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EFFECTS OF TOPICAL APPLICATION OF THE BIOLOGICAL CONTROL AGENT BIOTREK® ON PRODUCTION OF BAREROOT DOUGLAS-FIR AND WESTERN WHITE PINE SEEDLINGS USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

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ABSTRACT

A preliminary evaluation of the biological control formulation of *Trichoderma harzianum* marketed as BioTrek® was conducted on bareroot Douglas-fir and western white pine seedlings at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. The biocontrol material was applied topically on seed shortly after sowing and comparisons made between treated and untreated 1-0 and 2-0 seedlings. First-year Douglas-fir height and second-year white pine height and diameter were not affected by the treatment. BioTrek® significantly reduced white pine seedling root infection by *Cylindrocarpon* spp., but not by either *Fusarium* or *Trichoderma* spp. The major *Fusarium* species isolated from roots of non-diseased seedlings was *F. oxysporum*. Further tests of BioTrek® are underway at the nursery.

INTRODUCTION

Diseases caused by soil-borne pathogens are important limiting factors in the production of bareroot conifer seedlings at the USDA Forest Service Nursery in Coeur d'Alene, Idaho (James 1982, 1983, 1993, 1995; James and others 1991). Damping-off and root diseases have traditionally been controlled by pre-plant soil fumigation using general biocides such as methyl bromide/chloropicrin and dazomet (Basamid®) (James and others 1990, 1996). Although dazomet is currently used operationally as a pre-plant soil fumigant at the Coeur d'Alene Nursery, growers would like to develop ways of producing high-quality seedlings without soil fumigation.

Recent tests at the Coeur d'Alene nursery (James and others 1996; Stone and others 1997) have shown that bare fallowing with periodic soil cultivation for at least one year prior to sowing, a conifer seedling crop may be as effective as soil fumigation in controlling soilborne pathogens. However, large-scale, field-wide evaluations of bare fallowing have not yet been conducted at the nursery.

A possible way to enhance efficacy of bare fallowing is by introducing biological agents with proven antagonism toward the important nursery soil-borne pathogens at the time of sowing. One biocontrol formulation with potential efficacy is BioTrek® 22G, comprised of granules containing *Trichoderma harzianum* strain T-22 as the active ingredient. This biocontrol agent is registered for control of several plant pathogens including *Pythium*, *Rhizoctonia*, and *Fusarium* in nursery

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and greenhouse applications; it has mostly been evaluated on high-value agricultural crops (Elad and others 1982; Harman and others 1989; Wolffhechel and Jensen 1992). Therefore, a preliminary test was conducted to evaluate effects of BioTrek® on production of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and western white pine (*Pinus monticola* Dougl.) bareroot seedlings at the Coeur d' Alene Nursery.

MATERIALS AND METHODS

Standard crops of Douglas-fir and white pine seedlings were grown in fields 3 and 4, respectively, beginning in the spring of 1997. Eight different Douglas-fir seedlots and one white pine seedlot from an improved blister rust resistant seed orchard were sown. Following sowing, seeds were covered with standard hydro-mulch used to hold seeds in place and prevent dessication. One week after sowing, an aqueous formulation of BioTrek® was applied directly over seedbeds at the rate of 1lb./yd.^{2} (0.48 kg/m^2). Four passes were required by the sprayer to apply sufficient material over seed. All eight Douglas-fir seedlots were treated; beds of two seedlots were left untreated. Some beds of white pine were treated while others were not.

