Influence of Soil Fumigation and Fungicide Application on Outplanted Ponderosa Pine Seedlings

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Abstract — Ponderosa pine (Pinus ponderosa Laws.) was used as a model to determine the effects of soil fumigation and fungicide applications on outplanted stock in moderately-fertile soils of the Intermountain West, U.S.A. Five soil fumigation treatments (Vapam), singularly and in combination with fungicide (triadimefon) applications, were compared to a control. Growth characteristics, nutrition, and ectomycorrhizal colonization were evaluated. Seedling height growth at the end of the fourth growing season was best after spring fumigation. Greatest ectomycorrhizal colonization occurred in the non-fumigated treatment with fungicide applied before planting. Generally, spring or fall soil fumigation treatment, without fungicide applications, produced seedlings with the best growth. These results suggest that soil fumigation applications could improve early performance of ponderosa pine when reforesting sites with abundant, native inoculum. Ponderosa pine planted on moisture or nutrient limited sites, with adequate ectomycorrhizal inoculum, may benefit from elimination of ectomycorrhizae in the nursery.

INTRODUCTION

Ponderosa pine *(Pinus ponderosa* Laws.) is an important regeneration species on dry sites in the western U.S.A. (Linhart, 1988). Rapid achievement of full stocking following harvesting is usually a primary objective for managing ponderosa pine, but frequently is difficult to obtain. Most regeneration failures in this region result from long periods of evaporative demand, low soil moisture (Curtis and Lynch, 1957), root disease (Johnson and Zak, 1977), and poor ectomycorrhizal colonization (Harvey et al., 1992). Competition from invading or residual plants after harvest can further reduce amounts of water and nutrients available to conifer regeneration (Miller, 1987; Stewart, 1974). Nonconifer vegetation that competes with tree crops for moisture can increase the risk of regeneration failure and reduce growth of established stands (Boyd, 1985).

On sites with severe competition, common site preparation practices may not be adequate to reduce invading herbaceous species. These sites often must be replanted several times before stocking is adequate for stand development (Coffman, 1982). Alternative methods to reduce seedling mortality and/or increase growth during early years may give foresters valuable options in treating and regenerating such sites. Options could include soil fumigation before planting and/or seedling treatment with fungicides before planting.

In the western U.S.A., Vapam (sodium-N-methyl-dithiocarbamate) is sometimes used as a soil

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fumigant. Vapam is amenable for use in field situations since it does not require that the soil be covered with plastic after treatment. Vapam is less volatile than other fumigants, such as methyl bromide. If the soil is moist, tarping is not necessary. While results were preliminary (first year) fumigation with Vapam produced Douglas-fir *(Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) and white pine *(Pinus monticola* Dougl. ex. D.Don) seedlings with increased root growth and total weight as compared to seedlings planted in non-fumigated soil (Cornwall, 1985; Rainville, 1987). However, changes in plant nutrition or ectomycorrhizal function were not measured.

Soil fumigation has been shown to raise levels of available soil nitrogen (N) and phosphorus (P) and to increase nutrient uptake by seedlings (Benzian, 1965). The relationship of soil nutrient availability to plant uptake following fumigation is unclear, since addition of fertilizer does not give similar growth gains (Rovira and Simon, 1985; Bengtson and Smart, 1981; Rovira, 1976). However, increased availability of N and P may be due to loss of viability of microorganisms that tie up nutrients as well as increased nutrient levels from the dead organisms (Alexander, 1977). Soil organic matter content, cation exchange capacity, ectomycorrhizal colonization, and pH can all be affected by fumigation treatments (Sandler et al., 1988). Many fumigation studies in greenhouses and forest nurseries have shown increased numbers of ectomycorrhizal short roots, increased nutrient uptake (Henderson and Stone, 1970; Marx et al., 1978; Snyder and Davey, 1986) and increased water uptake (Menge, 1982; Zak 1964).

