

## Effects of Soil Fumigation and Cover Crops on Potential Pathogens, Microbial Activity, Nitrogen Availability, and Seedling Quality in Conifer Nurseries

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Accepted for publication 18 January 1990 (submitted for electronic processing).

### ABSTRACT

Hansen, E. M., Myrold, D. D., and Hamm, P. B. 1990. Effects of soil fumigation and cover crops on potential pathogens, microbial activity, nitrogen availability, and seedling quality in conifer nurseries. *Phytopathology* 80:698-704.

In three forest tree nurseries of the Pacific Northwest, fall fumigation with chloropicrin or dazomet led to an immediate increase in total microbial respiration and nitrogen availability, but levels were comparable to those in unfumigated plots when tree seedlings were sown the next spring. Population densities of *Fusarium* spp. and *Pythium* spp. in fumigated plots were reduced dramatically and stayed significantly below those in unfumigated plots throughout the 2-yr crop cycle. Grass or legume cover

crops increased pathogen population densities over those in bare, fallow plots before fumigation, and the trend continued throughout the crop cycle in unfumigated plots. At the end of the study, population densities of *Fusarium* in fallow, unfumigated plots were comparable to those in cover-cropped, fumigated plots. Seedling mortality was lower, and surviving seedlings were larger and more uniform on fallow and fumigated plots than on cover-cropped or unfumigated plots.

Forest tree nurseries play a vital role in the forest industry of Oregon and Washington. Nearly every harvested hectare of Douglas-fir is replanted by hand with Douglas-fir seedlings. Some 162 million seedlings grown in more than 6,000 ha of nurseries are used to reforest about 150,000 ha of forest land annually.

Addition of organic amendments and fumigation of soil in the fall before sowing the following spring are standard practices in northwest tree nurseries, although different schedules have evolved at the various nurseries through trial and error. Tree seedlings are grown 2 or 3 yr in the nursery before being transplanted to forest sites. The nurseries are productive in most years, and stock quality usually is high. Despite this success, current soil management practice is "more art than science." Nursery managers are concerned about unknown long-term effects of fumigants on soil productivity, and, conversely, about their ability

to grow healthy trees if fumigants are removed from the market because of environmental or health hazards. Our research was designed to measure, for the first time, certain effects of cover cropping and fumigation through the entire crop cycle of forest tree nurseries.

The benefits of soil fumigation to forest nursery production are well documented (2,12,13,28,30,31), although large and successful nurseries in British Columbia never fumigate (32). With one exception (33), all documentation has dealt with spring fumigation and annual crops, often with methyl bromide as the sole active ingredient. In most nurseries, pathogens are greatly reduced or eliminated, but they usually reestablish themselves by the end of the growing season. Addition of chloropicrin to the fumigant solution alters the mix of species during recolonization (27).

Previous researchers have shown that fumigation of soils resulted in an immediate decline in population densities of bacteria and fungi, and that this decline often is followed by increased

microbial activity as measured by CO<sub>2</sub> respiration or N mineralization (18,37). In fact, it is this observation that led to the development of the CHCl<sub>3</sub> fumigation-incubation method for determining microbial biomass in soil (15).

Observations of increased inorganic N after fumigation led to numerous studies designed to determine if this beneficial effect of fumigation was caused by the decline in numbers of plant pathogens or by an increased supply of N (9,23). Most of these studies were done with agronomic crops, such as wheat (14,21,29), and little is known about whether conifer seedlings are affected more by pathogen suppression or increased N availability after fumigation.

The influence of cover crops and other organic amendments on subsequent disease levels has been examined for many host-pathogen systems, but the results vary from case to case. Some cover crops increase subsequent disease severity, whereas others reduce losses (7,19,34,36). No basis for predicting the outcome of a particular situation has emerged. In general, higher levels of soil organic matter are correlated with higher total microbial activity (5,10).

Numerous investigators have shown that disease levels decrease as total microbial populations increase, presumably because of antagonistic and competitive interactions (6,20,22). A key determinant in the outcome of interactions between cover crop and pathogens seems to be initial colonization of the crop residue (4). Pathogens that can colonize cover crops before they are plowed down have a distinct advantage. Fumigation may neutralize this advantage, but those bacteria, and more rarely fungi, that survive fumigation, do so in crop residues in the soil (3).

To better understand the interactive effects of commonly used cover crops and fumigation practices on microbial population densities in forest nursery soils, we monitored total microbial activity, populations of *Pythium* and *Fusarium*, disease incidence, and nitrogen availability through the 2-yr crop cycle at three nurseries in western Oregon and Washington. We also compared effects of the combinations of cover crop and fumigation treatments on final seedling quality, as measured by seedling morphology, growth potential, and pathogen incidence.

## MATERIALS AND METHODS

**Nurseries.** The experiment was installed at three bareroot tree nurseries in the spring of 1985. Nursery A is located 19 km southwest of Olympia, WA, on Nisqually loamy sand (Pachic Xerumbrepts). The beds in which our experiment was installed had been in conifer seedling production for 17 yr. Nursery B is 30 km south of Portland, OR. The soil is a Willamette silt loam (Pachic Ultic Argixevalls). Our experiment was in beds that had been in conifer production for 25 yr. Nursery C is 70 km southwest of Eugene, OR. Two soil types, Chapman loam (Cumulic Ultic Haploxevolls) and Newburg loamy sand (Fluventic Haploxevolls), are present. The beds used in our experiment had been in seedling production for 9 yr.

