Managing Soilborne Pathogens of White Pine in a Forest Nursery

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ABSTRACT

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In a forest tree nursery, 100% methyl bromide (MB), 100% chloropicrin (CP), combinations of MB and CP, and dazomet significantly reduced populations of soil fungi. Captan, thiram, a combination of captan and thiram, and covering seed with silica sand had no effect on fungal populations in soil. Nine months after fumigation, populations of soil fungi remained lower only in plots treated with MB and/or CP. Preemergence mortality of eastern white pine (*Pinus strobus*) was greatest when seed was covered with silica sand (49%) and least when soil was treated with 100% MB (21%). Postemergence damping-off was greatest when soil was treated with captan (19%) and least when soil was treated with 67% MB and 33% CP (1%). Plots treated with MB and/or CP had the highest seedling stand densities, averaging 232 seedlings per 0.5 m^2, compared to 125 seedlings per 0.5 m^2 in plots treated with dazomet and an average of 53 seedlings per 0.5 m^2 in plots treated with captan, thiram, captan and thiram, or silica sand and in the control plots. Seedling taproots were significantly longer in soils treated with MB and/or CP than in all other treatments. The colonization of root tips by mycorrhizal fungi did not differ significantly among treatments on seedlings examined in June or July 1987.

Damping-off and root diseases that occur in the first year of growth are the primary causes of seedling mortality in forest nurseries in the north central United States. Soils in these nurseries are commonly fumigated with a formulation of 67% methyl bromide (MB) and 33% chloropicrin (CP) to control insects, pathogenic fungi, and weeds (13,16,18). This combination of MB and CP can dramatically increase the yield of nursery seedlings and also improves seedling growth and quality (20,24).

However, use of these fumigants has several drawbacks. In addition to reducing populations of harmful pests, treatment with MB-CP eliminates beneficial mycorrhizal fungi. Mycorrhizal deficiencies may result in stunted, nutrient-deficient seedlings and seedling mortality (3,11,23). Surviving stunted seedlings may not meet grading specifications, resulting in additional yield losses (4). In addition, fumigation is expensive—application costs exceed $2,400/ha.

Although many nursery managers believe that the value of bare-root seedlings justifies these costs, Sutherland (21) suggested a combination of alternative management strategies for bare-root nurseries. Such strategies, including both herbicidal weed control and cultural practices that suppress pests, may be more economical than fumigation with MB-CP. We evaluated the relative effectiveness of four MB-CP combinations and several alternative practices for managing diseases caused by soilborne fungi in a forest tree nursery.

MATERIALS AND METHODS

Study area and treatments. The experiment was conducted at the F. G. Wilson State Nursery in Grant County, WI. Damping-off caused by various species of *Fusarium, Rhizoctonia,* and *Pythium* is common in this nursery, and a severe root disease of eastern white pine (*Pinus strobus* L.) associated with *F. oxysporum* (S. A. Enebak, R. Camp, and R. G. Collett, *unpublished*) has also occurred for several years. Thus, we selected treatments that might be effective against these soilborne fungi, and white pine was the tree species used in the study.

A randomized block design was used, with six replications of each treatment. One cultural and eight chemical treatments were applied to plots measuring 2.7 × 5.4 m. The chemical treatments were as follows: 100% MB (MB-100), 67% MB and 33% CP (MB-67), and 33% MB and 67% CP (MB-33), applied at a rate of 392 kg/ha, and 100% CP (CP-100), applied at a rate of 196 kg/ha, each fumigant applied on 4 September 1986 and injected 30 cm into the soil, which was covered immediately with polypropylene and remained covered for 5 days; dazomet, applied on 4 September 1986 as a topdressing at a rate of 280 kg a.i./ha, tilled 30 cm into the soil, packed with a roller, and sealed with 2.5 cm of water from overhead irrigation; captan, applied as a soil drench at a rate of 6 kg a.i./ha on 26 March 1987; thiram, applied as a seed coat at 38 g a.i. and 50 ml of spreader-sticker per kilogram of seed; and captan applied in combination with thiram-treated seed (captan-thiram). Untreated plots served as controls.

For the cultural treatment, two seedbeds (1.2 × 165 m) were established on 19 October 1986, and the hand-sown white pine seed was covered with No. 40 (120 grit) washed silica sand. The plots were then packed with a roller. All other seed plots were then sown mechanically at a rate of 14 g/m^2. After sowing, seedbeds were covered with screens and left until spring.

Before germination, four permanent subplots (0.5 m^2) were established in each plot to evaluate the presence and density of soilborne fungi, preemergence mortality, postemergence damping-off, and seedling stand densities. Areas adjacent to the permanent subplots were designated for seedling collections. Standard nursery fertilization and insect and weed control practices were followed. Overhead irrigation was applied as needed to maintain seedling growth.

