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Ponderosa Pine Seedling Response to Planting-site Soil Fumigation and Fungicide Application

Abstract

Ponderosa pine (*Pinus ponderosa* Laws.) was used as a biological model to determine the effects of planting site soil fumigation and fungicide applications on stock planted in moderately-fertile soils of the Intermountain West of the United States. Five soil fumigation (Vapam) treatments, singularly and in combination with fungicide (triadimefon) applications, were compared to a control. Growth characteristics, nutrition, and ectomycorrhizal colonization were evaluated. Seedlings were significantly taller in the spring fumigation treatment at the end of four growing seasons. Greatest ectomycorrhizal colonization occurred in the non-fumigated treatment with fungicide applied before planting. Generally, spring or fall fumigation treatments, without fungicide applications, produced the tallest seedlings. These results suggest that soil fumigation applications before outplanting could improve early performance of ponderosa pine when reforesting sites with abundant, native ectomycorrhizal inoculum. It also suggests that if fumigation is impractical, reduction of vegetative competition may give similar growth gains. Elimination of non-native ectomycorrhizae in the nursery may improve colonization after outplanting.

Introduction

Ponderosa pine (*Pinus ponderosa* Laws.) is an important species on dry sites in the western U.S.A. (Linhart 1988). Rapid achievement of full stocking following harvesting is frequently difficult for ponderosa pine. Most regeneration failures in the western U.S.A. result from long periods of evaporative demand combined with low soil moisture during the growing season (Curtis and Lynch 1957). Low soil nutrient levels, particularly nitrogen (N), often compound moisture problems (Jurgensen et al. 1986). Competition from invading or residual plant regrowth 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 (i.e. scalping and burning) 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.

Soil fumigants are routinely used in bareroot forest tree nursery management to decrease weed competition, eliminate soil-borne pathogens, and reduce nematode infection (James et al. 1990; Tanaka et al. 1986). Fumigation usually results in superior seedling growth in nursery soils, which can continue after seedlings are outplanted (Benzian 1965; Danielson and Davey 1969; James et al. 1990; Thies and Patton 1971).

Klock (1980) used methyl bromide to fumigate planting spots during a field study in eastern Washington. Survival and growth of Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) and ponderosa pine planted on these dry sites more than doubled compared to controls after 5 years. He speculated that fumigant-induced changes in soil biology affected disease resistance, soil nutrient availability, or ectomycorrhizal development, but detailed measurements were not taken (Bengtson and Smart 1981; Klock 1980).

In the western U.S.A., Vapam (sodium-N-methyl-dithiocarbamate) is often used as a soil fumigant in forest nurseries and may be more amenable for use in field outplanting 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 and is registered for fumigation of planting sites. While results were preliminary (first year), fumigation with Vapam produced Douglas-fir and western 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. Vapam fumigation also eliminated competing vegetation (Cornwall 1985; Rainville 1987). However, changes in plant nutrition or ectomycorrhizal function were not measured.

Application of triadimefon, 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone, a systemic fungicide, inhibits ectomycorrhizal short root development in forest nurseries (South and Kelley 1982). However, Kelley (1987) noted that after 1 year, greenhouse-grown loblolly pine (*Pinus taeda* L.) seedlings treated with triadimefon had significantly more ectomycorrhizal short roots than control seedlings. Survival and growth of seedlings treated with triadimefon were similar to control seedlings after outplanting into plantations (Rowan and Kelley 1986).

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 to a forest site. Thus, soil sterilization as a method for competition control (both microbial and plant) and reduction of non-native ectomycorrhizae may have an additive effect and prove effective regeneration tools.

The objectives of this study were to: (1) examine the impact of planting-site fumigation with Vapam and fungicide applications of triadimefon on survival and growth ponderosa pine seedlings, and (2) determine seedling ectomycorrhizal colonization, height, diameter, and nutrition, and evaluate soil ammonium and nitrate levels in a moderately fertile, forested environment with high levels of native ectomycorrhizal inoculum.

Methods and Materials

Site Description

The study site is located at an elevation of 800 m on the Priest River Experimental Forest 19 km N of Priest River, ID, U.S.A. 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 precipitation and temperature are 84 cm and 6.6° C, respectively. Approximately 80% of the precipitation falls as snow (Wellner 1976).

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) fall soil fumigation with Vapam[1],

(2) spring 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 four additional times throughout the first growing season,

(5) no soil fumigation and triadimefon applied to seedlings in the greenhouse before outplanting.