**Experimental design.** Three cover-crop and two soil-fumigation treatments, each with four replicates, were installed at each of the nurseries. All treatments were applied according to standard nursery practices and utilized available nursery space; consequently, they varied in detail from nursery to nursery. No fungicides were applied to the plots in the experiment. Cover-crop treatments included a grass (Sudan grass, *Sorghum bicolor* (L.) Moench, or oats, *Avena sativa* L.), legume (bean, *Phaseolus vulgaris* L., or pea, *Pisum sativum* L.), and no cover crop (fallowing). Each plot either was fumigated or not fumigated. Cover crops were planted in June of 1985 and plowed under in August. Soil fumigant was applied in September. Beds lay fallow through the winter and were sown with Douglas-fir (*Pseudotsuga menziesii* (Mirb) Franco) seed in May of 1986. Seedlings were harvested in February of 1988.

At nursery A, a split-plot design was used to test the effects of soil fumigation and cover crops. Cover-crop treatments were applied to whole plots in a randomized complete block design, and fumigation treatments were applied to subplots. The fumigant

was methyl bromide-chloropicrin (MC33). Four cover-crop treatments were applied: oats, field peas, a 50:50 mixture of peas and oats, and fallowing. Plots with cover crops at nursery A were 7.3 m wide × 15.2 m long and encompassed three seedling beds.

At nursery B, dazomet, rather than MC33, was the fumigant. A split-plot design was used to compare fumigation with cover-crop treatments (Sudan grass, Blue Lake bush beans, and fallowing). Individual plots with cover crops were 6.1 m wide × 20.4 m long.

The split-plot design at nursery C was similar to that at nursery A, except that fumigation treatments (MC33) were replicated in adjacent strips three beds wide running the length of the field instead of in randomized blocks. Only fumigation effects were analyzed at nursery C because cover-crop plot boundaries could not be relocated after fumigation.

**Field sampling.** A sampling plot 1 m long × the width of the bed (1.2 m) was permanently located in the center of each treatment plot at each nursery. At 10 sampling dates, keyed to the phenology of the crop, a soil sample composited from five 30-ml subsamples was removed from each sampling plot. Only the upper 12 cm of soil were included in each sample. Samples were collected: 1) immediately before fumigation in the fall of 1985; 2) immediately after the tarps were removed following fumigation; 3) midwinter; 4) immediately before beds were formed and seed was sown in the spring of 1986; 5) immediately after sowing; 6) in the late summer, after symptoms of hypocotyl rot were expressed but before upper-stem canker was present (11); 7) in the late fall, after upper-stem canker was expressed; 8) in the spring of 1987, 1 yr after sowing; 9) in the summer of 1987; and 10) just before the crop was lifted in early 1988. An additional sample was collected at nursery B after the 1988 cover crop was plowed under, 3 yr after the first sample. Actual sample dates varied according to cultural schedules practiced at individual nurseries.

**Laboratory assays.** Each composited sample was stored at 4 C until processed. All soils were sifted through a 5-mm-mesh screen; very wet soils were first air-dried sufficiently to allow passage through the screen. Samples were mixed thoroughly, and separate subsamples were removed for the various microbial and nutrient assays.

**Water content and pH.** Water content of soil was determined gravimetrically by drying approximately 100 g fresh weight of soil at 105 C for 24 hr. Soil pH was measured in a 2:1 slurry (40 ml of distilled H<sub>2</sub>O/20 g fresh weight of soil) by a combination pH electrode.

**Extractable inorganic N.** Soil samples (about 12 g fresh weight) were extracted with 100 ml of 2 M KCl for 24 hr. Concentrations of NH<sub>4</sub><sup>+</sup> (salicylate/nitroprusside method) and NO<sub>3</sub><sup>-</sup> (diazotization following Cd reduction) in the KCl extract were determined colorimetrically with an autoanalyzer (16).

**Soil respiration.** Soil respiration rates were measured in the laboratory. Field-moist soil (about 25 g) was placed into 2.5-cm-diameter × 20-cm-long acrylic tubes (70 ml nominal volume). The tubes were capped with rubber septa and incubated at 25 C for 24–48 hr. Concentrations of CO<sub>2</sub> in the headspace were measured by gas chromatography on samples removed at the beginning and end of the incubation period.

**Microbial biomass C.** The fumigation-incubation method of Jenkinson and Powlson (15) was modified for use in determining microbial biomass C (25). About 12 g fresh weight of soil was placed in a glass scintillation vial and exposed to CHCl<sub>3</sub> vapor for 24 hr at room temperature. Unfumigated controls were not included. After removal of the CHCl<sub>3</sub>, the soils were transferred to stoppered acrylic tubes (70 ml nominal volume) and incubated for 10 days at 25 C. At the end of the incubation, a sample of the headspace gas was analyzed for CO<sub>2</sub> by gas chromatography. A k<sub>c</sub> of 0.41 (l) was used to calculate microbial biomass C from the CO<sub>2</sub> flush that followed fumigation.

