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EFFECTS OF PREPLANT SOIL TREATMENTS ON *FUSARIUM* AND *TRICHODERMA* POPULATIONS AND FUNGAL ROOT COLONIZATION OF 2-0 NONDISEASED WESTERN WHITE PINE SEEDLINGS – USDA FOREST SERVICE NURSERY COEUR D'ALENE, IDAHO

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ABSTRACT

Preplant soil treatments were implemented to determine effects on populations of potentially-pathogenic *Fusarium* and potentially disease-suppressive *Trichoderma* spp. as well as root colonization by these and other selected fungi on healthy-appearing, bare root 2-0 western white pine seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Soil treatments included fumigation with dazomet, bare fallowing with periodic cultivation, steam treatment, fallowing with amendments of *Trichoderma harzianum* biocontrol agents (BioTrek® and University of Idaho isolates [UI]), and incorporation of two cultivars of winter *Brassica* cover crops followed by biocontrol amendments (UI). Soil *Fusarium* populations were significantly reduced by dazomet fumigation; fallowing with biocontrol amendments and incorporating winter mustard crops with biocontrol amendments resulted in significantly higher *Fusarium* populations. *Trichoderma* populations were significantly decreased by dazomet fumigation, bare fallowing and steam treatment. Incorporation of *Brassica* crops did not significantly affect soil *Trichoderma* populations. Level of *Fusarium* root colonization was significantly reduced by dazomet fumigation and steam treatment. High levels of root colonization by rhizosphere-inhabiting isolates of *Cylindrocarpon* were common in some treatments. Assaying root colonization by selected fungi may supplement other parameters to evaluate effectiveness of soil treatments implemented to reduce pathogen populations and improve conifer seedling production.

INTRODUCTION

During recent years, efforts have been underway to develop alternatives to pre-plant soil fumigation for production of bare root seedlings in forest nurseries. These efforts have stemmed from the fact that methyl bromide, usually the most common and effective fumigant (James 1989), is a major potential destroyer of stratospheric ozone (World Meterological Association 1995) and will no longer be available for nonemergency use in the United States after 2005 (James et

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al. 1994b; Stone et al. 1995, 1997). During investigations concerning alternative soil treatments, various parameters have been used to evaluate treatment efficacy. Such factors as soil populations of potential plant pathogens (*Fusarium* and *Pythium*), potential disease antagonists (*Trichoderma*) seedling density, disease, height, diameter and root biomass have all been measured (James et al. 1994b; Stone et al. 1995, 1997). Several of these parameters may more closely reflect associated factors rather than treatment effects on seedling production. For example, seedling density may often be more related to seedling size (and vice versa) than to treatment effects, especially by the end of the second growing season in bare root nurseries.

Disease is not always a reliable indicator because of the nonuniform distribution of soil pathogens within fields and effects of environmental conditions on disease symptom expression (Bloomberg 1976; James and Beall 1999, 2000; James et al. 1991). Treatment effects on soil populations of *Fusarium* and *Pythium* spp. should give a rough estimate of efficacy. However, such populations may not necessarily reflect seedling disease potential because assayed populations include both pathogenic and non-pathogenic fungal strains, which are often morphologically similar.

A potentially good indicator of treatment efficacy should be root biomass. If seedlings are more adversely affected by soilborne pathogens, their roots are more readily attacked, decay is increased, and root growth and overall biomass is reduced. However, in bare root seedling production, roots are routinely pruned prior to lifting to encourage increased fibrous root growth and to make extraction from soil easier with less root damage. Under such conditions, it is difficult to properly compare root systems because pruning effects compound possible interactions with root pathogens.

Another possible way to evaluate soil treatment efficacy is to monitor root infection by organisms targeted in pre-plant treatments. Since *Fusarium* spp. are usually the major pathogens of concern in most bare root forest nurseries in western North America (Bloomberg 1976; Bloomberg and Lock 1972; James et al. 1991), determining extent of seedling root infection by these fungi may indirectly give an accurate estimate of soil treatment efficacy.

This report compares populations of *Fusarium* (potential pathogens) and *Trichoderma* (potential antagonists) before and after preplant soil treatments and documents how treatments affect first-year seedling root infection by these fungi.