Standard nursery growing regimes including irrigation, fertilization, and root pruning were performed throughout the 2-year growing cycle. At the end of the first growing season, 30 healthy-appearing Douglas-fir seedlings from each of two beds that had either been treated with BioTrek® or left untreated, and 30 white pine seedlings from treated and untreated beds were carefully excavated and analyzed for root infection by potentially pathogenic fungi as well as *Trichoderma* spp., some of which may have been the applied biocontrol agent. Because *Trichoderma* isolates were not differentiated, the proportion of the root colonizing population comprising the biocontrol agent was unknown. Seedling root systems were washed thoroughly to remove adhering soil and dissected into pieces approximately 5 mm in length. Ten root pieces per seedling were randomly selected, surface sterilized in a 10 percent bleach solution (0.525% aqueous sodium hypochlorite), and placed on a selective agar medium for *Fusarium* spp. and closely related fungi (Komada 1975). Plates were incubated under diurnal cycles of cool, fluorescent light at about 24°C for 7-10 days. Fungi emerging from root pieces were identified to genus and selected *Fusarium* isolates transferred to carnation leaf (Fisher and others 1982) and potato dextrose agar for species identification using the taxonomy of Nelson and others (1983). Height of the sampled 1-0 Douglas-fir seedlings, from the cotyledon scar to the tip of the terminal bud, was also recorded.

At the end of the second growing season, just before lifting, representative healthy-appearing Douglas-fir and white pine seedlings were excavated from treated and untreated beds. Sample sizes for Douglas-fir were 30 seedlings from each of the eight treated lots and 30 seedlings from each of the two untreated lots. For white pine, 60 seedlings were collected from both treated and untreated beds. Collected seedlings were processed for root colonization as described above. Height and diameter (caliper) of the white pine seedlings were also determined.

Morphological data (height and diameter) and percent seedling infection and colonization (based on percent of sampled root pieces colonized by particular fungi) were analyzed using a paired "t" test, comparing treated vs. untreated seedlings. Significant differences were reported at the P=0.05 level.

RESULTS AND DISCUSSION

The BioTrek® label recommends incorporating material directly into soil prior to planting to facilitate direct contact of *Trichoderma* with conifer seed during germination and root growth. *Trichoderma* was expected to colonize the rhizosphere on developing seedling roots, thus excluding infection by pathogens. Topical application over sown seed was also recommended by the pesticide representative, provided sufficient material was applied to reach recommended label rates. In this study, BioTrek® was applied over seed which had been sown in fields previously fumigated with dazomet. Therefore, it was expected that the biocontrol agent should quickly become established and dominate the fungal mycoflora near sown seeds.

Data from first-year Douglas-fir seedlings indicated that BioTrek® treatment had no significant effects on seedling height (table 1). There were also few significant differences comparing *Fusarium*, *Cylindrocarpon*, and *Trichoderma* root infection and colonization between treated and untreated seedlings (table 2). On first-year seedlings, *Trichoderma* infection and colonization was significantly (P=0.05) greater on treated than untreated white pine seedlings and *Fusarium* colonization was significantly greater on treated Douglas-fir seedlings. After 2 years' growth, white pine seedling height and diameter were not significantly affected by BioTrek® applications (table 3). BioTrek® significantly reduced infection and colonization of roots by *Cylindrocarpon* spp., but not the other assayed fungi, on 2-0 white pine seedlings (table 4). Although *Cylindrocarpon* spp. are potentially pathogenic on conifer seedlings (Dahm and Strzelczyk 1987; James 1991; James and others 1994), these fungi are usually not as pathogenic as *Fusarium* spp. On both 1-0 and 2-0 stock, BioTrek® did not significantly reduce infection and root colonization by *Fusarium* spp. on healthy-appearing Douglas-fir and white pine seedlings (tables 2 and 4).

The major *Fusarium* species consistently isolated from all seedling roots was *F.* oxysporum Schlecht. (tables 5 and 6). However, several other *Fusarium* spp., including *F. sporotrichioides* Sherb., *F. solani* (Mart.) Appel & Wollenw., and *F. acuminatum* (Ell. & Ev.), commonly colonized seedling roots as well. Although these other *Fusarium* species may be pathogenic on conifer seedlings (James 2000; James and Perez 1999; James and others 1989; Matuo and Chiba 1966; Vaatarja and Bumbieris 1967; Vaartaja and Cram 1956), they are not usually as pathogenic as *F. oxysporum*. Previous work at the Coeur d'Alene Nursery (James 1998; James and others 1990, 1996), indicated that *F. oxysporum* usually comprises more than 80 percent of the soil *Fusarium* population. If *F. oxysporum* made up such a large proportion of the *Fusarium* population in fields 3 and 4, BioTrek® may have slightly reduced *F. oxysporum* root infection (tables 5 and 6).