**Pathogen populations.** Population densities of species of *Fusarium* and *Pythium* were determined by dilution plating on selective media. Ten grams of soil was mixed with 90 ml of 0.1%

water agar and further diluted stepwise in water agar up to  $10^{-4}$  g/ml. A 0.5-ml sample of each appropriate dilution was spread across the surface of the medium in a petri dish. Three replicate dishes were prepared for each fungus at each dilution.

Population densities of *Fusarium* were determined on Komada's medium (17) amended with 1.0  $\mu$ g/ml benomyl. The addition of benomyl further restricted contaminating fungi without affecting propagules of *Fusarium* and resulted in consistently higher density counts (P. B. Hamm and E. M. Hansen, unpublished). Dishes were incubated for 6 days at room temperature under 16-hr photoperiods. The dilution yielding closest to 10–20 colonies per dish was used for propagule counts. Species in randomly selected colonies were determined according to the methods and keys of Nelson et al (26).

Population densities of *Pythium* were determined on a modified V-8 agar developed by Peninsu-Labs (Kingston, WA). For this procedure, clarified V-8 juice (354 ml) was mixed with 5.7 g of  $\text{CaCO}_3$  and centrifuged for 20 min at 2,000 rpm; the supernatant then was added to distilled water to make 1,500 ml. Then, 30 g of agar and 15  $\mu$ g/ml pimaricin were added, and the mixture was autoclaved. After it had cooled to 45 C, 15  $\mu$ g/ml rifamycin, 375  $\mu$ g/ml ampicillin, 30  $\mu$ g/ml rose bengal, and 180  $\mu$ g/ml PCNB were added. Dilution-dish procedures were as described for *Fusarium*. Once this procedure had been completed, dishes were incubated 3 days at room temperature in the dark. After random colony selection, species of fungi were identified according to the methods and keys of Van der Plaats-Niterink (35).

**Seedling assays.** The numbers of healthy and diseased seedlings were counted on each sample plot in late summer and late fall. All seedlings from the sample plots were removed and graded separately for quality when the crop was lifted in early 1988. The total number of seedlings and the number of cull seedlings were recorded for each plot; cull specifications were in accordance with the standards of the particular nursery.

Additional measurements were made on subsamples of 10 or 20 seedlings. Colonization by species of *Fusarium* and *Pythium* on seedling roots was determined by plating 10 1.0-cm sections of each carefully washed taproot (without lateral roots) on Komada's or Peninsu-Lab medium. Stem diameter at the ground-line was measured, and root and shoot dry weights were determined. A separate sample of 30 trees per treatment was tested for root growth capacity (RGC). In this standardized assay, seedlings were grown for 4 wk in hydroponic tanks at 20 C and 16-hr photoperiods; then the number of actively growing root tips (up to 50) was counted.

**Data analysis.** Data from each sample time at each nursery were analyzed separately by analysis of variance (ANOVA). Treatment means were compared with Fisher's protected LSD test ( $P = 0.05$ ). In cases with a significant interaction between fumigation and cover-crop treatments, the LSD test compared the fumigated and unfumigated treatments separately. Fumigated cover-crop treatments were compared with unfumigated cover-crop treatments with Bonferroni significances (24).

## RESULTS

**Nitrogen and microbial activity.** Chemical and biological properties of the soils in all three nurseries were similar (Table 1). Over the duration of the study, the ranges of the soil properties at the three nurseries overlapped, although microbial biomass

TABLE 1. Ranges of chemical and biological properties of soils (dry-weight basis) in three bareroot forest nurseries over a cropping cycle (10 sampling dates)

Nursery	pH	$\text{NH}_4^+$ N ( $\text{mg kg}^{-1}$ )	$\text{NO}_3^-$ N ( $\text{mg kg}^{-1}$ )	Biomass C ( $\text{mg kg}^{-1}$ )	Respiration rate ( $\text{mg-C kg}^{-1}$ $\text{day}^{-1}$ )
A	5.3–6.1	0.1–2.8	<0.1–6.9	90–250	9–88
B	5.2–6.1	1.1–9.7	<0.1–18	120–380	26–64
C	5.0–5.8	0.3–6.6	<0.1–16	85–340	17–45

C and concentrations of soil inorganic N tended to be somewhat lower at nursery A and pH was slightly lower at nursery C. These chemical and biological soil properties responded identically at all three nurseries to all treatments, and their temporal variation was similar. Consequently, more detailed data will be presented only for nursery A, which was representative (Fig. 1).

Cover cropping rarely had a significant effect on the soil chemical and biological properties measured at any nursery at any sampling period. One exception was the first sample at nursery A, for which pH and biomass C were significantly higher and  $\text{NO}_3^-$  significantly lower in the oat treatment (data not shown).

