

# Forest Health Protection



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## PREPLANT SOIL TREATMENT EFFECTS ON PRODUCTION OF BARE ROOT BITTERBRUSH SEEDLINGS LONE PEAK CONSERVATION NURSERY DRAPER, UTAH

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### ABSTRACT

Three preplant soil treatments were evaluated for their effects on the production of bare root bitterbrush seedlings at the Lone Peak Conservation Nursery in Draper, Utah. The two fumigation treatments (standard methyl bromide/chloropicrin [MBC] and chloropicrin alone) reduced levels of soilborne *Fusarium* spp. more than bare fallowing with periodic cultivation. At the end of the growing season, *Fusarium* spp. had reinvaded beds treated with MBC at higher levels than beds treated with chloropicrin alone. Soil fumigation treatments resulted in greater seedling density than bare fallowing. Seedlings from beds treated with chloropicrin had significantly greater heights and diameters at the end of the growing season than seedlings from beds fumigated with MBC. Results of this evaluation indicate that chloropicrin by itself is as effective as MBC in controlling soilborne pathogens, resulting in production of high-quality bitterbrush seedlings. Bare fallowing was not as effective as chemical soil fumigation in reducing pathogen inoculum in soil or controlling seedling disease.

### INTRODUCTION

Production of high-quality seedlings for ornamental and reforestation plantings is an important priority of the Lone Peak Nursery in Draper, Utah. Many different hardwood and conifer species are grown annually at the nursery; most of these are produced as bare root seedlings. In order to ensure high seedling quality by reducing damage from soilborne pathogens and competition from weeds, growers at the nursery have routinely implemented pre-

plant soil fumigation with methyl bromide/chloropicrin (MBC)(66% and 33%, respectively) for many years. Although this practice is expensive, it has generally assured predictable production of high-quality seedlings in sufficient numbers to meet demands. However, methyl bromide has been identified as an important chemical destroyer of atmospheric ozone (World Meteorological Association 1995), which limits ultraviolet light from reaching the earth's surface. As a result, methyl bromide is currently being phased out and, for the most part will no longer

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be produced or used in the United States after January 2005 (Shaheen 1996; Stone et al. 1995). Therefore, nursery growers need to develop alternatives to methyl bromide for preplant soil fumigation. Efforts have been widespread throughout the U.S. to develop effective alternatives to methyl bromide in forest nurseries (Chapman 1992; Hildebrand et al. 2004; James et al. 2004a, 2004b, 2004c; Stone et al. 1995). It has generally been found that each nursery is unique in its potential disease problems and suitable alternatives must be designed specifically for that nursery. For example, dazomet, a granular soil fumigant, has been widely used at several nurseries (Boone 1988; Chapman 1992; James et al. 2004a, 2004b). It is usually effective at some nurseries (James et al. 1996; Miller and Norris 1970) but not efficacious at others (James and Beall 1999). Bare fallowing for at least 1 year prior to sowing, accompanied by periodic tilling to keep weed populations low and mix soil is often effective because it leads to reduced organic matter in soil which tends to limit microbial survival (Hildebrand et al. 2004; James and Beall 2000; Stone et al. 1995). Often, potential plant pathogens are reduced in fallowed fields because of the lack of food sources (Hildebrand et al. 2004). When susceptible seedling crops are introduced into fallowed fields, pathogen levels may be too low to initiate substantial disease. The key to effective use of fallowing to control soilborne pathogens is to ensure that supplemental organic matter is not added to soil (Hamm and Hansen 1990; Hansen et al. 1990; Ramirez-Villapudua and Munnecke 1988). Since many potential pathogens are very good saprophytes, they are able to effectively colonize most sources of organic matter, resulting in buildup of populations to the point that they can be damaging to seedling crops (Hamm and Hansen 1990; James et al. 1996).

Bitterbrush (*Purshia tridentata* [Pursh] DC) is one of the most important seedling crops produced at the Lone Peak Nursery. This plant is in high demand for wildlife habitat restoration (Austin and Urness 1983). Seedlings are grown for 1 year in bare root beds; they are then lifted in the fall and kept in cold storage until being shipped to the field, usually the following year. Root diseases, primarily caused by *Fusarium* spp., are major limiting factors in the production of bare root seedlings, including bitterbrush, at

the nursery (James 2002a, 2002b). These pathogens initiate a series of diseases including pre- and postemergence damping-off of young germinants and root decay of older seedlings (James et al. 1991, 2004a, 2004b, 2004c). As a result, stands of seedlings may sometimes be greatly reduced and quality of surviving seedlings may be adversely affected (Hildebrand et al. 2004; James et al. 1990, 2004a). *Fusarium* spp. are well-adapted soilborne pathogens that produce resting spores that remain viable for long time periods in the absence of suitable host plants (James et al. 1991; Nelson et al. 1983; Smolinska et al. 2003). Their populations can quickly expand in the presence of organic matter (Hamm and Hansen 1990; Hansen et al. 1990; Ramirez-Villapudua and Munnecke 1988) to levels that can cause diseases on susceptible nursery crops.