MATERIALS AND METHODS

Soil treatments were applied during the growing season prior to planting. Seven treatments were conducted on plots that were replicated five times; plots were established within a production field in a complete randomized block design. The seven treatments were: A = standard soil fumigation with dazomet (Basamid® granular) at 350 lbs./acre (392 kg/ha); B = bare fallow for the year prior to sowing with periodic cultivation to keep weed populations low and turn soil; C = treatment of soil with aerated steam (steam injected at 7 in. [18 cm] at 77°C), D = bare fallow with biocontrol amendment (*Trichoderma harzianum* BioTrek® preparation), E = bare fallow with biocontrol amendment (*Trichoderma harzianum* University of Idaho isolates [UI]), F = cultivation of a rape variety of *Brassica* (as a winter crop: sown in the fall, grown during the fall and early spring, incorporated into soil at least 14 days prior to sowing) supplemented with the UI biocontrol agent, G = cultivation of a mustard variety of *Brassica* (grown and incorporated similar to the rape) supplemented with the UI biocontrol agent. In the spring following soil treatments, one

seedlot of western white pine (*Pinus monticola* Dougl.) was sown using standard nursery practices within all plots.

Prior to treatments, soil samples were collected from the tested field in a systematic manner. Seventy samples were collected along transects running through the field; 10 samples were collected from each of the seven different treatment areas (two per replicate plot). Following treatments, just prior to sowing, soil samples were collected from approximately the same locations as before treatment. At each sample point a soil core was taken to a depth of about 8 in. (20 cm). Soil was placed in plastic bags, kept refrigerated, and transported to the laboratory for analysis.

Standard soil dilutions (James and Beall 1999, 2000; James et al. 1996) were conducted to estimate populations of *Fusarium* and *Trichoderma*. Soil from each sample was initially sieved (2mm 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 hrs. until sample weight had 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 and thoroughly mixed. One ml of the solution was placed on each of three plates of selective agar medium (Komada 1975) and spread uniformly. Plates were incubated 5-7 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 colony-forming units (cfu) per g of oven-dried soil. Selected *Fusarium* isolates were transferred to potato dextrose (PDA) and carnation leaf agar (Fisher et al. 1982) for identification using the taxonomy of Nelson et al. (1983). For each treatment, pre-treatment populations were statistically compared with pre-sowing populations using paired T Tests. Populations among the four treatments were compared with an analysis of variance; significantly different ($P \leq 0.05$) means were located using Tukey's HSD.

At the end of the second growing season (November), healthy-appearing seedlings were extracted from treatment plots. A total of 25 seedlings were randomly selected from each treatment (5 from each replicate plot). Seedlings were carefully extracted from soil to include as much of their root system as possible and refrigerated during transport to the laboratory. Seedling roots were washed thoroughly under tap water to remove soil particles. Lateral and fine terminal roots were severed from seedlings and cut into pieces of varying length with sterile scissors. Seedling roots from each treatment were collected together and washed two more times in sterile, distilled water. Roots were chopped in a blender; pieces about 5 mm in length were randomly selected and surface sterilized in bleach (0.525% aqueous sodium hypochlorite). One hundred root pieces were sampled from seedlings from each treatment; sampled roots were placed on the *Fusarium* medium. Plates with roots were incubated as described above. Emerging fungi were identified to genus and selected *Fusarium* isolates were identified to species as described above. Percent of sampled root pieces (100 per treatment) colonized by particular fungi was calculated as "colonization." Average percent colonization by different groups of fungi were compared statistically with an analysis of variance. Significantly different ($P \leq 0.05$) means were located using Tukey's HSD. All percentages underwent arc-sin conversion prior to analysis.

RESULTS AND DISCUSSION

Soil populations of *Fusarium* were significantly reduced only by dazomet fumigation (table 1);

population reductions by steam treatment were not statistically significant. All other treatments, with the exception of treatment F (incorporation of winter rape with biocontrol amendments), resulted in significantly greater *Fusarium* populations. Some increases were quite large, up to a three-fold increase in treatment D (fallow with BioTrek® amendments). Changes in *Fusarium* populations may have affected white pine seedling responses, which were greatly affected by some treatments (Figure 1). Significant decreases of *Trichoderma* populations were found in the fumigated, bare fallowed and steam treated plots (table 1). Populations of these fungi were not significantly affected by the *Brassica* crop residues. Comparing the seven treatments, it was quite evident that dazomet fumigation and steam treatment most severely reduced *Fusarium* populations. Populations in fallowed plots were similar to those incorporated with *Brassica* residues and generally exceeded disease threshold levels (James and Beall 1999, 2000; James et al. 1996).

