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PREPLANT SOIL TREATMENT EFFECTS ON PRODUCTION OF DOUGLAS-FIR SEEDLINGS AT THE USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

Four preplant soil treatments were compared for their effects on presowing populations of potential soilborne pathogens (*Fusarium* and *Pythium*), potential antagonists (*Trichoderma*) and production of bare root western white pine seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. The most effective treatment in reducing pathogen populations and producing high-quality seedlings was dazomet fumigation. Fallowing with periodic cultivation was less effective than fumigation but superior to incorporating winter-grown *Brassica* cultivars (Dwarf Essex and Stonewall) prior to sowing the conifer crop. Applying a biological control formulation of *Trichoderma harzianum* did not have significant effects on seedling production. Dazomet fumigation provided the best means of ensuring production of high-quality seedlings at the nursery. *Brassica* cover/green manure crops tested thus far at the nursery have been much less effective than chemical soil fumigation for soilborne disease management.. Fallowing may be an effective alternative to fumigation, particularly if organic matter is not added to fields between seedling crops.

INTRODUCTION

Bare root forest seedling production in the United States has often depended on preplant soil fumigation with chemical biocides to ensure production of high-quality stock for reforestation. Most nurseries have relied on methyl bromide/chloropicrin (MBC) mixtures for fumigation (Boone 1988; Cordell 1982; James 1989; Miller and Norris 1970; Smith and Bega 1966). However, methyl bromide is currently being phased out and scheduled for elimination as a soil fumigant by January 2005, primarily because it significantly contributes to deterioration of the ultraviolet light-protective stratospheric ozone layer (Evans and Greczy 1995; Linderman et al. 1994; Shaheen 1996; World Meterological Association 1995). Potential alternatives to methyl bromide and/or preplant chemical soil fumigation have recently been evaluated at some forest nurseries.

The USDA Forest Service Nursery in Coeur d'Alene, Idaho has a history of using preplant soil fumigants to control soilborne plant pathogens and weeds. MBC had been the fumigant of choice (Boyd 1971; Williams 1976), but was replaced with dazomet (Basamid® granular) several years ago because it was as effective as MBC (James et al.

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1990, 1996), but caused less environmental concerns. Dazomet is usually applied in the late summer or early fall prior to sowing the following spring (Kelpsas and Campbell 1994; Tanaka et al. 1986). It is applied topically, cultivated into the soil and activated/sealed with overhead irrigation (Barnard et al. 1994; Chapman 1992; Shugert 1989; Stenlund et al. 1997). The chemical becomes volatile when wetted and does not require covering with plastic polyethylene sheets like MBC (Chapman 1992; McIntyre et al. 1990; Shugert 1989).

Although dazomet is effective at Coeur d'Alene (James et al. 1990, 1996), it is expensive, requires expert application, and still presents some potential environmental risks at the nursery. Therefore, growers have encouraged development of possible alternatives to all chemical soil fumigation. A series of tests have been conducted at the nursery to evaluate cost-effective, efficacious alternatives to chemical soil fumigation (James et al. 1993, 1994, 1996, 2004; Stone et al. 1995). This report summarizes findings of tests involving four different soil treatments on the production of western white pine (*Pinus monticola* Dougl.) seedlings at the nursery.

MATERIALS AND METHODS

Tests were initiated during the summer of 2000. Each treatment was replicated five times; treatment blocks were located within two sections of Field 9 in a complete randomized block design (figure 1). Each treatment block was 50 ft. in length and one seedling bed wide, with the exception of the dazomet treatments that were two bed widths because of application machinery requirements. The treatments were: standard dazomet soil fumigation (300 lbs./acre [335 kg/ha]) in September 2000, bare fallowing with periodic cultivation, and two *Brassica* green manure crops (cultivars "Dwarf Essex" and "Stonewall") that were sown in the fall of 2000, grown over the winter, and incorporated into the soil 10 days before sowing in the spring of 2001. To evaluate potential effects of a biological control agent on these treatments, a formulation of *Trichoderma harzianum* (strain ThzID1) was added to half of the dazomet, fallow, and *Brassica* plots (resulting in 8 total treatments) just prior to sowing.

Soil populations of potential pathogens in the genera *Fusarium* and *Pythium* and potential antagonists in the genus *Trichoderma* were assayed prior to sowing. Samples were collected from within each replicate plot; three collections within each plot were collected and mixed together to represent a single sample. Each collection consisted of a soil core taken to a depth of about 8 inches (20 cm). Soil was placed in plastic bags, kept refrigerated and transported to the laboratory for analysis.