Seedlings treated with fungicide before planting (treatments 3, 4, and 5) had triadimefon applied three times at 2-week intervals, while actively growing in the greenhouse. Triadimefon was selected because of its suppressive effect on ectomycorrhizae development on pines (Marx and Cordell 1987). 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 with 450 ml/m^2 of Vapam after the soil had been soaked to a depth of 30 cm by rain. Vapam was applied using a 10 liter watering can. Treated areas were water-sealed (surface soil soaked with water) to move the Vapam into the soil. Fall fumigation plots were treated the second week of October (1988). The soil was covered with black plastic until 4 weeks before spring planting. Spring fumigation plots were treated approximately 6

[1] The use of trade or firm names in this paper is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

weeks before planting (1989). The plots were covered with black plastic for 2 weeks, but were uncovered for 4 weeks before planting to allow the fumigant to dissipate. Control plots were covered at the same time spring fumigation plots were covered. Plots were covered with black plastic so they would be uncovered a uniform time before planting. Plots were tested for any remaining fumigant by planting highly sensitive tomato plants as an indicator 1 week before seedlings were outplanted. Plots were planted with 100 1-yr-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 in each treatment except the triadimefon treated seedlings which had none. After budset of 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. Seedling shoots (stem, branches, and needles combined) were prepared for analysis of Total N and phosphorus (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 II Model 300. 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 were counted with a 20x binocular microscope. Roots were subsequently dried at 60° C for 24 h and weighed.

Soil Sampling and Analysis

Five 20 g random soil core samples were collected to a depth of 15 cm in each treatment replication 1 d before fumigation, and 14 and 45 d after fumigation. They were sieved to pass a 2 mm screen. Control soil samples were collected at the same time as the fall fumigation samples. Undried soil samples were extracted using 1N KCl and analyzed for ammonium-nitrogen and nitrate-nitrogen using an Alpkem Rapid Flow Analyzer II Model 300 (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 $p \leq 0.05$ 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 through year four was unaffected by these treatments (data not shown). Survival after the first growing season was 70% in the control, 71% in the no fumigation-triadimefon treatment, 85% in the fall fumigation and continuous triadimefon treatment, 87% in the fall fumigation pre-plant triadimefon treatment, 90% in the fall fumigation treatment, and 96% in the spring fumigation treatment. 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 growth, diameter, and biomass than the other treatments, but, as with the first-year, the differences were not always significant. By the end of the fourth season the seedlings growing in the spring fumigation plots had significantly greater height than those in the other treatments (Table 2).

Spring fumigation before outplanting may provide the necessary environment (i.e. control of resident fungi adversely affecting root systems) for improved seedling growth. Fall fumigation may allow recolonization of the soil before spring outplanting. Other investigators have noted striking increases in seedling height and biomass in fumigated soil compared to controls that were not fumigated (Anderson and Liberta 1992; Cornwall 1985; Henderson and Stone 1970; James et al. 1990; Klock 1980; Rainville 1987).

The cost of using Vapam across an entire site may be prohibitive ($2470/ha) (Landis and Campbell 1989). However, soil fumigation as a

TABLE 1. Growth characteristics of ponderosa pine seedlings as affected by site treatment one and two years after treatment. (n=20 for year 1 and n=20 for year 2)

Treatment	Year 1			Year 2		
	Height (cm)	Caliper (mm)	Total Biomass (g)	Height (cm)	Caliper (mm)	Total Biomass (g)
Spring fumigation	18.1a (0.3)[B]	4.7ab (0.2)	6.0ab (0.4)	36.1a (0.9)	12.7a (0.4)	49.9a (3.2)
Fall fumigation	18.1a (0.3)	5.2a (0.2)	6.1a (0.4)	33.5ab (0.9)	11.9a (0.4)	45.7ab (3.2)
Fall fumigation and triadimefon	17.6ab (0.3)	4.5abc (0.2)	6.2a (0.4)	32.3b (0.9)	11.7a (0.4)	41.4ab (3.3)
Fall fumigation and continuous triadimefon[A]	16.8b (0.3)	4.6ab (0.2)	5.7ab (0.4)	32.7ab (0.9)	11.8a (0.4)	42.1ab (3.2)
No fumigation and triadimefon	17.4ab (0.3)	3.8c (0.3)	5.1b (0.3)	30.8b (0.9)	10.0b (0.4)	34.9b (3.3)
Control	17.6ab (0.4)	4.4bc (0.3)	5.2b (0.4)	33.2ab (0.9)	11.8a (0.4)	42.9b (3.2)

[A]Continuous triadimefon during year 1 only.