Fumigation also changes the microbial equilibrium of the soil. Parr (1968) suggested the pattern or sequence of reestablishment of fungi in fumigated soil varies considerably and depends on soil chemical and physical properties, as well as on the fumigant itself. In general, microbial numbers decrease after fumigation, but certain species quickly reinhabit the soil, and shortly after treatment overall numbers are usually in excess of untreated controls (Martin and Pratt 1958). Fumigation alters microbe species in favor of fungi normally suppressed by *Aspergilli* and *Penicilli*a. Actinomycetes have a greater tolerance for fumigation than either fungi or bacteria (Wensley, 1953). One principal recolonizing group in fumigated surface soils is *Trichoderma* spp. (Danielson and Davey 1969). The antagonism of *Trichoderma* to certain detrimental fungi may improve growth of outplanted seedlings. For example, *Trichoderma viride* is antagonistic to *Phytophtora, Pythium, Armillaria, Rhizoctonia,* and other parasitic fungi (Garrett, 1956; Wright, 1956; Weindling, 1938).

Broad-spectrum fumigants like Vapam have been shown to inhibit the growth of ectomycorrhizal fungi in forest nurseries (Johnson and Zak, 1977; Hacskaylo and Palmer, 1957; Trappe and Strand, 1969). Some fungicides may be more selective than broad-spectrum fumigants. They are generally believed to be less harmful to ectomycorrhizal fungi (Marx and Barnett, 1974). Application of triadimefon, 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone (Bayleton®), a systemic fungicide, does not inhibit short root development (South and Kelley, 1982). In fact, Kelley (1987) noted that after 1 year, greenhouse-grown loblolly pine *(Pinus taeda* L.) seedlings treated with triadimefon had significantly more ectomycor-rhizal short roots that control seedlings. Survival and growth of seedlings treated with triadimefon were similar to control seedlings after outplanting into plantations (Rowan and Kelley, 1986). Two-year-old loblolly pine seedlings had a similar response (Marx, 1987). These greenhouse studies differ from field nursery tests. Marx et al. (1986) noted that growth of loblolly pine in South Carolina was inhibited by the application of triadimefon.

Increases in growth that continue even after competing vegetation has reoccupied the site indicate a basic change in soil/tree root biology. Applying triadimefon in the nursery may encourage faster seedling root colonization by native ectomycorrhizal symbionts after outplanting in a forest site.

Field studies, using planting spot

methyl bromide soil fumigation in the western U.S.A., have shown a doubling of survival and growth of outplanted Douglas-fir and ponderosa pine on dry sites in eastern Washington (Klock, 1980). Fumigant-induced changes in soil biology may affect disease resistance, soil nutrient availability, or ectomycorrhizal development (Bengtson and Smart, 1981; Klock, 1980).

This paper examines the impact of planting-site fumigation with Vapam and fungicide application (triadimefon) on ponderosa pine. It considers ectomycorrhizal colonization, seedling growth and nutrition, and soil N transformations in a moderately fertile, forested environment with high levels of native ectomycorrhizal inoculum.

METHODS AND MATERIALS

Site Description

The study was located on the Priest River Experimental Forest 19 km N of Priest River, ID, U.S.A. at an elevation of 1550 m. The previous stand, which consisted of western white pine, Douglas-fir, lodgepole pine *(Pinus contorta* Dougl. ex Loud.), and western larch *(Larix occidentalis* Nutt.), was cleared in 1985 and the soil rototilled to a depth of 30 cm. The soil is a coarse loamy, mixed, frigid Typic Xerochrept (Soil Survey Staff, 1975). Mean annual average precipitation and temperature is 84 cm and 6.6°C, respectively.