Because of the importance of bitterbrush and the prevalence of *Fusarium*-associated diseases at the Lone Peak Nursery, an evaluation was conducted to determine efficacy of different preplant soil treatments on production of bitterbrush seedlings.

## MATERIALS AND METHODS

This test was conducted in the production field at the farthest southern end of the nursery. Three treatments were compared: standard operational soil fumigation with MBC at 350 lbs./acre; chloropicin alone at 300 lbs./acre; and bare fallowing with periodic cultivation for one growing season prior to sowing. Preplant treatments were conducted within plots that were 50 feet in length and the equivalent of one seedbed in width (approximately 4-5 feet). Treatments were implemented in a complete randomized block design and replicated four times.

Three sets of soil samples were taken to evaluate effects of soil treatments on populations of *Fusarium*, *Trichoderma*, a group of common saprophytes and potential antagonists of *Fusarium* (Knudsen and Bin 1990; Knudsen et al. 1991; Papavizas 1985), and *Pythium*, another important group of soilborne pathogens that are known to cause disease problems at some nurseries (James 1982, 1989). The first set was taken prior to treatment to give an overall assessment of resident

populations of these groups of fungi. The second set was taken just prior to sowing in approximately the same locations as the first set to evaluate preplant treatment effects on fungal populations. A final set of soil samples was collected and processed at the end of the growing season to determine fungal population response to presence of the bitterbrush crop.

Samples were collected from within each replicate plot; two samples from near the center of each plot were collected. Each sample consisted of three cores of soil which were mixed together. Each collection consisted of soil cores 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.

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 milliliter 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 which readily supports growth of this fungus unless the medium is amended with benomyl or lithium chloride (James et al. 1990, 1996). Plates were incubated at least 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 gram 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 species identification using the taxonomy of Nelson et al. (1983).

Ratios of *Trichoderma* to *Fusarium* populations were calculated for each treatment; these ratios may indicate very rough estimates of potential disease suppressiveness of the soil since *Trichoderma* spp. are known antagonists of a wide range of soilborne plant pathogens, including *Fusarium* spp. (Knudsen and Bin 1990; Knudsen et al. 1991; Papavizas 1985).

For assays of *Pythium* populations, 0.5 g of soil was combined with 10 ml of 0.3% WA. One milliliter of solution was placed on each of three plates containing another selective medium consisting of V-8 juice agar amended with the antibiotics pimarcin, rifamycin, and ampicillin and the fungicide pentachloronitrobenzene (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.

To determine background levels of potentially pathogenic fungi on bitterbrush seed, two lots [98-310-01 and 00-310-01; 98 and 00 refer to years of collection], which were used to sow the test area, were analyzed. Sampled seed was collected from bulk storage and transferred to the laboratory for analysis. Three hundred seeds from each seedlot were incubated on Komada's medium and emerging fungi identified to genus or species using the taxonomy of Barnett and Hunter (1998) and Booth (1966).

Postemergence disease was evaluated by collecting samples of recently emerged seedlings displaying disease symptoms, such as leaf necrosis. A total of 106 seedlings were sampled from within the three treatment areas. Seedlings were transported to the laboratory and rated for disease severity based on level of above-ground leaf necrosis. Five numerical ratings were used: 1 = no leaf necrosis; 2 = 1%-25% leaf area necrosis; 3 = 26%-50% leaf area necrosis; 4 = 51-75% leaf area necrosis; 5 = greater than 75% leaf area necrosis. Seedling roots were washed thoroughly and dissected into pieces about 5 mm in length. Four root pieces per seedling were randomly selected, surface sterilized in a 10% bleach solution (0.525% aqueous sodium hypochlorite),

rinsed in sterile, distilled water, and placed on the *Fusarium* selective agar medium. Plates were incubated and emerging *Fusarium* spp. identified as described previously. Percent of sampled seedlings and extent of seedling root colonization by particular *Fusarium* spp. was calculated.

At the end of the growing season (November), seedling density within each replicated block for each treatment was measured and expressed as number of seedlings per square meter. Means for each treatment were compared with a one-way analysis of variance. Significant differences among the means were located with the LSD statistical test. After density measurements, fifty "average" seedlings from each of the three treatment areas were collected for analysis of morphological characters. Seedling heights (from the groundline to the tip the terminal bud on the longest stem), diameter (just above the groundline) and root biomass (oven-dry weight of all roots below the groundline) were measured. Means for each treatment were statistically compared with a one-way analysis of variance and LSD multiple comparison test. Significant differences were expressed at  $P=0.05$ .

A final sampling of diseased seedlings was conducted at the end of the growing season. Six seedlings displaying root disease symptoms (leaf necrosis and branch dieback) were collected from within the test area. Seedling roots were washed thoroughly and dissected into pieces about 5 mm in length. Fifteen root pieces per seedling were selected, surface sterilized, rinsed and incubated on the selective *Fusarium* agar medium. Plates were incubated and emerging *Fusarium* spp. identified as described previously. Percent of sampled seedlings and extent of seedling root colonization by particular *Fusarium* spp. was calculated.

