 °C) for 5 weeks prior to planting. The day before planting, we randomly sampled 20 seedlings per treatment for pathology analysis conducted by Robert James (U.S. Department of Agriculture, Forest Service, retired, Vancouver, WA). For pathology methods, see Khadduri (2010). Table 1 details cull, morphology, and pathology parameters.

On March 6, 2009, we planted 60 seedlings from each treatment on 2 sites (table 2, figure 1), in a randomized complete block design with 4 rows of 15 trees per treatment at each site. We interplanted an additional 20 trees per treatment (5 within each row) for destructive pathology sampling in the first season. We incorporated replications from the nursery trial plots in the field design. We spaced seedlings 8 ft (2.4 m) within rows and 10 ft (3 m) apart between rows (figure 2).

On June 6 and October 10 of the first growing season, we destructively sampled five interplanted trees per replicate and sent to Robert James' laboratory for analy-

Table 1. Douglas-fir seedling parameters for those grown with and without fumigation used in the 2009 nursery to woods evaluation. Morphology and pathology data are only for packable seedlings, i.e., seedlings that were not culled at time of harvest.

Seedling parameter	Methyl bromide fumigation	No fumigation
Cull rate at nursery harvest (%)	4.5	14.7
Average height at outplant (cm)	49.4	42.6
Average stem diameter at outplant (mm)	7.7	7.1
Fusarium root infection end of storage (%)	0	16
Pythium root infection (%)	4	19
Cylindrocarpon root infection (%)	0	32
Trichoderma (beneficial) colonization (%)	83	41

Table 2. Site locations for the 2009 and 2010 nursery to woods trials.

Year planted	Site	Latitude	Longitude
2009	Point Blank	46.78831	– 123.06697
2009	Coyote	46.95106	– 123.1337
2010	Norseman	46.83047	– 122.74584
2010	Silver Spring	46.90321	– 123.3429

sis. James examined 50 current-growth root pieces per replicate for *Fusarium/Cylindrocarpon* and *Trichoderma* spp. and 25 root pieces per replicate for *Pythium*.

We evaluated seedling survival through year 3 and height and basal stem diameter (and corresponding volume measurements) through year 7. We calculated stem volume using the volume of a cone: stem volume = π (diameter/2)^2(height/3).

Figure 1. Seedlings were planted on the (a) Coyote and (b) Point Blank units in March 2009 to evaluate nursery fumigation on field performance. (Photos by Lucy Winter, Washington Department of Natural Resources)

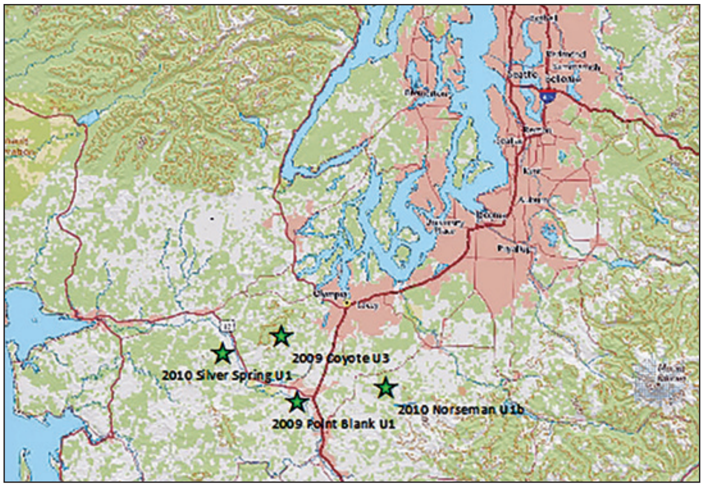

Figure 2. Four sites were planted on Washington Department of Natural Resources lands for the 2009 and 2010 outplant trials to compare seedlings grown with and without fumigation in the nursery.