[B]Different letters indicate significant differences ($p \leq 0.05$) among treatments. Values in parentheses are standard error of the mean.

TABLE 2. Height of 4-year-old ponderosa pine seedlings as affected by site treatment (n=250).

Treatment	Height (cm)
Spring fumigation	74.8a (0.8)[B]
Fall fumigation	68.5b (0.9)
Fall fumigation and triadimefon	68.5b (0.9)
Fall fumigation and continuous triadimefon[A]	64.9b (0.9)
No fumigation and triadimefon	60.6c (0.9)
Control	68.1b (0.9)

[A]Continuous triadimefon during year 1 only.

[B]Different letters indicate significant differences ($p \leq 0.05$) among treatments. Values in parentheses are standard error of the mean

site preparation technique in the western U.S.A. could be used on certain problem sites which have a long history of regeneration failure or inadequate seedling establishment.

The lack of many significant differences between treatments and controls during the first growing season may be due to outplanting nursery stock with pre-set buds and a high nutrient status (Kozlowski et al. 1973). The growth changes in fumigated soil treatments may be at least partially associated with altered populations of soil microbes or a reduced level of weakly pathogenic fungi (fungi that slowly deteriorate root systems) (James et al. 1990; Munnecke et al. 1978; Smith and Bega 1966). Although no biomass measurements of herbaceous competition were taken, visual assessment indicated the fumigated plots had substantially less competition than plots that were not fumigated. This may also contribute to growth increases. More moisture may have been available for seedling uptake.

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 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 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.

Ectomycorrhizae Colonization

There were no significant treatment differences during the first year in total ectomycorrhizal root tips between treatments (Table 3). This is consistent with results from the southeastern U.S.A.

TABLE 3. Total ectomycorrhizal short root (EMSR) colonization and EMSR/gram of dry root of ponderosa pine seedlings one and two years after site treatment. (n=20 for year 1 and n=20 for year 2)

Treatment	Year 1 Total EMSR	Year 1 EMSR/gram of dry root	Year 2 Total EMSR	Year 2 EMSR/gram of dry root
Spring fumigation	30a (4)[B]	15b (3)	32b (6)	3a (2)
Fall fumigation	28a (4)	13b (2)	42ab (6)	8a (2)
Fall fumigation and triadimefon	31a (4)	20b (2)	39ab (6)	4a (2)
Fall fumigation and continuous triadimefon	24a (4)	10b (30)	59a (6)	8a (2)
No fumigation and triadimefon	49a (4)	36a (3)	62a (6)	9a (2)
Control	32a (4)	22ab (3)	42ab (6)	5a (2)

[A]Continuous triadimefon during year 1 only.

[B]Different letters indicate significant differences ($p \leq 0.05$) among treatments. Values in parentheses are standard error of the mean.

where increasing rates of triadimefon did not alter first year ectomycorrhizal colonization of feeder roots (Kelley 1987). However, ectomycorrhizal root tips per gram of dry root were significantly greater in the no fumigation, before planting, triadimefon application treatment compared to the others. This is probably related to the slightly greater number of total ectomycorrhizal tips on these seedlings. In this study, fumigation alone did not improve ectomycorrhizal colonization of ponderosa pine. In fact, soil fumigation has been shown to reduce populations of ectomycorrhizal fungi (Johnson and Zak 1977). Interestingly, suppression of ectomycorrhizae in the nursery and during the first growing season by triadimefon resulted in a general increase in total ectomycorrhizal tips the second growing season. However, on seedlings that were not reinoculated with ectomycorrhizae or without large native populations of ectomycorrhizae available, colonization can be slow (Henderson and Stone 1970).

Triadimefon may rid seedlings of non-site-specific ectomycorrhizae early in the first growing season and allow site-specific ectomycorrhizae to colonize roots more vigorously (Harvey et al. 1991; Page-Dumroese et al. 1990). Although triadimefon suppresses ectomycorrhizae development on pines (Marx and Cordell 1987), it appears that maximum seedling growth can be achieved with a moderate number of native ectomycorrhizae. In this study, greater ectomycorrhizal colonization did not translate into greater seedling biomass.

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 except for movement 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).

While there were significant differences in first year total seedling P, the actual values differed by very little and are probably not biologically significant. By the end of the second year differences were no longer important. Because endemic ectomycorrhizae were likely eliminated from the fumigation plots, we expected that seedling P would be depressed (Anderson and Liberta 1992; Henderson and Stone 1970). Apparently, in this soil type, P was available in sufficient quantity for normal seedling uptake.