Study Design

A randomized complete block design was established on this site in 1988, with three replications of five treatments and an untreated control. Treatments consisted of:

(1) spring soil fumigation with Vapam,
(2) fall soil fumigation with Vapam,
(3) fall soil fumigation with Vapam and triadimefon applied to seedlings in the greenhouse before outplanting,
(4) fall soil fumigation with Vapam and triadimefon applied to seedlings in the greenhouse before outplanting, plus 4 additional times during the first growing season,
(5) no soil fumigation and triadimefon applied to seedlings in the greenhouse before outplanting.

Seedlings treated with fungicide before planting had triadimefon applied 3 times at 2-week intervals, while actively growing in the greenhouse. The concentration was 1.8 mg active ingredient/seedling mixed with a surfactant (Marx, 1987). Plots were 30 m * 30 m in size with a 1 m buffer.

Soil Treatment Application

Fall and spring soil fumigations were achieved by applying 450 ml/m^2 Vapam after the soil had been soaked to a depth of 30 cm by rain. Treated areas were water-sealed to move the Vapam into the soil. Fall fumigation plots were treated the second week of October (1988) and the soil covered with black plastic until 4 weeks before spring planting. Spring fumigation plots were treated with Vapam, at the same rate as the fall fumigation treatments, approximately 6 weeks before planting (1989). The plots were covered with black plastic for 2 weeks, then uncovered for 4 weeks prior to planting to allow dissipation of the fumigant. Plots were tested for remaining fumigant by planting highly sensitive tomato plants as an indicator 1 week before seedlings were outplanted. Plots were then planted with 1-year-old container-grown ponderosa pine seedlings on a 1-by-1 m spacing.

Seedling Sampling and Measurements

Before outplanting, 5 seedling root systems from each treatment were examined for ectomycorrhizal root tips. Active ectomycorrhizal root tips comprised approximately 10 percent of the root system, except the triadimefon treated seedlings which had no active ectomycorrhizal root tips. Monthly during the first and second growing seasons, 5 seedlings from each treatment and replication were carefully excavated. Top height, rooting depth, and root-collar diameter were measured. Tops of seedlings were severed from the roots, dried at 60° C for 24 h, weighed, and ground to pass a 20-mesh sieve. Entire seedling tops were analyzed for total N and P by Kjeldahl digestion methods using the salicylic acid-sodium thiosulfate modification (Bremner and Mulvaney, 1982). They were analyzed on an Alpkem Rapid Flow Analyzer. Calcium (Ca) and magnesium (Mg) were analyzed by atomic absorption spectroscopy, potassium (K) was analyzed by flame emission after samples had been dry ashed at 450° C and leached with 2N HNO_3. After seedling roots were washed, total ectomycorrhizal tips counted with a 20x binocular microscope. Roots were then dried at 60° C for 24 h and weighed.

Soil Sampling and Analysis

Five 20 g random soil core samples were collected in each treatment 1 d before fumigation, and 14 and 45 d after fumigation. They were sieved to pass a 2 mm sieve. Control soil samples were collected at the same time as the fall fumigation samples. Ammonium - nitrogen (NH_4-N) and nitrate-nitrogen (NO_3-N) were analyzed on undried samples in a 1N KCl extract using and Alpkem Rapid Flow Analyzer (Keeney and Nelson, 1982).

Statistical Analysis

The significance of difference among treatments was tested using a one-way analysis of variance (ANOVA) for a randomized complete block design followed by Scheffe's multiple range test (Scheffe, 1953). A type I error rate of 0.05 was applied.

RESULTS AND DISCUSSION

Seedling growth characteristics

After the first growing season, height growth was greatest in the fumigation only treatments, and least in the fall fumigation treatment with continuous triadimefon application (Table 1). Rooting depth was unaffected by these treatments (data not shown). Root-collar diameter was greatest in the fumigation only treatments. Second- year seedling growth was similar to the first year. Seedlings growing in the fumigated treatments had greater height growth, root-collar diameter, and biomass than the other treatments, but, also as with the first-year, the differences were not always significant. By the end of the fourth growing season, seedlings growing in the spring fumigation plots had significantly greater height than those in any other treatments (Table 2).