Study 2: 2010 Nursery to Woods Evaluation

In early February 2010, we lifted bareroot 1+1 Douglas-fir seedlings from a separate trial and field, again evaluating the standard methyl bromide:chloropicrin 67:33 350 lb/ac (392 kg/ha) tarped with HDPE plastic treatment against a nontreated control. After culling, we analyzed seedlings for height, stem diameter, and shoot and root volume, as well as seedling roots for Fusarium and Pythium root infection at time of harvest (table 3; see Weiland et al. 2011 for pathology assessment details).

We planted seedlings on two sites (table 2) on March 16, 2010. We used the same experimental design as study 1, again incorporating nursery replicate plots to the field (figure 3). We did not track field pathology

Figure 3. Seedlings were planted on the Silver Spring unit in March 2010 to evaluate nursery fumigation on field performance. (Photo by Lucy Winter, Washington Department of Natural Resources)

Table 3. Douglas-fir seedling parameters for those grown with and without fumigation used in the 2010 nursery to woods evaluation. Morphology and pathology data are only for packable seedlings, i.e., seedlings that were not culled at time of harvest.

	Methyl bromide fumigation	No fumigation
Cull rate at harvest (%)	7	12
Average height at outplant (cm)	49	42.6
Average stem diameter at outplant (mm)	7.4	6.9
Shoot volume (cm3)	50	37
Root volume (g)	21.7	20.1
Fusarium root infection at harvest (%)	3	20
Pythium root infection at harvest (%)	1	5

for this study but again evaluated survival through year 3 and height and stem diameter (and corresponding volume measurements) through year 5.

Study 3: Nursery to Large Container Evaluation

In 2017, we again evaluated bareroot 1+1 Douglas-fir seedlings from a fumigation trial, comparing the current operational standard MB:pic 67:33 at 250 lb/ac (280 kg/ha), tarped with totally impermeable, or TIF, plastic (Raven Industries, Sioux Falls, SD) with a nontreated control. In contrast to the first two studies evaluated, cull rates and initial morphology were similar between treatments, despite differences in root pathology (table 4).

Table 4. Douglas-fir seedling parameters for those grown with and without fumigation used in the 2017 transplant evaluation. Morphology and pathology data are only for packable seedlings, i.e., seedlings that were not culled at time of harvest.

	Methyl bromide fumigation	No fumigation
Cull rate at nursery harvest (%)	4.3	5.6
Average height (cm)	52	50
Stem diameter (mm)	8.6	8.3
Fusarium root infection (%)	6	27
Cylindrocarpon root infection (%)	0	2

Figure 4. Seedlings were transplanted into large containers in the greenhouse to evaluate pathology and drought effects. (Photo by Nabil Khadduri, April 2017)

We lifted seedlings in early February 2017, then stored them at 34 °F (1.5 °C) for 7 weeks. On April 6, we transplanted seedlings into tall 1-gal (3.8-L) containers (CP512, Stuewe and Sons, Tangent, OR), containing a soilless medium mixture of 80:20 peat:perlite with a 4-to-6 month 18-12-6 N:P:K complete slow-release fertilizer. We tested two levels of pathology (low and high, corresponding to 2017 bareroot plots with and without fumigation; see table 4) and two levels of drought stress (wet and dry) in ambient greenhouse conditions (figure 4). Wet and dry treatments were achieved by allowing for block weights to drop to 70 or 50 percent, respectively, of saturated weight (volume/volume) before rewatering. In addition, we monitored seedlings in each treatment with a plant moisture stress chamber (PMS Instruments, Corvallis, OR). If seedlings in the wet treatment reached -0.5 Mpa of stress, they were rewatered regardless of block weight. Similarly, if stress levels were -1.0 to -1.5 Mpa for seedlings in the dry treatment, they were rewatered.

We evaluated 96 seedlings per pathology by drought stress combination in a randomized complete block design. Four replications in the greenhouse study continued from the four replications established in the bareroot trial. The study lasted 20 weeks. In addition to baseline seedling pathology at the time of transplant, we destructively sampled soil and seedling roots for Fusarium, Pythium, and Cylindrocarpon analyses at weeks 9 and 20. We measured height and stem diameter at weeks 0 and 20 and final shoot and root volumes at week 20 (figure 5).