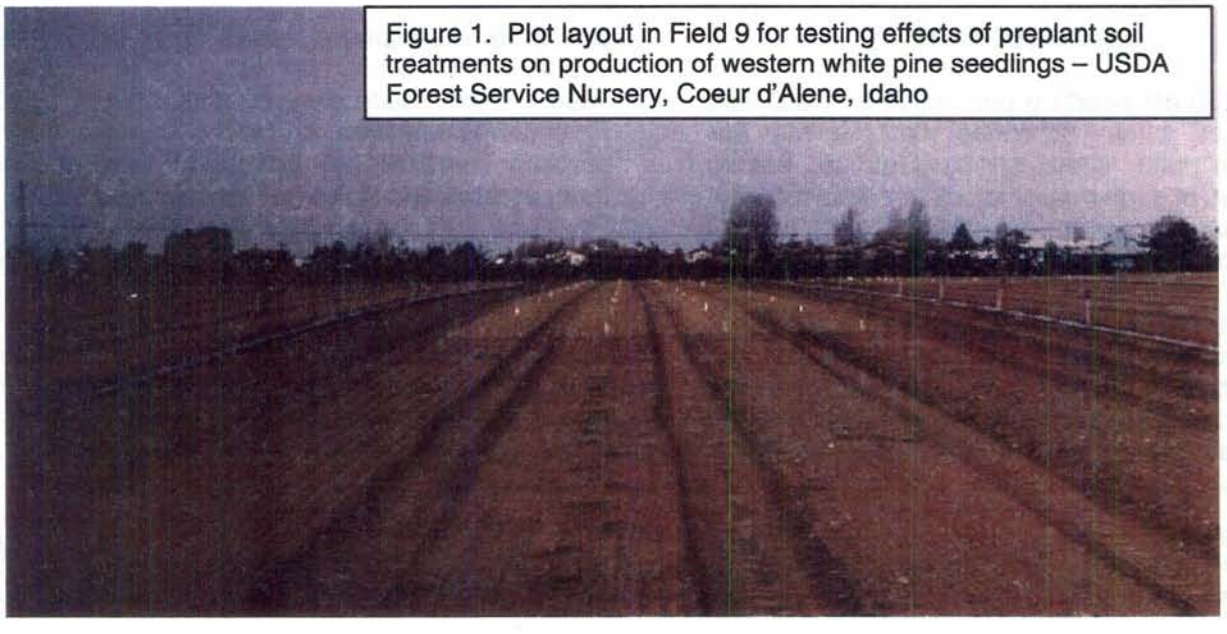

Figure 1. Plot layout in Field 9 for testing effects of preplant soil treatments on production of western white pine seedlings – USDA Forest Service Nursery, Coeur d'Alene, Idaho

Standard soil dilutions (Hildebrand and Dinkel 1988, James et al. 1990, 1996, Stone et al. 1995) were conducted to estimate populations of *Fusarium, Trichoderma*, and *Pythium* spp. Soil from each sample was initially sieved (2 mm sieve) to remove rocks, pieces of organic matter, and soil aggregates. From each sample, an approximate 5 g subsample was oven-dried at about 100°C for at least 24 h until sample weight stabilized. Oven-dry weight was then calculated to provide a standard for sample comparison. For assays of *Fusarium* and *Trichoderma* populations, 0.05 g of field-moist soil was combined with 10 ml of 0.3% water agar (WA) and thoroughly mixed. One mililiter of solution was placed on each of three plates of selective agar medium for *Fusarium* and closely-related fungi (Komada 1975) and spread uniformly. *Trichoderma* propagules were also enumerated on Komada's medium because it readily supports growth of this fungus unless the medium is amended with benomyl or lithium chloride. Plates were incubated for 7 days at about 24°C under diurnal cycles of cool, fluorescent light. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium; populations were expressed as number of colony-forming units (cfu) per g of oven-dried soil (it was assumed that each fungal colony originated from one propagule). Selected *Fusarium* isolates were transferred to carnation leaf agar (Fisher et al. 1982) and potato dextrose agar (PDA) for identification to species using the taxonomy of Nelson et al. (1983). Isolates of *Trichoderma* were not identified to species.