In this evaluation, soil fumigation prior to sowing probably greatly reduced most soil pathogen populations. The high *Fusarium* levels detected on roots by the end of the first growing season may have been due to seed infection (James 1987) or reintroductions from adjacent, non-fumigated fields or from soil below the effective fumigation level (Marois and others 1983; Miller and Norris 1970).

Effects of BioTrek® on soil pathogen populations and seedling disease incidence was not assessed. Since most bareroot diseases from soil-borne pathogens occur during the first during the first growing season (James 1996; James and Beall 1999, 2000), it is possible that BioTrek® may have improved seed germination and seedling establishment. An additional test in a different field at the Coeur d'Alene Nursery is currently underway to evaluate BioTrek®, along with several other treatments, on disease and production of white pine seedlings in non-fumigated, fallowed soil. Perhaps more definitive conclusions regarding BioTrek® efficacy in reducing soil-borne diseases will result from this other evaluation.

Table 1. Effects of Biotrek® application on height of 1-0 Douglas-fir seedlings – USDA Forest Service Nursery, Coeur d' Alene, Idaho.

Treatment	Average Height (cm)[1]
Biotrek® Treated	
Bed 1	9.9 (1.9)
Bed 2	7.4 (2.3)
Both Beds	8.7 (2.1)[2]
Untreated	
Bed 1	10.2 (2.5)
Bed 2	10.1 (2.0)
Both Beds	10.2 (2.3)[2]
All Seedlings	9.4 (2.2)

[1] Values in parentheses are standard deviations.
[2] Comparing treated vs. untreated seedlings, means are not significantly different (P=0.05) using a paired "t" test.

Table 2. Effects of Biotrek® application on 1-0 Douglas-fir and western white pine seedling root infection and colonization by *Fusarium, Cylindrocarpon,* and *Trichoderma* spp. – USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

	Douglas-fir		White Pine	
	Treated	Untreated	Treated	Untreated
Infection[2]				
Fusarium	98.3 A[4]	66.7 A	96.7 A	96.7 A
Cylindrocarpon	10.0 A	16.7 A	15.5 A	19.7 A
Trichoderma	34.5 A	22.3 A	50.0 A	6.7 B
Colonization[3]				
Fusarium	47.8 A	13.0 B	39.7 A	30.7 A
Cylindrocarpon	1.8 A	2.5 A	2.1 A	2.7 A
Trichoderma	8.3 A	5.4 A	11.7 A	1.3 B

[1] Sample sizes: Douglas-fir: 60 seedlings each for treated and untreated beds; white pine: 30 seedlings each for treated and untreated beds.

[2] Percent of sampled seedlings with roots infected by particular fungi.

[3] Percent of root pieces (10 sampled per seedling) colonized by particular fungi.

[4] For each conifer species, within each row, means followed by the same capital letter are not significantly different (P=0.05) using a paired "t" test. All percentages underwent arc-sin conversion prior to analyses.

Table 3. Effects of Biotrek® application on height and diameter of 2-0 western white pine seedlings – USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Treatment	Average Height (cm)[1]	Average Diameter (mm)[1]
Biotrek® Treated		
Bed 1	24.7 (4.3)	4.42 (0.88)
Bed 2	31.2 (4.8)	5.58 (1.14)
Both Beds	27.9 (4.5)[2]	5.00 (1.01)[2]
Untreated		
Bed 1	27.3 (5.4)	5.42 (1.27)
Bed 2	23.2 (4.3)	4.95 (0.65)
Both Beds	25.2 (4.8)[2]	5.18 (0.96)[2]
All Seedlings	26.6 (4.7)	5.09 (0.99)

[1] Values in parentheses are standard deviations.