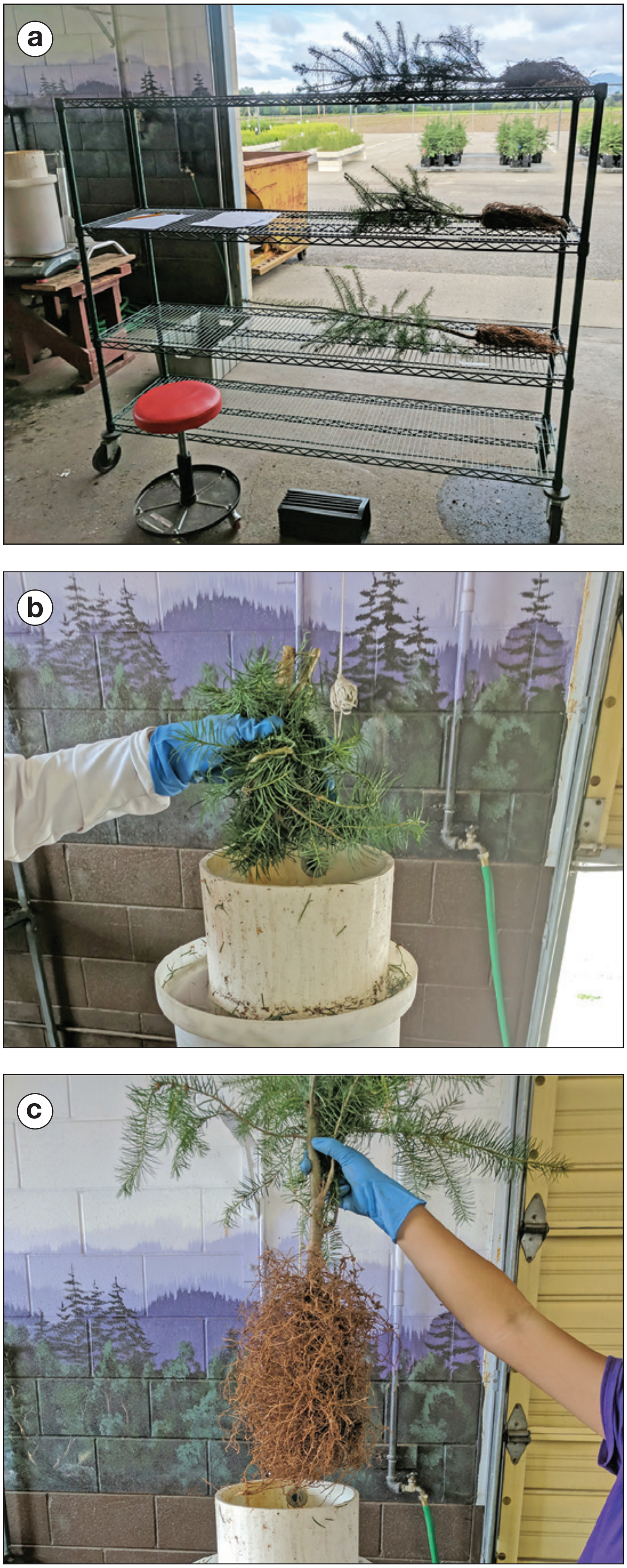

Figure 5. Seedlings in the greenhouse trial to evaluate pathology and drought effects were measured at the end of the study for (a) height and stem diameter, (b) shoot volume, and (c) root volume. (Photos by Nabil Khadduri, August 2017)

Data Analyses

Data from all three studies were analyzed using SAS statistical software to run analysis of variance and response variables. Means are separated with Tukey's test of least significant difference, and p values are considered significant at the 0.05 level. In study 1, no interactions occurred with site, so pathology data from both sites were combined.

Results

Study 1: 2009 Nursery to Woods Evaluation

Fusarium root colonization differed significantly between treatments throughout the season (figure 6a). Fusarium levels for seedlings from fumigated ground were negligible coming out of storage and stayed very low at the June and October sampling points. Seedlings from nontreated nursery ground started out at 15 percent colonization, then rose to more than 35 percent mid-season (when Fusarium tends to be most active), and fell to 10 percent by October.