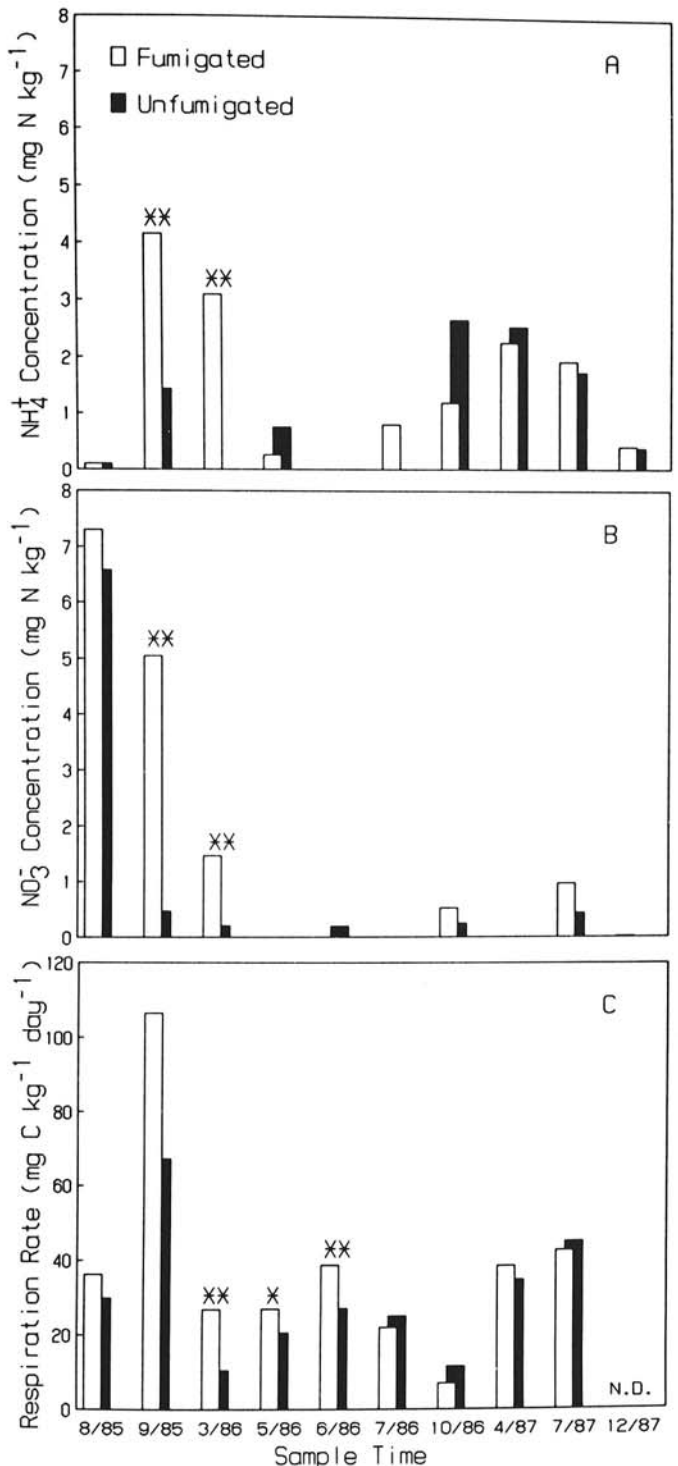


Fig. 1. Temporal response of soil biological and chemical properties to fumigation at bareroot nursery A over a 2-yr cropping cycle. A,  $\text{NH}_4^+$ . B,  $\text{NO}_3^-$ . C, soil respiration rate. Significant differences denoted by \* ( $P < 0.05$ ) or \*\* ( $P < 0.01$ ); N.D. = not determined.

These effects of cover cropping were not observed after fumigation.

Fumigation had no significant effect on soil pH or microbial biomass C. Soil pH increased over time, from 5.3 to 6.1. Microbial biomass C fluctuated among sampling dates in a pattern that did not appear associated with seasonal changes or differences in other soil properties.

At the first sampling date after fumigation, inorganic soil N was significantly greater on fumigated than on unfumigated plots

(Fig. 1A and B). This pulse in inorganic N disappeared by the fourth sampling date at nursery A, but increased concentrations of  $\text{NH}_4^+$  continued through the sixth sampling date at nurseries B and C. Nitrate concentrations were elevated in fumigated plots from the third through the fifth sampling at nursery B, but there was no effect of fumigation on  $\text{NO}_3^-$  concentrations at nursery C.

At all sites, soil respiration responded the same to fumigation as did inorganic N concentrations (Fig. 1C). At nurseries A and B, there was an initial flush of C mineralization for the first

TABLE 2. Population densities of *Fusarium* spp. (colony-forming units per gram of dry weight of soil) at representative times during a 2-yr crop cycle in soils of three Douglas-fir seedling nurseries subjected to various combinations of cover crops and fumigation treatments<sup>2</sup>