[2] Comparing treated vs. untreated seedlings, means are not significantly different (P=0.05using a paired "t" test.

Table 4. Effects of Biotrek® application on 2-0 Douglas-fir and western white pine seedling root infection and colonization by *Fusarium, Cylindrocarpon,* and *Trichoderma* spp. – USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

	Douglas-fir		White Pine	
	Treated	Untreated	Treated	Untreated
Infection[2]				
Fusarium	56.3 A[4]	73.3 A	80.0 A	71.7 A
Cylindrocarpon	85.8 A	96.7A	35.0 B	58.3 A
Trichoderma	69.2 A	75.0 A	100.0 A	100.0 A
Colonization[3]				
Fusarium	10.7 A	15.0 A	17.3 A	12.7 A
Cylindrocarpon	30.0 A	45.5 A	5.3 B	15.8 A
Trichoderma	22.3 A	17.7 A	69.5 A	77.3 A

[1] Sample sizes: Douglas-fir: treated = 8 lots @ 30 seedlings/lot = 240 seedlings; untreated = 2 lots @ 30 seedlings/lot = 60 seedlings; white pine: 60 seedlings each for treated and untreated beds.

[2] Percent of sampled seedlings with roots infected by particular fungi.

[3] Percent of root pieces (10 sampled per seedling) colonized by particular fungi.

[4] For each conifer species, within each row, means followed by the same capital letter are not significantly different (P=0.05) using a paired "t" test. All percentages underwent arc-sin conversion before analyses.

Table 5. Effects of Biotrek® application on 1-0 Douglas-fir and western white pine seedling root colonization by different *Fusarium* spp. USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

	Douglas-fir		White Pine		
***Fusarium*[2]**	**Treated**	**Untreated**	**Treated**	**Untreated**	**All Isolates**
FOXY	51.0	16.7	87.5	90.5	60.3
FACU	43.8	57.7	2.5	2.1	30.5
FSOL	0	1.3	9.2	7.4	3.2
FAVE	4.2	2.6	0.8	0	2.6
FSPO	0	16.7	0	0	2.2
FPRO	0	3.7	0	0	0.5
FSAM	1.0	0	0	0	0.5
FEQU	0	1.3	0	0	0.5

[1] Numbers are percent of *Fusarium* isolates from sampled root pieces colonized by the particular species. All sampled seedlings appeared healthy.
[2] FOXY = *F. oxysporum*; FACU = *F. acuminatum*; FSOL = *F. solani*; FAVE = *F. avenaceum*; FSPO = *F. sporotrichioides*; FPRO = *F. proliferatum*: FSAM = *F. sambucinum;* FEQU = *F. equiseti.*

Table 6. Effects of Biotrek® application on 2-0 Douglas-fir and western white pine seedling root colonization by different *Fusarium* spp. USDA Forest Service Nursery, Coeur d'Alene, Idaho[1].

	Douglas-fir		White Pine		
***Fusarium*[2]**	**Treated**	**Untreated**	**Treated**	**Untreated**	**All Isolates**
FOXY	67.3	68.0	63.5	73.7	67.5
FSPO	20.6	18.7	0	1.3	13.3
FSOL	0	0	30.7	17.1	8.5
FACU	3.7	11.3	0	7.9	4.9
FAVE	7.5	0	2.9	0	4.2
FSAM	0.9	0	0	0	0.6
FEQU	0	0	2.9	0	0.6
FCUL	0	2.0	0	0	0.4

[1] Numbers are percent of *Fusarium* isolates from sampled root pieces colonized by the particular species. All sampled seedlings appeared healthy.
[2] FOXY = *F. oxysporum*; FSPO = *F. sporotrichioides*; FSOL = *F. solani*; FACU = *F. acuminatum*; FAVE = *F. avenaceum*; FSAM = *F. sambucinum;* FEQU = *F. equiseti*; FCUL = *F. culmorum.*

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