Pythium root colonization also differed by treatment. Seedlings from the fumigated treatment had low levels coming out of storage and undetectable levels for the remainder of the season. Seedlings from nontreated nursery ground started out near 20 percent colonization and fell to 5 percent by season's end (figure 6b).

Cylindrocarpon root colonization was significantly higher coming out of storage for seedlings grown in nontreated ground compared with those grown in fumigated ground (figure 6c). Thereafter, colonization levels increased but did not differ between treatments.

Trichoderma (a beneficial fungal genus) root colonization was significantly higher on seedlings from the fumigated treatment compared with those in the nontreated treatment (figure 6d). Levels decreased during the growing season and were no longer different between treatments.

Despite initial differences in initial size and root pathology, survival was high (> 94 percent) for both treatments at both sites in years 1 through 3. Initial stem volume was significantly larger for seedlings that were grown in fumigated ground at the nursery

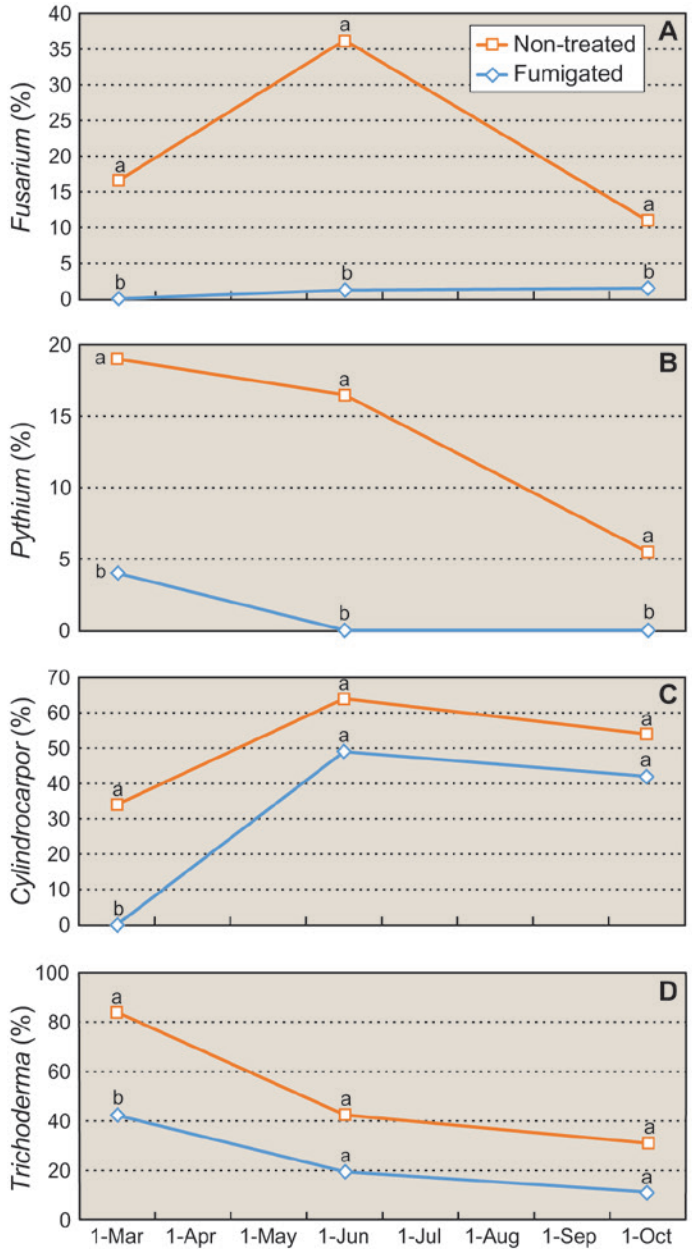

Figure 6. Douglas-fir seedlings grown in fumigated or nontreated (control) nursery soils and outplanted in 2009 varied in root colonization by (a) *Fusarium* spp., (b) *Pythium* spp., (c) *Cylindrocarpon* spp., and (d) *Trichoderma* (beneficial) spp.

compared with those grown in nontreated ground. Stem-volume differences continued through the study. At the end of year 7, seedlings from fumigated nursery ground had 37 percent greater stem volume than the control seedlings (figure 7).