TABLE 4. Nutrient concentrations of combined ponderosa pine seedling foliage and stems one and two years after site treatment. (n=20 for year 1 and n=20 for year 2).

Treatment	Year 1					Year 2				
	Total N	Total P	Ca	Mg	K	Total N	Total P	Ca	Mg	K
	------(percent)------		---------(up/g)--------			------(percent)------		---------(ug/g)---------		
Spring fumigation	0.79a (0.05)[B]	0.10ab (0.06)	2168a (192)	894b (87)	5464a (55)	1.78a (0.32)	0.35a (0.03)	1863c (112)	813a (40)	5757a (280)
Fall fumigation	0.81a (0.05)	0.10ab (0.06)	2325ab (174)	905b (79)	5076a (50)	1.73a (0.31)	0.45a (0.03)	1982bc (106)	823a (40)	5279a (265)
Fall fumigation and triadimefon	0.76ab (0.04)	0.09b (0.05)	2565a (161)	1145a (73)	4937a (46)	1.72a (0.32)	0.36a (0.03)	2042bc (111)	848a (42)	5416a (276)
Fall fumigation and continuous tridimefon[A]	0.78ab (0.05)	0.10ab (0.06)	2124b (174)	884b (79)	5386a (50)	1.55b (0.35)	0.36a (0.03)	2241a (121)	849a (45)	5528a (301)
No fumigation and triadimefon	0.69b (0.05)	0.10ab (0.06)	2358ab (192)	982ab (87)	5009a (55)	1.68ab (0.33)	0.37a (0.03)	2153ab (112)	809b (42)	5484a (281)
Control	0.76ab (0.04)	0.11a (0.05)	2045b (165)	859b (75)	5146a (47)	1.77a (0.31)	0.39a (0.03)	2021bc (107)	836a (40)	5694a (268)

[A]Continuous triadimefon during year 1 only.

[B]Different letters indicate significant differences ($p \leq 0.05$) among treatments. Values in parentheses are standard error of the mean.

Ponderosa pine total N was greatest in the two fumigation-only treatments. Second-year fall fumigation with continuous first year triadimefon had the lowest total N values. This may be due to the plant growth-regulating properties of triadimefon (Davis 1991; Fletcher and Nath 1984). Wang et al. (1986) reported that triadimefon applications increased the mineral nutrition of apple seedlings. Most differences associated with triadimefon applications have likely been caused by variations in application rates and frequencies or by variations in environmental conditions. Soil fumigation has been shown to increase tree nutrient uptake (Benzian 1965). In this study, fumigation alone slightly increased seedling N concentrations during the first growing season. However, second year results indicate that total N was not different from the controls.

Soil N Transformations

Although there was an immediate increase in ammonium-N in fumigated soils, levels had dropped off to near those before fumigation levels by 45 d after fumigation (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 may have allowed seedlings to capitalize on N releases. This likely contributed to the improved seedling growth in this treatment. Similar results are reported elsewhere (Munnecke and Ferguson 1960; Singha et al. 1979).

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 (Munnecke and Ferguson 1960; Singha et al. 1979). The toxic effect of Vapam on nitrifiers has been attributed to the highly toxic compound methyl isothiocyanate (Tu 1972). This was apparently not the case here since fumigation rates were low and Vapam was watered into the soil after application and its toxicity may have been reduced.

Conclusions

Fumigation offers an effective way to rid a site of all competition by controlling both vegetation and microorganisms in the rooting zone. This treatment alone provides an environment conducive for enhanced regeneration performance. Spring fumigation provides the greatest opportunity for improving ponderosa pine seedling growth,