## RESULTS

Soil in the field selected for this evaluation was populated with relatively high levels of *Fusarium*

and corresponding low levels of *Trichoderma* (table 1). The low T/F ratios probably indicated low disease suppressiveness. Populations of *Pythium* were low throughout the field.

At the time of sowing, *Fusarium* populations had been greatly reduced in plots treated with MBC and completely eliminated in chloropicrin-treated plots (table 2). Bare fallowing also reduced *Fusarium* populations, but not nearly as much as chemical fumigation and surviving populations still exceeded expected disease thresholds (Hildebrand and Dinkel 1988; James et al. 1990, 1996). *Trichoderma* levels were likewise greatly reduced by chemical fumigation and either increased or did not change in fallowed plots. *Pythium* populations were eliminated by chemical fumigation and greatly reduced by fallowing.

By the end of the growing season, *Fusarium* levels had increased to much higher levels in MBC-treated plots than in those treated only with chloropicrin (table 3). Fallowed plots also had higher *Fusarium* populations by the end of the growing season. *Trichoderma* levels also increased more in MBC-treated plots compared to chloropicrin-treated plots but did not change much in fallowed plots. *Pythium* levels increased much more in fallowed plots but were still at very low levels in fumigated plots.

Six different *Fusarium* species were identified from soil isolates within the test area (table 4). Initially, *F. solani* (Mart.) Appel & Wollenw. was by far the most prominent species; following treatment, *F. solani* and *F. oxysporum* Schlecht. were detected at similar levels. By the end of the growing season, *F. solani*, *F. oxysporum* and *F. equiseti* (Corda) Sacc. were all isolated at relatively similar levels. *Fusarium chlamydosporum* Wollenw. & Reinking was initially detected at higher levels than was found following soil treatments or at the end of the growing season. The other two species, *F. acuminatum* Ell. & Ev. and *F. scirpi* var. *compactum* (Lambotte & Fautr.) Wollenw. were detected at very low levels during each assay.

Table 1. Average pre-treatment soil populations of *Fusarium*, *Trichoderma* and *Pythium* spp. at the Lone Peak Nursery, Draper, Utah<sup>1</sup>.

Treatment	Fungus			
	<i>Fusarium</i>	<i>Trichoderma</i>	T/F Ratio <sup>2</sup>	<i>Pythium</i>
MBC <sup>3</sup>	2180	324	0.149	21
Chloropicrin <sup>4</sup>	2651	776	0.079	35
Fallow - 1	2585	254	0.098	30
Fallow - 2	2901	481	0.166	28
All Treatments	2579	460	0.178	28

<sup>1</sup>Values in table are colony-forming units per gram of oven-dry soil.

<sup>2</sup>Ratio of *Trichoderma* to *Fusarium* populations; higher numbers denote more potential for disease suppressiveness.

<sup>3</sup>Methyl bromide-chloropicrin applied at 350 lbs./acre.

<sup>4</sup>Applied at 300 lbs./acre.

Table 2. Average pre-sowing soil populations of *Fusarium*, *Trichoderma* and *Pythium* spp. at the Lone Peak Nursery, Draper, Utah<sup>1</sup>.

Treatment	Fungus			
	<i>Fusarium</i>	<i>Trichoderma</i>	T/F Ratio <sup>2</sup>	<i>Pythium</i>
MBC <sup>3</sup>	67	126	0.125	0
Chloropicrin <sup>4</sup>	0	8	0	0
Fallow - 1	1486	667	0.593	3
Fallow - 2	1612	465	0.287	7
All Treatments	791	317	0.399	2

<sup>1</sup>Values in table are colony-forming units per gram of oven-dry soil.

<sup>2</sup>Ratio of *Trichoderma* to *Fusarium* populations; higher numbers denote more potential for disease suppressiveness.

<sup>3</sup>Methyl bromide-chloropicrin applied at 350 lbs./acre.

<sup>4</sup>Applied at 300 lbs./acre.

Table 3. Average end-of-first-growing-season populations of *Fusarium*, *Trichoderma* and *Pythium* spp. at the Lone Peak Nursery, Draper, Utah<sup>1</sup>.

Treatment	Fungus			
	<i>Fusarium</i>	<i>Trichoderma</i>	T/F Ratio <sup>2</sup>	<i>Pythium</i>
MBC <sup>3</sup>	605	485	0.802	19
Chloropicrin <sup>4</sup>	108	149	1.378	9
Fallow	2163	498	0.2302	149
All Treatments	1159	397	0.343	74

<sup>1</sup>Values in table are colony-forming units per gram of oven-dry soil.

<sup>2</sup>Ratio of *Trichoderma* to *Fusarium* populations; higher numbers denote more potential for disease suppressiveness.

<sup>3</sup>Methyl bromide-chloropicrin applied at 350 lbs./acre.

<sup>4</sup>Applied at 300 lbs./acre.