Spring fumigation before outplanting provided the environment for improved seedling growth. In Vapam treated soil, *Tricho-Derma viride* and *Penicillium* sp. are the dominant recolonizers (Singha, et al., 1979; Corden and Young, 1965). These fungi may act as antagonists against potential pathogens. Fall fumigation may allow recolonization of all species of microbes in the rooting zone before seedling outplanting the following spring. Other investigators have also noted striking increases in seedling height and biomass in fumigated soil, compared to controls that were not fumigated (James et al., 1990; Rainville, 1987; Cornwall, 1985; Klock, 1980; Henderson and Stone, 1970).

The lack of many significant differences between treatments and controls during the first growing season may be due to outplanting containerized nursery stock with pre-set buds and a high nutrient status (Kozlowski et al., 1973). Although no biomass measurements of herbaceous competition were taken, visual assessment indicated the fumigated plots has substantially less competition than plots that were not fumigated. This may also contribute to growth differences.

By the end of the second year, seedlings that had triadimefon applied, either before planting or continuously, had reduced height growth, root-collar diameter and total biomass compared to the fumigation only treatments. Marx (1987) and Rowan and Kelley (1986) found no significant effects of triadimefon on first-year growth of nursery-grown bareroot loblolly or slash pine *(Pinus elliottii* Engelm.) seedlings compared to control seedlings. However, Davis (1991) found that triadimefon can act as a potent inhibitor of shoot growth with effects persisting for several years.

Table 1. Growth characteristics of ponderosa pine as affected by site treatment.

TREATMENT	Year 1			Year 2		
	Height - cm -	Caliper - mm -	Biomass - g -	Height - cm -	Caliper - mm -	Biomass - g -
Spring fumigation	18.1a [B]	4.7ab	6.0ab	36.1a	12.7a	49.9a
Fall fumigation	18.1a	5.2a	6.1a	33.5ab	11.9a	45.7ab
Fall fumigation and triadimefon	17.6ab	4.5abc	6.2a	32.3b	11.7a	41.4ab
Fall fumigation and continuous triadimefon [A]	16.8b	4.6ab	5.7ab	32.7ab	11.8a	42.1ab
No fumigation and triadimefon	17.4ab	3.8c	5.1b	30.8b	10.0b	34.9b
Control	17.6ab	4.4bc	5.2b	33.2ab	11.8a	42.9b

[A] Continuous triadimefon during year 1 only.
[B] Different letters indicate significant differences (P<0.05) among treatments.

Ectomycorrhizae colonization

There were no significant treatment differences during the first year in total ectomycor-rhizal root tips (Table 3). This is consistent with results from the southeastern U.S.A. where increasing rates of triadimefon did not alter first year ectomycorrhizal colonization of feeder roots (Kelley, 1987). However, ectomycor-rhizal root tips per gram of dry roots were significantly greater in the no fumigation, before planting triadimefon application treatment compared to the others. This is probably related to slightly greater number of total ectomycorrhizal tips on these seedlings. In this study, fumigation alone did not improve ectomycorrhizal colonization of ponderosa pine.

By the end of the second growing season, ectomycorrhizal colonization was greatest in the fall fumigation treatment that had triadimefon applied continuously during the first growing season and the no fumigation treatments with triadimefon applied before planting. Fall fumigation alone and fall fumigation treatments with triadimefon applied before planting were not significantly different from these treatments. Soil fumigation has been shown to reduce populations of ectomycorrhizal fungi (Johnson and Zak, 1977). Without reinoculation of seedling root systems, or ample native ectomycorrhizae present, colonization can be slow (Henderson and

Table 2. Height of 4-year-old ponderosa pine seedlings as affected by site treatment.