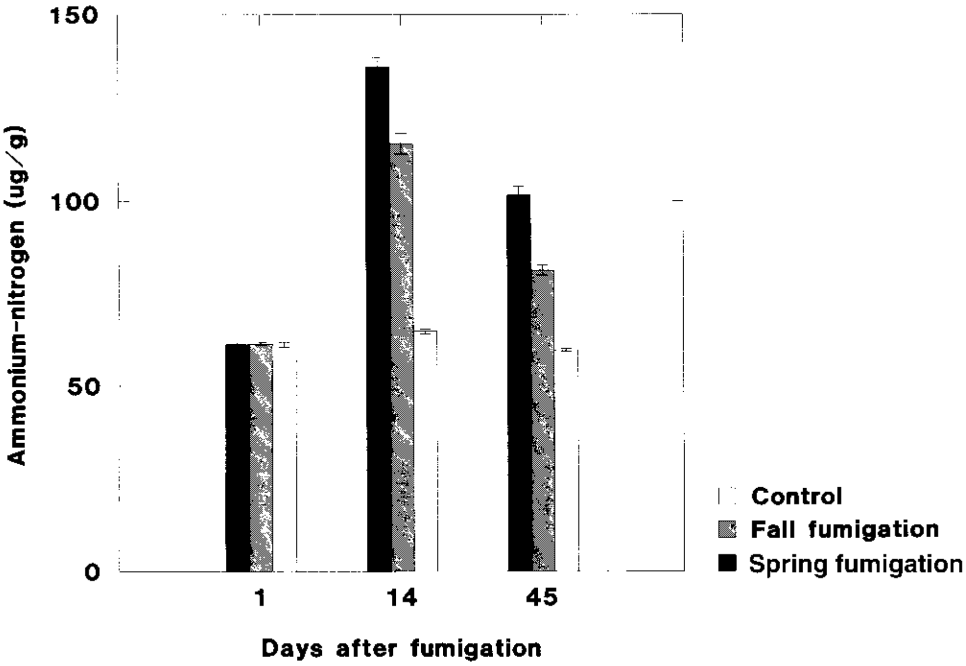

Figure 1. Change in soil ammonium-N levels 1, 15, and 45 days after fumigation with Vapam. Error bars indicate 95% confidence intervals around the mean.

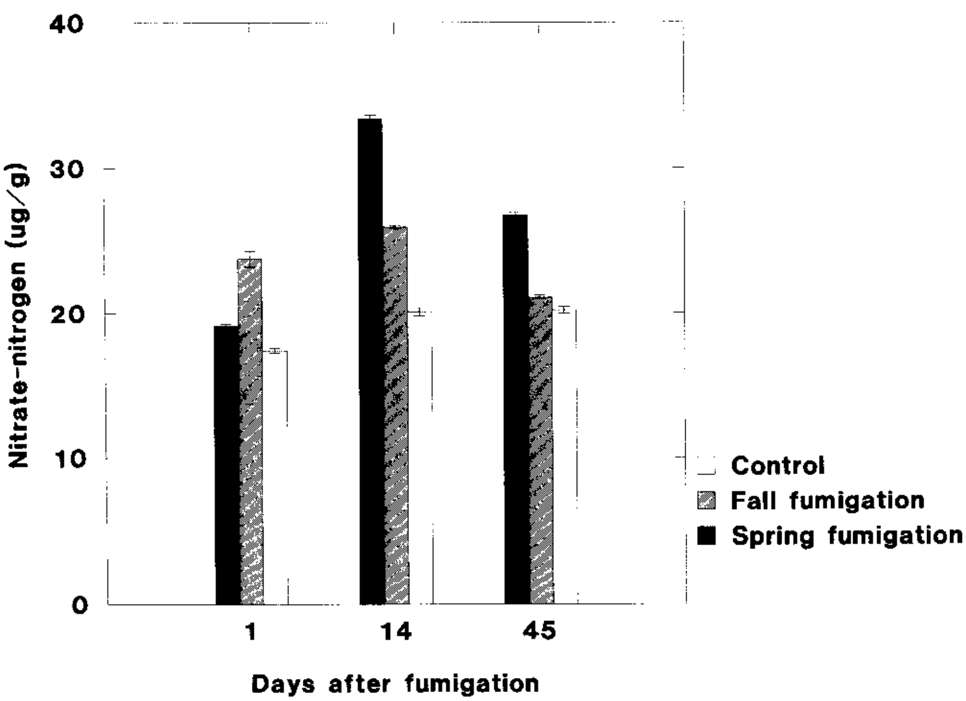

Figure 2. Change in soil nitrate-N levels 1, 15, and 45 days after fumigation with Vapam. Error bars indicate 95% confidence intervals around the mean.

however fall fumigation was also successful. Fall fumigation may be more effective if seedlings are planted 4 to 6 weeks after the fumigation instead of the following spring. However, fall weather in the western U.S.A. is often unfavorable to either fumigation or planting. Initial growth increases from fumigation, particularly if conducted in the spring prior to planting, appear to last well beyond the first growing season.

While mechanical site preparation and burning may be the standard tools of a land manager, fumigation might provide a viable alternative for successful regeneration on harsh sites with abundant competition. Fumigation is a method that can increase seedling survival and growth immediately after planting, a characteristic potentially extremely valuable when reforesting harsh sites.

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 ectomycorrhizae in the nursery is likely to be helpful if rapid colonization by native ectomycorrhizae is essential for seedling survival and if there is abundant, native inoculum.

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