Study 2: 2010 Nursery to Woods Evaluation

Despite differences in initial size and pathogen load, survival did not differ between treatments at either site. Overall survival exceeded 96 percent at year 1 and 93 percent at year 3. As in study 1, stem volumes of seedlings from fumigated nursery ground were significantly larger from the onset and continued to be larger throughout the study. After 5 years, seedlings from fumigated nursery ground had 45 percent greater stem volume than the control seedlings (figure 8).

Study 3: Nursery to Large Container Evaluation

Similar to studies 1 and 2, we observed high survival (> 97 percent), with no differences among treatments. Initial pathology differences did not affect final height or height growth. Seedlings grown in the wet treatment had significantly greater height growth, stem diameter growth, and final height compared with those in the dry treatment regardless of initial pathogen load (figures 9a and 9b). Low-pathogen and high-moisture seedlings had the largest final shoot and root volumes after one growing season (figure 9c).

Although end-of-season root infection levels were low overall, two significant differences stood out. The high-pathogen, wet seedlings had the highest levels of *Cylindrocarpon* root infection across treatments,

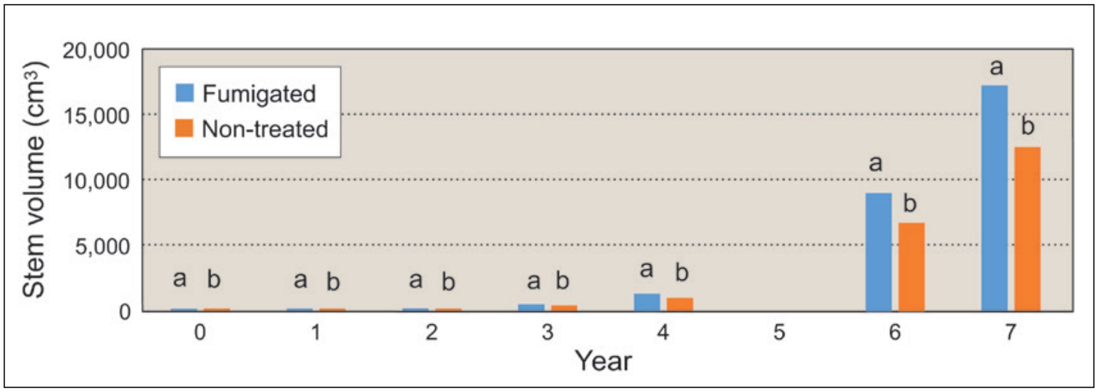

Figure 7. For the 2009 trial, average stem volume of seedlings growing in fumigated nursery ground was significantly greater throughout 7 years of field evaluation compared with seedlings that had been grown in nontreated (control) nursery ground.