For assays of *Pythium* populations, 0.5 g of soil was combined with 10 ml of 0.3% WA. One mililiter of solution was placed on each of three plates containing another selective medium consisting of V-8 juice agar amended with the antibiotics pimaricin, rifamycin, and ampicillin and the fungicide penta-chloronitrobenzene (James et al. 1990, 1996; Stone et al. 1995). Plates were incubated in the dark at about 24°C for 3 days. *Pythium* colonies were identified on the basis of their diameter after 3 days (15-20 mm), feathery margin, and growth within rather than superficially on the agar surface. Populations were expressed as cfu/g of oven-dried soil. Selected *Pythium* isolates were transferred to PDA for identification using the taxonomy of Waterhouse (1968). All plots were sown during early May, 2001 with the same seedlot of western white pine using standard nursery procedures and covered with hydro-mulch. After seedling emergence was deemed nearly complete (late July), three sampling sub-plots (0.5 m^2) were installed within each replicate-plot; these subplots were located approximately equidistant from each other and concentrated within the center of each replicate-plot. Seedling emergence and post-emergence damping-off were determined in each sub-plot in July; selected damped-off seedlings were collected for laboratory analysis of associated pathogens. At the end of the first growing season (October), seedling density and disease were determined within each of the sub-plots. Selected diseased seedlings were again collected for laboratory analysis, which included thoroughly washing roots of diseased seedlings and incubating them on Komada's medium and identifying associated organisms as described above for soil samples.

At the end of the second growing season (November 2002), sample seedlings were carefully extracted from beds by hand for morphology measurements. Sample seedlings were located within inner seedling rows (to eliminate edge effects) and entire root systems were included with each seedling. Fifty "average" seedlings were collected from each replicate plot. Seedlings were transported to the laboratory for measurement. Seedling height (from basal cotyledon scar to the tip of the terminal bud), diameter (just above the groundline) and root mass (oven-dry weight of all roots below the groundline) were determined for each sample seedling. Seedling heights, diameters, and root masses were compared among the treatments with an analysis of variance. Significant differences (P=0.05) in these three morphology categories were located using the LSD procedure.

RESULTS

Presowing soil populations of *Fusarium, Pythium* and *Trichoderma* spp. within treatment plots are summarized in table 1. The lowest *Fusarium* populations were in the dazomet treatment areas, followed by fallow plots; much higher populations were in the two *Brassica* treatment areas, with Dwarf Essex plots being the highest. *Pythium* populations had similar trends with highest populations in the *Brassica* plots. *Trichoderma* pop-ulations, including those isolates added just prior to sowing, were generally high in all plots except the dazomet treatments. Ratios of *Trichoderma* to *Fusarium* populations, which may roughly estimate potential disease suppressiveness (James et al. 1990, 1996; Papavizas 1985), were highest for the dazomet-treated plots (table 1).

Table 1. Effects of selected preplant soil treatments on presowing soil populations of *Fusarium*, *Trichoderma*, and *Pythium* spp. at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Treatment	Colony-forming Units/g Oven-dry Soil[1]		
	Fusarium	**Trichoderma[2]**	**Pythium**
Dazomet	**56** [0-134]	**638** [67-2223]	**4** [0-33]
Fallow/Cultivation	**527** [338-803]	**1350** [737-1744]	**173** [0-408]
Dwarf Essex	**1211** [471-1965]	**1568** [479-2479]	**290** [0-481]
Stonewall	**889** [67-1814]	**1620** [942-2160]	**445** [216-612]

[1]Values in **bold** are means; ranges are in brackets; means are from 5 replicate plots per treatment.
[2] *Trichoderma/Fusarium* ratios: Dazomet [11.40], Fallow [2.56], Dwarf Essex [1.29], Stonewall [1.82]; the higher the ratio the greater potential for disease suppressiveness.

Four *Fusarium* species were isolated from soil in pre-sowing assays (table 2). By far the most prevalent species was *F. oxysporum* Schlecht., which comprised more than 75% of the population. Other species included *F. equiseti* (Corda) Sacc., *F. sporotrichioides* Sherb. and *F. solani* (Mart.) Appel & Wollenw. Two species of *Pythium* were routinely isolated from nursery soil: *P. irregulare* Buisman and *P. ultimum* Trow.

Emergence, survival, first-year height growth and disease severity of seedlings are summarized in table 3. The two *Brassica* treatments had deleterious effects on seedling production during the first growing season. Fewer and smaller seedlings were produced in plots with incorporated *Brassica* crops. More and taller seedlings were produced in dazomet-treated plots. First-year disease was low in all treatments. Diseased seedlings initially turned chlorotic and later necrotic (figure 2). *Fusarium oxysporum* was isolated from all sampled diseased seedlings.