Nursery and treatment	Sampling date				
	Prefumigation	Postfumigation	Presowing		
	9/85 Sample 1	11/85 Sample 2	6/86 Sample 4	8/86 Sample 6	8/87 Sample 9
<b>Nursery A</b>					
Fumigated					
Fallow	1,670 a	15 a	40 a	1,170 a	6,390 a
Oats	10,570 b	0 a	40 a	1,130 a	2,910 a
Peas & oats	5,700 b	0 a	20 a	820 a	3,510 a
Peas	3,750 b	3 a	40 a	1,000 a	5,400 a
Unfumigated					
Fallow	1,820 A	1,260 A	430 A	1,260 A	17,060 A
Oats	6,090 B	10,040 B	2,460 BC	6,570 B	53,440 A
Peas & oats	5,820 B	8,270 B	3,640 C	9,100 B	43,340 A
Peas	11,420 B	9,550 B	1,920 B	6,720 B	39,910 A
<b>Nursery B</b>					
Fumigated					
Fallow	13,690 a	90 a	40 a	80 a	760 a
Sudan	32,910 b	1 a	330 b	530 b	2,710 b
Beans	48,340 b	160 a	1,170 c	1,670 c	9,180 b
Unfumigated					
Fallow	1,920 A	8,510 A	1,370 A	3,590 A	2,460 A
Sudan	17,120 B	8,710 A	4,520 B	11,820 B	8,990 B
Beans	31,660 B	8,680 A	13,130 C	18,030 C	33,780 B
<b>Nursery C</b>					
Fumigated					
Fallow	16,440 a	0 a	640 a	640 a	1,170 a
Unfumigated	17,220 a	21,910 b	40,640 b	4,900 a	12,390 a

<sup>2</sup> Within a column segment for a single nursery and fumigation treatment, population densities followed by the same letter (fumigated, lowercase; unfumigated, uppercase) are not significantly different by Fisher's protected LSD ( $P = 0.05$ ).

TABLE 3. Population densities of *Pythium* spp. (colony-forming units per gram of dry weight of soil) at representative times during a 2-yr crop cycle in soils of three Douglas-fir seedling nurseries subjected to various combinations of cover crops and fumigation<sup>2</sup>

Nursery and treatment	Sampling date				
	Prefumigation	Postfumigation	Presowing		
	9/85 Sample 1	11/85 Sample 2	6/86 Sample 4	8/86 Sample 6	8/87 Sample 9
<b>Nursery A</b>					
Fumigated					
Fallow	280 a	4 a	4 a	2 a	10 a
Oats	640 b	0 a	4 a	6 a	20 a
Peas & oats	680 b	0 a	30 a	10 a	10 a
Peas	940 b	1 a	2 a	20 a	30 a
Unfumigated					
Fallow	160 A	260 A	150 A	130 A	100 A
Oats	780 B	790 A	200 A	150 AB	230 A
Peas & oats	630 B	560 A	400 A	170 AB	250 A
Peas	1,060 B	1,100 A	240 A	530 B	380 A
<b>Nursery B</b>					
Fumigated					
Fallow	2 a	0 a	0 a	0 a	0 a
Sudan	20 b	1 a	0 a	0 a	0 a
Beans	80 b	0 a	0 a	0 a	0 a
Unfumigated					
Fallow	0 A	100 A	10 A	0 A	4 A
Sudan	30 B	60 A	10 A	20 B	20 B
Beans	80 B	110 A	30 A	20 B	20 B
<b>Nursery C</b>					
Fumigated					
Fallow	400 a	0 a	40 a	70 a	130 a
Unfumigated	300 a	500 b	490 a	330 a	310 a

<sup>2</sup> Within a column segment for a single nursery and fumigation treatment, population densities followed by the same letter (fumigated, lowercase; unfumigated, uppercase) are not significantly different by Fisher's protected LSD ( $P = 0.05$ ).

three or four sampling dates after fumigation. This flush was not observed at nursery C.

**Population densities of *Fusarium* and *Pythium*.** Before fumigation, propagules of *Fusarium* were abundant in all nursery soils; among the various treatments, they ranged from  $1.7 \times 10^3$  to  $4.8 \times 10^4$  colony-forming units (cfu)/g dry weight of soil (Table 2). Twenty-four months later, the range in unfumigated beds was similar, from  $2.4 \times 10^3$  to  $5.3 \times 10^4$  cfu (Table 2). Propagules of *Pythium* spp. were less abundant, ranging from 0 to  $1.1 \times 10^3$  cfu before fumigation and from 0 to  $3.8 \times 10^2$  cfu in unfumigated plots 2 yr later (Table 3). Soil population densities of *Pythium* were especially low at nursery B.

*Fusarium oxysporum* Schlecht. made up >50% of all isolates of *Fusarium* at all sampling dates and at all nurseries. It represented >80% of all isolates in all samples, except at three sampling dates from nursery A when *F. sambucinum* Fuckel was common. *P. mamillatum* Meurs predominated among *Pythium* spp. (>70% of isolates) at nursery C and was regularly recovered at nursery A; *P. irregulare* Buisman formed >60% of all samples at nursery A, 43% of all isolates at nursery B, and about 25% of the isolates at nursery C. *P. erinaceum* Robertson and *P. echinulatum* Matthews were regularly encountered at nursery B, but not at the other nurseries.

Soil fumigation dramatically reduced populations of both *Fusarium* and *Pythium* at all nurseries, and they remained lower than in unfumigated soil at all 10 sample dates at each nursery (Tables 2 and 3). All differences were significant at nurseries A and B but not at nursery C. Only after a third year and the intervening growth and incorporation of a new cover crop did population densities of these fungi in previously fumigated soil approach those in unfumigated soil (data available for only nursery B, because cultural practices at other nurseries prevented this direct comparison).