TREATMENT	Height - cm -
Spring fumigation	74.8a [B]
Fall fumigation	68.5b
Fall fumigation and triadimefon	68.5b
Fall fumigation and continuous triadimefon [A]	64.9b
No fumigation and triadimefon	60.6c
Control	68.1b

[A] Continuous triadimefon during year 1 only.
[B] Different letters indicate significant differences (P<0.05) among treatments

Table 3. Ectomycorrhizal short root (EMSR) colonization of ponderosa pine as affected by site treatment.

TREATMENT	Year 1		Year 2	
	Total Tips	Tips/g dry root	Total Tips	Tips/g dry root
Spring fumigation	30a [B]	15b	32b	3a
Fall fumigation	28a	13b	42ab	8a
Fall fumigation and triadimefon	31a	20b	39ab	4a
Fall fumigation and continuous triadimefon [A]	24a	10b	59a	8a
No fumigation and triadimefon	49a	36a	62a	9a
Control	32a	22ab	42ab	5a

[A] Continuous triadimefon during year 1 only.
[B] Different letters indicate significant differences ($P<0.05$) among treatments.

Stone 1970). In this study, fall fumigation likely allowed mycorrhizal inoculum to recover before planting.

Triadimefon may rid seedlings of non-site-specific ectomycorrhizae early in the first growing season and allow site-specific ectomycorrhizae to colonize roots vigorously (Harvey et al., 1991; Page-Dumroese et al., 1990). It appears that maximum seedling growth can be achieved with a moderate number of native ectomycorrhizae.

Seedling nutrition

Seedling nutrient concentrations did not always correspond to seedling growth characteristics. After the first growing season, seedling K concentration among treatments was unchanged (Table 4). In the fall fumigation treatment with triadimefon applied before planting, Mg concentrations were greatest compared to most other treatments, except the no fumigation triadimefon treatment before planting. Results were similar for Ca. Greater concentrations of Ca and Mg in triadimefon-treated seedlings may be caused by residual triadimefon and its metabolite triadimenol in the needles. After foliar application of triadimefon, it remains in the sprayed portion of the plant and is usually not redistributed through it, but may move to the leaf margins (Davidse and DeWaard, 1984). Triadimefon has been shown to reduce transpiration and produce thicker, darker leaves on many plants (Fletcher and Nath, 1984).

Although significant differences in seedling total P were found during the first-growing season, the actual values differed by very little. By the end of the second year, differences were no longer significant. Because endemic ectomycorrhizae were likely eliminated from the fumigation plots, we expected seedling P to be depressed (Henderson and Stone, 1970). Apparently, in this soil type, P was available in sufficient quantity for normal seedling uptake.

Total seedling N was greatest in the two fumigation-only treatments. During the second-year, fall fumigation treatments with triadimefon applied before planting had the lowest total N values. This may be due to the plant growth-regulating properties of triadimefon. Wang et al. (1986) reported that triadimefon applications increased the N nutrition of applied seedlings. However, most differences associated with triadimefon applications have been caused by variations in application rates and frequencies and by variations in environmental

Table 4. Nutrient concentrations of ponderosa pine foliage and stems as affected by site treatment.

TREATMENT	Year 1					Year 2				
	Total N	Total P	Ca	Mg	K	Total N	Total P	Ca	Mg	K
	-- percent --		------- mg/kg -------			-- percent --		------- mg/kg -------		
Spring fumigation	0.79a [B]	0.10ab	2168a	894b	5464a	1.78a	0.35a	1863c	813a	5757a
Fall fumigation	0.81a	0.10ab	2325ab	905b	5076a	1.73a	0.45a	1982bc	823a	5279a
Fall fumigation and triadimefon	0.76ab	0.09b	2565a	1145a	4937a	1.72a	0.36a	2042bc	848a	5416a
Fall fumigation and continuous triadimefon [A]	0.78ab	0.10ab	2124b	884b	5386a	1.55b	0.36a	2241a	849a	5528a
No fumigation and triadimefon	0.69b	0.10ab	2358ab	982ab	5009a	1.68ab	0.37a	2153ab	809b	5484a
Control	0.76ab	0.11a	2045b	859b	5146a	1.77a	0.39a	2021bc	836a	5694a

[A] Continuous triadimefon during year 1 only.
[B] Different letters indicate significant differences (P<0.05) among treatments.

conditions.