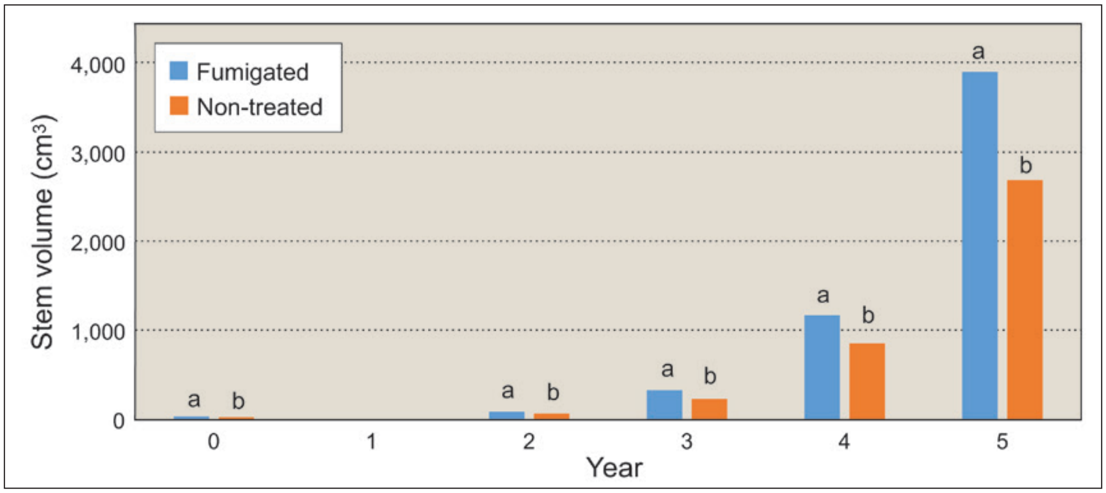

Figure 8. For the 2010 trial, average stem volume of seedlings growing in fumigated nursery ground was significantly greater throughout 5 years of field evaluation compared with seedlings that had been grown in nontreated (control) nursery ground.

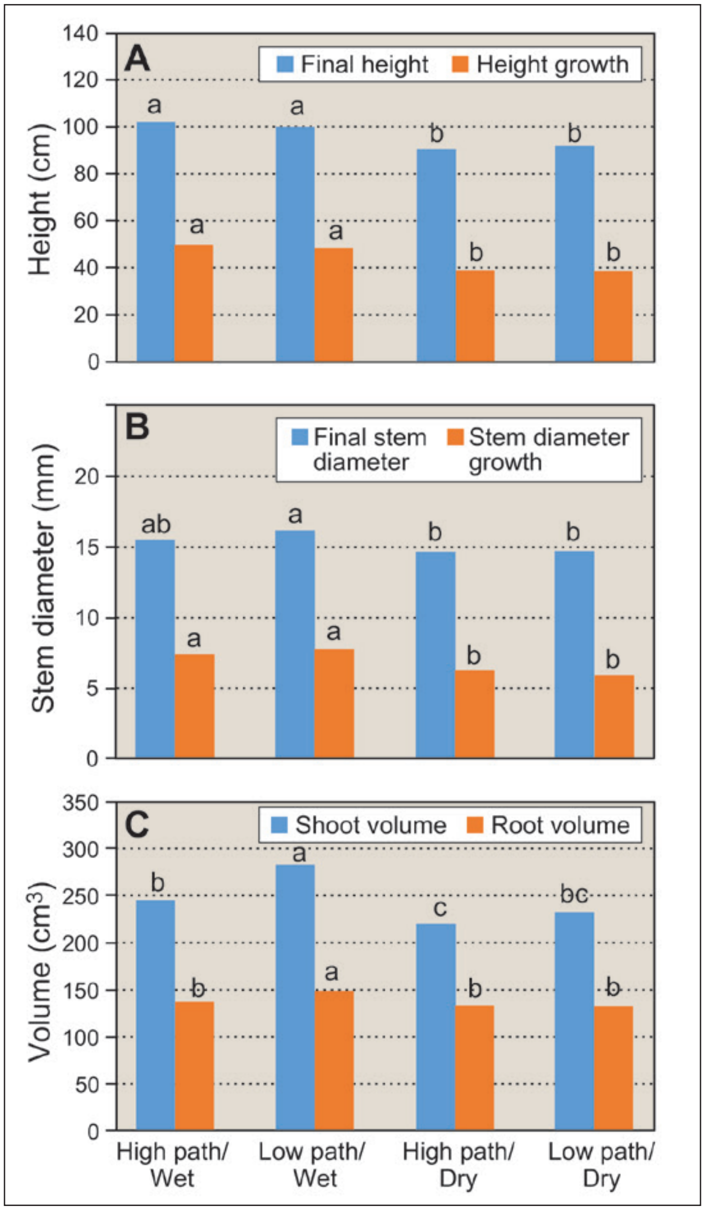

Figure 9. Evaluation of seedlings grown in the 2017 container trial to evaluate pathology and drought showed several differences among treatments for (a) height, (b) stem diameter, and (c) volume.