The type of cover cropping affected populations of both *Fusarium* and *Pythium* at nurseries A and B. Differences in the densities of *Fusarium* between cover-crop treatments were significant before fumigation at nurseries A and B, and at eight of nine subsequent sampling dates in unfumigated plots (Table 2). In fumigated plots, the effects of cover cropping were significant in two of nine postfumigation samples at nursery A and seven

of nine at nursery B. When population densities of *Fusarium* differed significantly among cover crops, fallowing consistently resulted in the lowest densities, and, at nursery B, the bean treatment almost always supported the highest densities (Table 2). The differences persisted in unfumigated treatments until seedlings were lifted. Unfumigated fallow treatments were not significantly different (Bonferroni significance) from the various fumigated treatments at all but one sample date after sample five at nursery A and after sample two at nursery B.

The effects of cover cropping on population densities of *Pythium* were most evident before fumigation at nurseries A and B (Table 3). Bare fallowing resulted in the lowest population densities, and residues of the bean or pea cover crops supported the highest densities, although not significantly higher than the grass cover crop. Fumigation nearly eliminated *Pythium* spp. at both nurseries, and subsequent effects of cover cropping could not be detected. In unfumigated plots, however, fallowing regularly resulted in the lowest population densities, and the legume treatment often had the highest, although differences usually were not significant (Table 3).

**Seedling quality.** The number and quality of seedlings harvested at the end of the 2-yr crop cycle differed significantly among cover-crop treatments only at nursery B (Table 4). More live trees and more trees meeting nursery size standards (packable) were present in fumigated plots than in unfumigated plots at all three nurseries. On fumigated plots, the trees had greater stem caliper and greater shoot-to-root ratios. All differences were significant except at nursery C and for shoot/root ratio at nursery B. Although more seedlings were produced in fumigated beds than in unfumigated beds, there were no real differences in root growth potential of the trees, as measured by the standard test (data not shown). Seedlings from unfumigated beds were smaller and more variable in size than those from fumigated beds, and fewer of them met packing standards for this reason. *F. oxysporum* was recovered significantly less frequently from roots of seedlings harvested from fumigated plots than from unfumigated plots at nurseries A and B (not tested at nursery C). *Pythium* rarely was recovered from seedlings of any treatment at any nursery (data not shown).

Few disease symptoms were observed at nurseries A and C

TABLE 4. Number and quality of Douglas-fir seedlings grown in three nurseries with and without cover cropping and fumigation<sup>w</sup>

Nursery and treatment	Packable seedlings <sup>x</sup>	Shoot/root ratio	<i>Fusarium</i> isolation <sup>y</sup>	Seedling count <sup>z</sup>	
				6/86	8/86
Nursery A					
Fumigated					
Fallow	195 a	2.7 a	3 a	28.3 a	27.1 a
Oats	200 a	...	...	28.7 a	26.3 a
Peas & oats	193 a	2.7 a	3 a	29.7 a	26.9 a
Peas	204 a	...	...	28.8 a	27.5 a
Unfumigated					
Fallow	186 A	2.2 A	11 A	28.3 A	26.9 A
Oats	180 A	...	...	28.1 A	25.3 A
Peas & oats	205 A	2.5 A	27 B	28.5 A	26.3 A
Peas	197 A	...	...	26.4 A	24.9 A
Nursery B					
Fumigated					
Fallow	313 a	1.5 a	7 a	38.0 a	32.0 a
Sudan	283 b	1.8 a	17 a	32.8 a	26.3 ab
Beans	223 b	1.9 a	48 b	34.8 a	23.3 b
Unfumigated					
Fallow	197 A	1.6 A	44 A	29.5 A	18.1 A
Sudan	161 B	1.6 A	73 B	28.8 A	15.4 AB
Beans	143 B	1.4 A	72 B	30.5 A	14.6 B
Nursery C					
Fumigated					
Fallow	334 a	...	...	31.6 a	31.8 a
Unfumigated					
Fallow	212 a	...	...	24.9 a	24.5 a

<sup>w</sup> Within a column segment for a single nursery and fumigation treatment, numbers followed by the same letter are not significantly different by Fisher's protected LSD ( $P = 0.05$ ).

<sup>x</sup> Average number of seedlings per 1- × 1.2-m plot) meeting nursery standards at final harvest.

<sup>y</sup> Average frequency (%) of isolations of *Fusarium* from 10 1-cm sections of taproot on Komada's medium (17).

<sup>z</sup> Number of healthy seedlings in 0.093 m<sup>2</sup> of bed at the indicated dates.

during this study, regardless of whether or not the plots were fumigated. *Fusarium* hypocotyl rot caused serious losses at nursery B, however, as evidenced by differences in seedling count between June and August (Table 4). Seedling mortality in unfumigated beds (45%) was significantly greater than that in fumigated beds (25%).

At nursery B, more packable trees, with less infection by *Fusarium*, were produced with bare fallowing than with either cover crop, regardless of whether or not the plots were fumigated. There also was less hypocotyl rot after fallowing than after either type of cover cropping.

## DISCUSSION

The significantly higher concentrations of inorganic N in the fumigated as compared with the unfumigated plots probably reflect the mineralization of N from microorganisms killed by the fumigation (14,20,22,28). Similarly, the increase in soil respiratory activity was probably a result of greater C availability from killed microorganisms (15).