Six Fusarium species were isolated from nursery soil (table 2). By far the most common species was *F. oxysporum* Schlect.; it comprised about 80% of the Fusarium population sampled both before and after treatments. Four morphologically distinct isolates (morphotypes) of *F. oxysporum* were routinely isolated from soil. These were differentiated primarily on their typical colony morphology on both Komada's medium and PDA. Although both colony types exhibited profuse aerial mycelium, hyphal texture and pigmentation were consistently different between the two morphotypes. This cultural diversity may not necessarily be related to pathogenic potential, but was useful in segregating these two morphotypes, which were consistently isolated from seedling roots. This species is a very common soil inhabitant in forest nurseries and is often causes serious seedling diseases (Bloomberg 1976; James et al. 1989a, 1991). *Fusarium oxysporum* is a common colonizer of root cortical tissues in both healthy-appearing and diseased seedlings (Bloomberg 1976; James and Gilligan 1988a, 1988b). When it initiates root tissue deterioration and necrosis, the fungus acts as a pathogen. However, some isolates colonize root tissues without eliciting host disease responses and thus act like non-pathogenic endophytes (Dumroese et al. 1993; Gordon and Martyn 1997; Gordon and Okamoto 1992a).

The other *Fusarium* species were generally isolated at very low levels. These included *F. avenaceum* (Fr.). Sacc. ; *F. scirpi* Lambotte & Fautr., *F. acuminatum* Ell. & Ev., *F. sporotrichioides* Sherb and *F solani* (Mart.) Appel & Wollenw.

Fusarium spp. were isolated from a large proportion of roots sampled from most treatments (table 3). Significantly less root colonization was detected on seedling roots from the dazomet-fumigated and stream-treated plots. Seedling root colonization from the other treatments were not statistically different. Extensive root colonization by *Fusarium* spp. was evident on many sampled seedlings even though these seedlings appeared healthy and did not display either above- or below-ground disease symptoms.

Level of root colonization by *Cylindrocarpon* spp., which are common inhabitants of plant rhizospheres (Booth 1966) and usually either saprophytes or very weak pathogens (Booth 1966; Dumroese et al. 2000; James et al. 1994a) varied widely among the treatments (table 3). These fungi apparently were quite sensitive to dazomet fumigation but not as sensitive as *Fusarium* spp. to steam treatment.

Table 1. Effects of pre-plant treatments on soil populations of *Fusarium* and *Trichoderma* - USDA Forest Service Nursery, Coeur d'Alene, Idaho.[1]

Treatment2	Replication	**Fusarium**		**Trichoderma**	
		Pre-Treat	Pre-Sow	Pre-Treat	Pre-Sow
A	1	103	350	5275	1045
	2	823	480	2186	632
	3	823	220	612	715
	4	823	110	1968	705
	5	1087	370	1485	803
	Average	732bc	306a*	2305a	780a*
	% Change	- 58.2		- 66.1	
B	1	557	810	2437	435
	2	308	2200	2155	715
	3	103	1200	1150	1244
	4	1190	950	1820	1550
	5	528	370	3058	1431
	Average	537a	1106c*	2124a	1075ab*
	% Change	+ 106.0		- 49.4	
C	1	340	67	2155	435
	2	308	126	7553	368
	3	476	67	1045	2160
	4	205	335	3058	4101
	5	205	335	4605	301
	Average	307a	186a	3683bc	1473b*
	% Change	- 39.4		- 60.0	
D	1	103	510	1045	7315
	2	103	1250	8223	6052
	3	410	2010	612	5814
	4	557	810	3058	7449
	5	103	870	7553	3695
	Average	255a	1090c*	4098c	6065e
	% Change	+ 327.4		+ 48.0	
E	1	721	1250	1420	4450
	2	692	1110	692	2160
	3	410	950	2655	3515
	4	557	1350	1501	2935
	5	205	1405	4605	4005
	Average	517a	1213c*	2175a	3413cd*
	%Change	+ 134.6		+ 56.9	

F	1	680	910	2655	5845
	2	721	1140	1954	4410
	3	680	750	8223	2911
	4	2173	610	1150	4625
	5	1087	455	2437	3879
	Average	1068c	773b	3284b	4334de
	%Change	- 27.6		+ 32.0	
G	1	557	1040	1619	3510
	2	205	1230	1887	1782
	3	476	910	2090	2415
	4	1087	1100	3058	2609
	5	823	1450	2856	4124
	Average	630ab	1146c*	2302a	2878bc
	%Change	+ 81.9		+ 25.0	

[1] Values in table are colony-forming units per gram of oven-dry soil. Pre-sowing averages followed by an asterisk are significantly different (P≤0.05) using a paired T Test. Percent change reflects changes from pre-treatment to pre-sowing population counts.