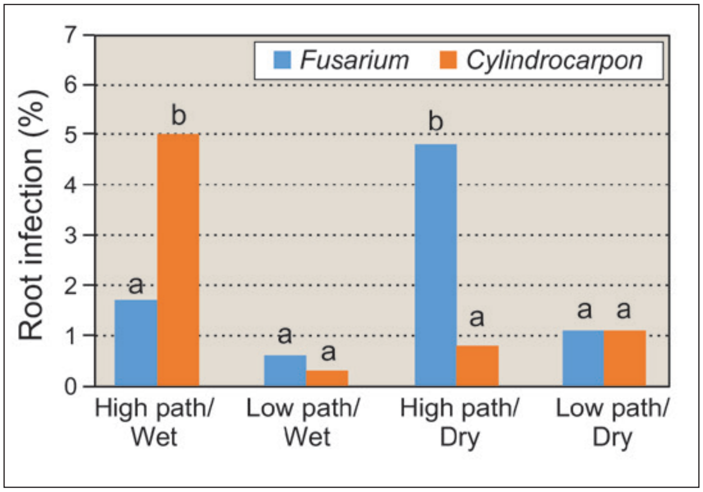

Figure 10. Seedlings transplanted into large containers with initially high pathology levels continued to have high levels after one growing season.

and the high-pathogen, dry seedlings had the highest levels of *Fusarium* root infection (figure 10). No *Pythium* root infection in any treatment was noted at the end of the study.

Discussion

Despite a higher cull rate, seedlings grown in nonfumigated ground for studies 1 and 2 still had smaller height, stem diameter, and shoot volume, and higher root pathogen levels before planting. Nevertheless, after nursery culling, these seedlings met minimum nursery packing standards for size and form. Seedlings from fumigated ground continued to be larger after outplanting, but survival did not differ among treatments. Axelrood et al. (1998), Dumroese et al. (2000), and Hansen et al. (1980) did not find growth or survival differences when comparing diseased, yet apparently packable, seedlings (no visible symptoms) with healthy seedlings. Due to confounding of initial size and root pathogen levels, it is difficult to determine the primary factor influencing subsequent field growth from these first two studies.

The 2017 large container study provided an opportunity to evaluate seedlings with very similar initial morphology but different levels of root pathology. Results from outplant studies in the woods are sometimes overwhelmed by climactic conditions (Dumroese and James 2005). For example, a growing season with favorable conditions may overcome initial stocktype differences. For this reason, we chose to evaluate in a greenhouse setting with two levels of moisture in addition to the two levels of initial root pathology. The combination of initial low root pathogen and high moisture growing environment led to the largest shoot and root volumes at the end of the growing season evaluation. We can infer that lower initial root pathogen levels directly led to larger seedlings at the end of one growing season.

Our 2017 large container evaluation findings, where distinct pathogen differences in the absence of initial size differences led to outplant performance differences, contrasts with earlier outplant studies. Both Axelrood (1991) and Hansen et al. (1980) evaluated Douglas-fir seedlings with significantly different initial pathogen loads but without significant initial size differences and saw no impact on early outplant performance. Whereas Hansen and Axelrood evaluated Douglas-fir in a forest setting, we ran this evaluation in a soilless, peat-based media. Perhaps the nursery pathogens continued to thrive in the artificial medium. Dumroese and James (2005) note that organisms pathogenic to seedlings in nurseries compete poorly in the rhizosphere of new roots penetrating into forest soil.

Soil fumigation is one in a number of tools that nursery managers employ but can be an integral component of the bareroot nursery program. These studies indicate that we must continue to actively pursue alternatives to current soil fumigation practices to ensure seedling quality as current soil fumigation regulations continue to evolve. We must also look at what long-term effects a transition away from MBC use might have, particularly with regard to the consistency of seedling quality after several seedling rotations in an alternative nursery pest management system.

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