The dramatic effect of fumigation on population densities of *F. oxysporum* and *Pythium* spp. was not surprising, although it has only been documented in one other study involving western conifer nurseries and current nursery practices (33). The duration of the effect was surprising, however. Not only were population densities low at the time of sowing 8 mo after fumigation, but they also increased very slowly and remained significantly lower than in unfumigated beds throughout the entire crop cycle. Differences in population densities were maintained despite the immediate proximity of unfumigated beds and the repeated movement of tractors and irrigation water across the plots. Not until a new cover crop was plowed under nearly 3 yr later did population densities approach prefumigation levels.

The importance of the preceding cover crop in affecting population densities of both *Fusarium* and *Pythium* was evident in the fall of the first year, even before the ground was fumigated. Differences persisted through the entire crop cycle in unfumigated treatments. Although legume cover crops tended to support higher population densities than did grass cover crops, the most significant differences were between no cover crop (fallowing) and the other treatments. These differences still were present 30 mo after the cover crop was plowed under in unfumigated plots. Population densities of *Fusarium* in fallow, unfumigated plots were often within the range found among fumigated plots with cover crops. There is very little experimental basis for cover cropping in the Northwest (8). Benefits cited include disease control from crop rotation, soil stabilization, and increased levels of soil organic matter, with supposed improvements in soil structure. Actual plant species used for cover cropping vary from nursery to nursery, depending on the experience of local managers.

Predicting seedling losses to *Fusarium* in bareroot Douglas-fir nurseries requires more than an initial estimate of soil population densities of *F. oxysporum* (P. B. Hamm and E. M. Hansen, unpublished). In this study, *Fusarium* disease levels differed dramatically between nurseries A and B, despite high soil population densities of *Fusarium* at both nurseries. *Fusarium* was recovered regularly from healthy seedlings at the end of the study, especially from unfumigated beds with a previous cover crop. It has been suggested that nonpathogenic as well as pathogenic isolates of *F. oxysporum* are present in nursery soils (3). Differences in pathogenicity between the populations of *Fusarium* at the two nurseries might explain the differences in disease incidence, although nursery A has had severe epidemics in previous years. Alternatively, pathogenesis, as distinct from infection, may require some stress to the seedling. Observed differences in disease may reflect different environmental conditions in the two nurseries.

This study confirms the value of fumigating nursery beds of forest trees before sowing. Soil fumigation is costly, however, and the chemicals used are extremely hazardous. Both economic and environmental factors are stimulating interest in alternative strategies for disease suppression. The influence of cover crops

on pathogen populations in nursery soils will be an important factor in proposed programs of integrated biological and cultural control.

## LITERATURE CITED

1. Anderson, J. P. E., and Domsch, K. M. 1978. Mineralization of bacteria and fungi in chloroform-fumigated soils. *Soil Biol. Biochem.* 10:207-213.
2. Bloomberg, W. J. 1965. The effect of chemical sterilization on the fungus population of soil in relation to root disease of Douglas-fir seedlings. *For. Chron.* 41:182-187.
3. Bloomberg, W. J. 1976. Distribution and pathogenicity of *Fusarium oxysporum* in a forest nursery soil. *Phytopathology* 66:1090-1092.
4. Bruehl, G. W., and Lai, P. 1966. Prior colonization as a factor in the saprophytic survival of several fungi in wheat straw. *Phytopathology* 56:766-768.
5. Chesters, C. G. 1949. Concerning fungi inhabiting soil. *Trans. Br. Mycol. Soc.* 32:197-216.
6. Cook, R. J., and Baker, K. F. 1983. The Nature and Practice of Biological Control of Plant Pathogens. American Phytopathological Society, St. Paul, MN. 539 pp.
7. Davey, C. B., and Krause, H. H. 1980. Functions and maintenance of organic matter in forest nursery soils. Pages 130-165 in: *Proc. North Am. For. Tree Nursery Soils Workshop*. State Univ., New York, Coll. Environ. Sci. For., Syracuse.
8. Duryea, M. L., and Landis, T. D., eds. 1984. *Forest Nursery Manual: Production of Bareroot Seedlings*. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, Boston, Lancaster. 386 pp.
9. Ebbels, D. L. 1971. Effects of soil fumigation on soil nitrogen and on disease incidence in winter wheat. *Ann. Appl. Biol.* 67:235-243.
10. Ferguson, J. 1957. Beneficial soil microorganisms. Pages 237-254 in: *The UC System for Producing Healthy Container-Grown Plants*. K. F. Baker, ed. Calif. Agric. Exp. Stn. Manual 23. 332 pp.
11. Hansen, E. M., and Hamm, P. B. 1988. Canker diseases of Douglas-fir seedlings in Oregon and Washington bareroot nurseries. *Can. J. For. Res.* 18:1053-1058.
12. Henry, E. 1901. Action du sulfure de carbone sur la vegetation de quelques plants forestiers. *Bull. Soc. Sci. Nancy, Ser. 3*, 2:27-33.
13. Hodges, C. S. 1962. Diseases in Southeastern forest nurseries and their control. U.S. Dep. Agric. For. Serv. Southeast. For. Exp. Stn., Stn. Pap. 142. 16 pp.
14. Jenkinson, D. S., Nowakowski, T. Z., and Mitchell, J. D. D. 1972. Growth and uptake of nitrogen by wheat and ryegrass in fumigated and irradiated soil. *Plant Soil* 36:149-158.
15. Jenkinson, D. S., and Powlson, D. S. 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. *Soil Biol. Biochem.* 8:209-213.
16. Keeney, D. R., and Nelson, D. W. 1982. Nitrogen-inorganic forms. Pages 643-698 in: *Methods of Soil Analysis*. Part 2. 2nd ed. A. L. Page, R. H. Miller, and D. R. Keeney, eds. Agron. Monogr. 9. American Society of Agronomy, Madison, WI.
17. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Rev. Plant Prot. Res.* 8:114-125.
18. Ladd, J. N., Brisbane, P. G., Butler, J. H. A., and Amato, M. 1976. Studies on soil fumigation. III. Effects on enzyme activities, bacterial numbers and extractable ninhydrin reactive compounds. *Soil Biol. Biochem.* 8:255-260.
19. Lewis, J. A., and Papavizas, G. C. 1975. Survival and multiplication of soil-borne plant pathogens as affected by plant tissue amendments. Pages 84-89 in: *Biology and Control of Soil-Borne Plant Pathogens*. G. W. Bruehl, ed. American Phytopathological Society, St. Paul, MN.
20. Lu, K. C. 1968. Effect of organic nursery amendments on soil microflora in relation to *Fusarium* root rot of ponderosa pine seedlings. Pages 40-45 in: *Proc. West. For. Conserv. Assoc., Western Forestry Nursery Council*, Portland, OR.
21. MacNish, G. C. 1986. Effects of fumigation on soil nitrogen, plant nitrogen and root disease incidence in wheat at Wangan Hills, Western Australia. *Aust. J. Soil Res.* 24:81-93.
22. Marois, J. J., and Mitchell, D. J. 1981. Effects of fungal communities on the pathogenic and saprophytic activities of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Phytopathology* 71:1251-1256.
23. Millhouse, D. E., and Munnecke, D. E. 1979. Increased growth of *Nicotiana glutinosa* as partially related to accumulation of ammonium-nitrogen in soil fumigated with methyl bromide. *Phytopathology* 69:793-797.
24. Milliken, G. A., and Johnson, D. E. 1984. *Analysis on Messy Data*. Vol. 1. Designed Experiments. Van Nostrand and Reinhold, New