[2] A = dazomet fumigation; B = bare fallow with periodic cultivation; C = stream treatment; D = bare fallow with biological control (*Trichoderma harzianum* – BioTrek) amendment; E = bare fallow with biological control (*Trichoderma harzianum* – UI isolate) amendment; F = incorporation of winter rape (*Brassica*) crop and biological control (*Trichoderma harzianum* – UI isolate) amendment; G = incorporation of winter mustard (*Brassica*) crop and biological control (*Trichoderma harzianum* – UI isolate) amendment. Comparing average population counts for each group of fungi among the four treatments, means followed by the same letters are not significantly different (P≤0.05) using Tukey's HSD.

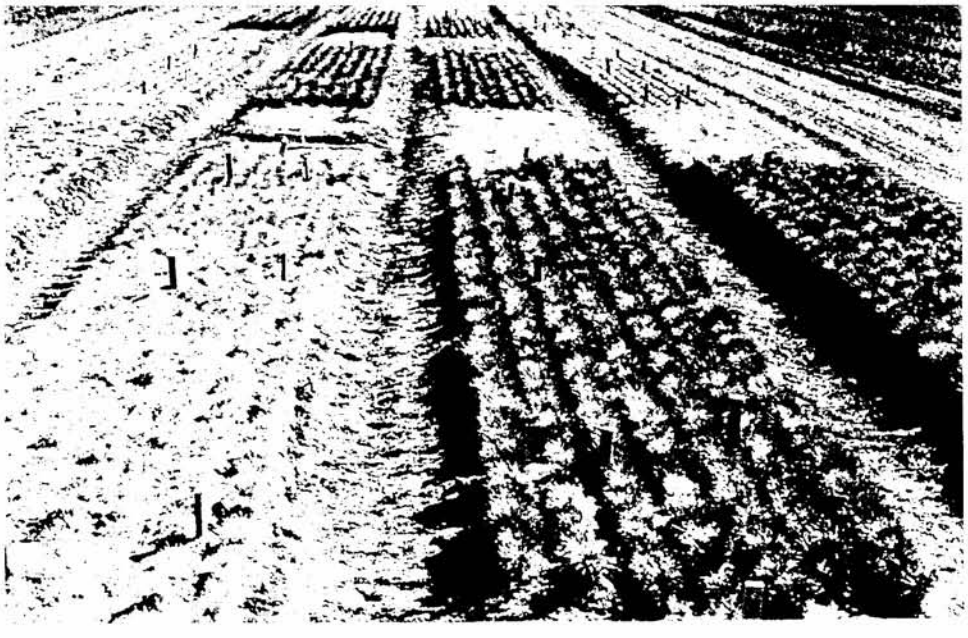

Figure 1. Preplant soil treatment effects on production of 2-0 bare root western white pine seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho

Table 2. *Fusarium* spp. isolated from soil undergoing pre-sowing treatments - USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

Fungal Isolate[2]	Samples[3]		
	Pre-Treatment	Pre-Sowing	Both Samples
FOXY1	54.8	49.5	52.2
FOXY2	20.2	28.2	24.2
FOXY3	2.4	0	1.2
FOXY4	5.9	1.4	3.6
All FOXY	83.3	79.1	81.2
FAVE	15.5	2.3	8.9
FSCI	1.2	0	0.6
FACU	0	7.6	3.8
FSPO	0	2.4	1.2
FSOL	0	8.6	4.3

[1] Numbers in table are percent of *Fusarium* isolates recovered from soil samples that were within the appropriate species.

[2] FOXY= *Fusarium oxysporum* (1 - 4 delineate different morphotypes); FAVE = F. *avenaceum*; FSCI = *F. scirpi*; FACU = *F. acuminatum*; FSPO = *F. sporotrichioides* FSOL = *F. solani*.

[3] Samples collected prior to (pre-treatment) and after (pre-sowing) treatments. *Fusarium* spp. collated for all treatments.