Soil N transformations

Although there was an immediate increase in ammonium-N in fumigated soils, levels had dropped offto before fumigation levels by 45 d after treatment (Fig. 1). Spring fumigation was the only treatment with significantly higher ammonium at planting time. Higher concentrations in the spring fumigation treatment may be attributed to warm soil temperatures immediately after fumigation. The short elapsed time between fumigation and planting in this treatment likely allowed seedlings to capitalize on N releases. This may have contributed to improved seedling growth in this treatment. Similar results are reported elsewhere (Singha et al., 1979; Munnecke and Ferguson, 1960).

Soil nitrate-N levels followed the same trend as ammonium-N, with the spring fumigation treatment having the highest concentration at planting time (Fig. 2). Other studies have shown that fumigation had a strong, initial effect of depressing nitrification (Singhaetal.,1979; Munnecke and Ferguson, 1960). This was apparently not the case here. The toxic effect of Vapam on nitrifiers has been attributed to the highly toxic compound methyl isothiocyanate. Since fumigation rates were relatively low and Vapam was watered into the soil after application,toxicitymayhave been reduced for this study.

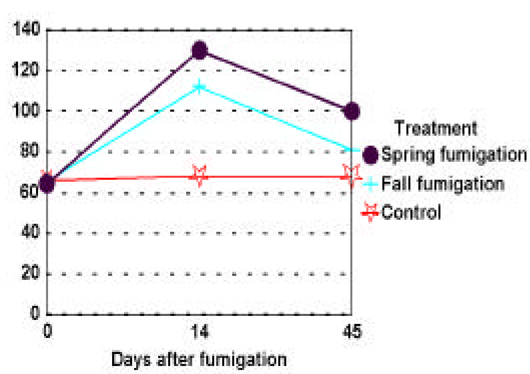

Figure 1. Effect of soil fumigation with Vapam on ammonium-nitrogen levels (mg/kg).

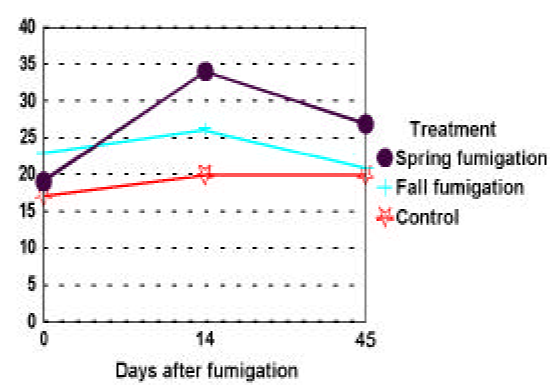

Figure 2. Effect of soil fumigation with Vapam on nitrate-nitrogen levels (mg/kg).

CONCLUSION

Fumigation offers an effective way to rid a site of competition by controlling both vegetation and microorganisms in the rooting zone. This treatment alone may provide an environment for enhanced regeneration performance. Spring fumigation provides the greatest opportunity for improving ponderosa pine seedling growth after outplanting, however fall fumigation was also fairly successful. Fall fumigation may be more effective if seedlings are planted 4 to 6 weeks after fumigation, depending on timing and weather, instead of the following spring. Initial growth increases from fumigation, particularly if conducted in the spring just before planting, appear to last well beyond the first growing season. Eliminating ectomycorrhizae before planting, or during the first growing season, enhanced second year colonization, but seedling growth was not improved over moderate colonization levels. Use of triadimefon to eliminate nursery produced ectomycorrhizae may only be useful if rapid colonization by native ectomycorrhizae is assured by abundant, native inoculum and required for seedling survival.

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