Effects of the soil treatments on height, diameter and root mass of 2-0 seedlings are summarized in table 4. Data comparisons for the four preplant soil treatments without considering bio-control amendments are collated in table 5. The only significant treatment effects were taller seedlings in the dazomet-treated plots. No differences were found among other treatments and biocontrol amendments did not significantly affect seedling growth. However, treatments may have affected seedling density (data not taken) among plots by the end of two growing seasons (figure 3).

Table 2. *Fusarium* species isolated from soil during population assays at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Fusarium Species	Number of Isolates Assayed	Percentage of Isolates
Fusarium oxysporum	186	77.8
Fusarium equiseti	32	13.4
Fusarium sporotrichioides	20	8.4
Fusarium solani	1	0.4

Table 3. Effects of selected preplant soil treatments on white pine seedling emergence and first-year survival, disease and height at the USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Treatment[2]	Number of seedlings per 0.5m^2						Seedling Height (cm)	
	Emergence		Survival		Disease			
Dazomet [NoBC]	**81.8**	[53-119]	**80.8**	[51-122]	**3.1**	[0-5]	**4.4**	[3.9-6.0]
Dazomet [+ BC]	**50.5**	[16-105]	**50.5**	[15-107]	**3.0**	[1-5]	**4.3**	[3.4-5.1]
Fallow [NoBC]	**21.0**	[14-31]	**19.4**	[12-28]	**1.0**	[1-7]	**3.0**	[2.8-3.3]
Fallow [+ BC]	**29.2**	[19-52]	**27.4**	[17-49]	**1.3**	[1-7]	**3.3**	[2.8-3.7]
DEssex [NoBC]	**9.3**	[4-18]	**9.0**	[3-17]	**0.5**	[0-3]	**2.7**	[2.4-3.3]
DEssex [+ BC]	**9.0**	[3-14]	**8.2**	[3-14]	**0.7**	[0-2]	**2.9**	[2.5-3.3]
Stonewall [NoBC]	**13.3**	[5-19]	**12.2**	[4-17]	**0.5**	[0-4]	**2.9**	[2.5-3.3]
Stonewall [+BC]	**17.0**	[4-49]	**17.5**	[3-46]	**0.7**	[1-3]	**3.0**	[2.6-3.3]

[1] Values in bold are means; ranges are in brackets. Density based on number of seedlings within subplots measuring 0.5m^2 located within each replicate plot.

[2] BC denotes biological control agent Trichoderma harzianum

Figure 2. Mortality of 1-0 western white pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. Post-emergence damping-off symptoms are indicated by arrows.

Table 4. Effects of selected preplant soil treatments on height, diameter, and root mass of 2-0 western white pine seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Treatment	Height[2]	Diameter[3]	Root Mass[4]
Dazomet [NoBC]	7.4A	2.4A	0.43A
Dazomet [+BC]	7.0A	2.4A	0.49A
Fallow [NoBC]	4.9B	2.3A	0.46A
Fallow [+BC]	6.4B	2.5A	0.52A
DEssex [NoBC]	4.5B	2.5A	0.40A
DEssex [+BC]	4.8B	2.4A	0.47A
Stonewall [NoBC]	4.5B	2.2A	0.41A
Stonewall [+BC]	5.1B	2.4A	0.38A

[1] Based on measuring 50 seedlings per replicated plot for each treatment. Within each column, means followed by the same capital letter are not significantly different (P=0.05) using LSD.

[2] Measured from the ground line to the tip of the terminal bud (cm).

[3] Measured just above the ground line (mm).

[4] Based on oven-dry weight of roots at lifting (g).

Table 5. Collated comparisons of western white pine seedling height, diameter, and root mass among different preplant soil treatments - USDA Forest Service Nursery, Coeur d'Alene, Idaho

Treatment	Height[2]	Diameter[3]	Root Mass[4]
Dazomet	7.2A	2.4A	0.46A
Fallow	5.7B	2.4A	0.49A
Dwarf Essex	4.6B	2.5A	0.44A
Stonewall	5.1B	2.3A	0.39A
All No BC	5.4A	2.4A	0.43A
All +BC	5.8A	2.4A	0.47A

[1] Averages in the top part of the table compare collated values for treatments with and without addition of the biological control agent. Averages in the bottom part of the table compare collated values for all treatments with and without biological control agent. Within each column, means followed by the same capital letter are not significantly different (P=0.05) using LSD.