York. 473 pp.

25. Myrold, D. D. 1987. Relationship between microbial biomass nitrogen and a nitrogen availability index. *Soil Sci. Soc. Am. J.* 51:1047-1049.
26. Nelson, P. E., Tousson, T. A., and Marases, W. F. O. 1983. *Fusarium Species: An Illustrated Manual for Identification*. The Pennsylvania State University Press, State College. 193 pp.
27. Ridge, E. H. 1976. Studies on soil fumigation: II. Effects on bacteria. *Soil Biol. Biochem.* 8:249-253.
28. Ridge, E. H., and Theodorou, C. 1972. The effect of soil fumigation on microbial recolonization and mycorrhizal infection. *Soil. Biol. Biochem.* 4:295-305.
29. Rovira, A. D. 1976. Studies on soil fumigation. I. Effects on ammonium, nitrate and phosphate in soil and on the growth, nutrition and yield of wheat. *Soil Biol. Biochem.* 8:241-247.
30. Sinclair, W. A., Cowles, D. P., and Hee, S. M. 1975. *Fusarium* root rot of Douglas-fir seedlings: Suppression by soil fumigation, fertility management, and inoculation with spores of the fungal symbiont *Laccaria laccata*. *For. Sci.* 21:390-399.
31. Smith, R. S., Jr., and Bega, R. V. 1966. Root disease control by fumigation in forest nurseries. *Plant Dis. Rep.* 50:245-248.
32. Sutherland, J. R. 1984. Pest management in Northwest bareroot nurseries. Pages 203-210 in: *Forest Nursery Manual: Production of Bareroot Seedlings*. M. L. Duryea and T. D. Landis, eds. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, Boston, Lancaster.
33. Tanaka, Y., Russell, K. W., and Linderman, R. G. 1986. Fumigation effect on soilborne pathogens, mycorrhizae, and growth of Douglas-fir seedlings. Pages 147-152 in: *Proc. Combined Western Forest Nursery Council and Intermountain Nursery Association Meeting*. T. D. Landis, ed. USDA For. Serv. Gen. Tech. Rep. RM-137. 164 pp.
34. Thies, W. G., and Patton, R. F. 1970. The biology of *Cylindrocladium scoparium* in Wisconsin forest tree nurseries. *Phytopathology* 60:1662-1668.
35. Van der Plaats-Niterink, A. J. 1981. Monograph of the Genus *Pythium*. *Studies in Mycology* No. 21. Centralbureau voor Schimmelcultures. 242 pp.
36. Williams, L. E., and Schmitthenner, A. F. 1960. Effect of growing crops and crop residues on soil fungi and seedling blights. *Phytopathology* 50:22-25.
37. Wolcott, A. R., Liao, F. H., and Kirkwood, J. I. 1967. Effects of fumigation, temperature, and level of nitrate on microbial numbers, CO<sub>2</sub> production, and N transformations in an organic soil. *Soil Sci.* 103:131-138.