Table 3. Effects of preplant soil treatments on colonization of 2-0 western white pine seedling roots by selected fungi - USDA Forest Service Nursery, Coeur d'Alene, Idaho.[1]

Fungus[2]	Treatment[3]						
	A	B	C	D	E	F	G
FOXY1	9	19	18	25	24	20	13
FOXY2	6	0	0	0	0	0	0
FOXY3	16	31	17	28	32	46	39
FOXY4	1	0	0	0	0	0	0
All FOXY	32	50	35	53	56	66	52
FSOL	0	10	0	3	1	6	2
FACU	1	1	3	1	2	0	3
FSPO	1	0	0	0	0	0	0
FEQU	0	0	0	0	0	0	1
All *Fusarium*	34a	61b	38a	57b	59b	72b	58b
Cylindrocarpon	46a	54ab	63bc	75d	66bcd	55ab	70cd
Trichoderma	1	3	8	1	3	2	1

[1] Values in table are percent of sampled root pieces (100 sampled from 25 seedlings per treatment) colonized by the appropriate fungus.

[2] FOXY = *Fusarium oxysporum* (1-4 are different morphotypes); FSOL = *F. solani*; FACU = *F. acuminatum*; FSPO = *F. sporotrichioides*; FEQU = *F. equiseti*.

[3] Treatments: A = Fumigation with dazomet; B = Bare fallow with monthly cultivation; C = Steam treatment; D = Bare fallow with biological control (*Trichoderma harzianum* - BioTrek) amendment; E = Bare fallow with biological control (*Trichoderma harzianum* – UI isolate) amendment; F = Incorporation of winter rape (*Brassica*) and biological control (*Trichoderma harzianum* – UI isolate) amendment; G = Incorporation of winter mustard (*Brassica*) and biological control (*Trichoderma harzianum* – UI isolate).

Interestingly, levels of root colonization by *Trichoderma* spp., which were introduced in four of the seven treatments (treatments D-G), were very low when sampled 2 years after treatment. Previous work (James 2002) indicated that Trichoderma spp. might readily colonize conifer seedling roots after the first growing season. However, in the current test, these fungi were not detected at high levels at the end of the second growing season. Perhaps they were replaced by other mycoflora, particularly mycorrhizal symbionts which are common on 2-0 seedlings at the Coeur d'Alene Nursery.

By far the most commonly isolated *Fusarium* species from the roots of seedlings was *F. oxysporum*. The same four morphotypes detected in soil assays (table 2) were isolated from white pine seedling roots (table 3). The current evaluation did not differentiate between potentially pathogenic and non-pathogenic isolates of *F. oxysporum*. Previous experience (Bloomberg 1976; James and Beall 1999, 2000; James et al. 1989b, 1991, 1996, 2000) indicated that *F. oxysporum* is likely the most important potentially pathogenic species of *Fusarium* associated with seedling roots. Extent of root colonization by this fungus (not necessarily associated with disease symptom production) is a good indicator of overall soil populations because *F. oxysporum* so readily colonizes plant root tissues (Dumroese et al. 1993; Gordon and Martyn 1997; James et al. 1991; Smith 1967).

Several other Fusarium species were also isolated from seedling roots, including *F. solani, F. acuminatum, F. sporotrichioides,* and *F. equiseti* (Corda) Sacc. Some of these species may include isolates that are pathogenic on conifer seedlings (James 2000, James and Perez 2000; James et al. 1989a, 1989b, 1991), but many are common soilborne saprophytes on seedling roots.

Characterizing the *Fusarium* population within forest nursery soils is difficult without implementing molecular methods, which may often differentiate pathogenic from non-pathogenic isolates (Appel and Gordon 1995; Gordon and Okamota 1992a, 199b, 1992c). Standard soil and root assays only reveal presence of particular species, but do not predict disease potential. Either molecular characterization or elaborate pathogenicity tests are required to determine level of disease potential within particular Fusarium populations (James 2000; James and Perez 2000; James et al. 1989b, 2000).

The primary goal of nursery soil treatment is to reduce potential pathogen populations to levels where diseases do not adversely affect seedling production or quality (Stone et al. 1995, 1997). Unless target organisms are capable of infecting seedling root tissues, they will not be able to initiate disease. Therefore, determining level of root infection by potential pathogens may be more effective in evaluating treatment efficacy than typical soil assays.

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