[2] Measured from the ground line to the tip of the terminal bud (cm).

[3] Measured just above the ground line (mm).

[4] Based on oven-dry weight of roots at lifting (g).

Figure 3. Preplant soil treatment effects on 2-0 western white pine seedlings—USDA Forest Service Nursery, Coeur d'Alene, Idaho. Orange plastic stakes locate subplots used to determine seedling emergency, disease and first-year height. Seedling density may have been affected by treatments.

DISCUSSION

Preplant soil fumigation with chemical biocides has been important in production of bare root conifer seedlings at the USDA Forest Service Nursery in Coeur d'Alene, Idaho for many years (Boyd 1971; James et al. 1990, 1996; Williams 1976). Initially, methyl bromide/chloropicrin (MBC) mixtures were the fumigants of choice (Boyd 1971; Williams 1976), but dazomet subsequently replaced MBC. Dazomet provides excellent disease control (James et al. 1990, 1996), but is expensive and may cause environmental concerns. Recent efforts to develop alternatives to chemical fumigation at the nursery have resulted in mixed results. Bare fallowing for at least one year, especially coupled with periodic cultivation, has often provided a viable alternative to fumigation, but only if organic soil amendments are not added to soil prior to sowing (James et al. 1993, 1994, 1996; Stone et al. 1995). Tested organic amendments have included sawdust or composted sewage sludge (James et al. 1993, 1994, 1996, 2004; Stone et al. 1995) and cover/green manure crops that are grown for a few months, chopped and tilled into the soil (James et al. 1996, 2004). All of these tend to result in soil population increases of potential pathogens and adversely affect seedling production. In addition, growers have recently grown sweet and field corn to maturity, and incorporated this biomass into soil to add organic matter needed to maintain desired soil tilth (James 2000). When organic matter is added to nursery soil, soil microorganism activity greatly increases (Bloomberg 1963; Borken et al. 2002; Chen et al. 1988; Linderman 1970). Unfortunately, enhanced microbial activity may result in large population increases of potential pathogenic fungi (Hamm and Hansen 1990; Hansen et al. 1990; James et al. 1993, 1994, 1996, 2004; Stone et al. 1995). Although not all population increases may involve pathogens, sufficient pathogen buildup occurs to

usually adversely affect subsequent seedling production (Hansen et al. 1990; Hamm and Hansen 1990; James et al. 1996, 2004). With organic matter additions, preplant chemical soil fumigation is required to sufficiently lower pathogen populations and improve seedling production (Hansen et al. 1990; James 2000).

Both cultivars of *Brassica* (Dwarf Essex and Stonewall) tested in this study caused buildup of pathogen populations when incorporated into soil. They also adversely affected production of western white pine seedlings. Apparently, the glucosinolates associated *Brassica* tissues were not sufficient to overcome the deleterious effects of enhanced organic matter on soil pathogens. In addition, amending soil with *Trichoderma harzianum* did not improve treatment effects on seedling production. The isolate of *T. harzianum* used in this evaluation was previously effective in agricultural systems (Knudsen and Bin 1990; Knudsen et al. 1991) and has shown promise against *F. oxysporum* in forest seedling nurseries (Mousseaux et al. 1998). However, in our evaluation, this biocontrol agent apparently did not sufficiently suppress soil *Fusarium* activity enough to ameliorate the adverse effects of organic amendments on seedling production.

To date, amending forest nursery soils with *Brassica* plant residues to control soilborne diseases has been disappointing. Several evaluations have consistently shown similar results: *Brassica* plant residues result in increased soil populations of pathogens that adversely affect conifer seedling production (Hansen et al. 1990; Hamm and Hansen 1990; James et al. 1996, 2004). Efforts to improve efficacy of *Brassica* spp. by using different cultivars, covering with plastic tarps to concentrate pathogen-toxic chemicals, or adding biocontrol agents have not yet been effective in forest nurseries (James et al. 1996, 2004). It is possible that other *Brassica* cultivars will prove more efficacious. Perhaps amending soil directly with *Brassica* meal containing very high glucosinolate concentrations may improve effectiveness (Chung et al. 2003). In any case, use of *Brassica* green manure crops cannot currently be recommended in forest nurseries in western North America. If other *Brassica* cultivars or materials show improved efficacy in the future, this recommendation may be changed.

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