Quantification of soilborne fungi. Four soil samples were collected over 9 mo. The first and second samples were collected on 10 August and 19 October 1986, 4 wk before and 4 wk after fumigation, respectively. Soil samples consisted of 20 soil cores (2.5 × 30.5 cm) collected arbitrarily within each plot. The third soil sample was collected just before seedling germination (25 March 1987), and the fourth sample was collected when

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Table 1. Populations (colony-forming units per gram of dry soil) of three fungi and of total soilborne fungi at the F. G. Wilson Nursery before sowing seed (10 August 1986) (BS), at sowing (19 October 1986) (S), before emergence (25 March 1987) (BE), and during emergence (19 April 1987) (DE)

	Fusarium spp.^{x,y}				*Rhizoctonia* spp.^{x,y}				*Pythium* spp.^{x,y}				Total soilborne fungi^{x,y}			
Treatment^{w}	**BS**	**S**	**BE**	**DE**	**BS**	**S**	**BE**	**DE**	**BS**	**S**	**BE**	**DE**	**BS**^{z}	**S**	**BE**	**DE**
MB-100	1,260 aA	— aB	40 aB	40 aB	11 aA	3 aB	6 abAB	6 aAB	6 aAB	— aB	— aB	— aB	2,730 aA	400 aB	75 aC	97 aC
MB-67	990 aA	33 aB	7 aB	50 aB	10 aA	3 aB	4 aB	5 aAB	7 aA	— aB	— aB	— aB	2,219 aA	268 aB	63 aC	98 aC
MB-33	1,450 aA	— aB	15 aB	90 aB	12 aA	5 aB	5 aB	8 aAB	8 aA	— aB	— aB	— aB	2,077 aA	503 aB	97 aC	150 aB
CP-100	1,550 aA	— aB	— aB	50 aB	11 aA	7 abB	3 aB	4 aB	4 aA	— aB	— aB	— aB	2,118 aA	342 abB	52 aC	129 aB
Dazomet	1,490 aA	220 bB	760 bAB	810 bAB	9 aA	11 bcA	9 bcA	10 abA	5 aA	3 aA	— aB	2 aB	3,039 aA	577 bcA	959 bcB	2,345 bA
Captan	1,230 aA	440 cA	940 bcA	980 bA	12 aA	11 bcA	11 cA	14 bA	7 aA	3 aAB	2 aB	— aB	1,971 aA	548 bcA	1,322 cA	2,962 bA
Thiram	1,650 aA	590 dB	910 bAB	730 bAB	10 aA	12 cA	12 cA	10 abA	7 aA	4 aAB	1 aB	2 aB	2,286 aA	637 cA	894 bcAB	1,798 bAB
Captan and thiram	1,760 aA	580 dB	881 bA	1,070 bAB	11 aA	12 cA	11 cA	14 bA	7 aA	2 aB	5 bA	1 aB	2,256 aA	379 cA	785 bcAB	2,788 bA
Silica sand	1,330 aA	520 cdB	1,450 cA	790 bAB	12 aA	11 bcA	9 bcA	15 bA	10 aA	3 aB	6 bAB	3 aB	2,091 aA	566 bcA	1,674 cA	2,224 bA
Control	1,420 aA	550 dB	670 bB	1,120 bAB	12 aA	11 cA	10 bcA	13 bA	2 aA	2 aA	1 abA	1 aB	2,310 aA	405 cA	1,014 bcA	2,664 bA

[w] MB-100 = 100% methyl bromide; MB-67 = 67% methyl bromide and 33% chloropicrin; MB-33 = 33% methyl bromide and 67% chloropicrin; CP-100 = 100% chloropicrin.

[x] Treatment means followed by the same letter (lowercase for columns and uppercase for rows) do not differ significantly ($P = 0.05$) according to the Student-Newman-Keuls mean separation test.

[y] A dash indicates less than 1 cfu/g.

[z] Colony-forming units per gram in hundreds.

Table 2. Preemergence mortality and postemergence damping-off of white pine seedlings caused by species of *Fusarium, Pythium,* and *Rhizoctonia* at the F. G. Wilson Nursery in 1987

	Preemergence mortality^{x}		Postemergence damping-off^{x}	
Treatment^{w}	**Number**^{y}	**Percentage**^{z}	**Number**^{y}	**Percentage**^{z}
MB-100	75 a	21 a	13 ab	4 a
MB-67	77 a	21 a	5 a	1 a
MB-33	78 a	22 a	19 abc	5 a
CP-100	85 a	24 a	9 a	3 a
Dazomet	99 ab	27 a	17 ab	5 a
Captan	123 b	34 ab	70 d	19 c
Thiram	158 c	43 c	37 c	10 bc
Captan and thiram	156 c	43 c	25 abc	7 ab
Silica sand	177 c	49 c	29 bc	8 ab
Control	112 b	31 ab	64 d	17 c

[w] MB-100 = 100% methyl bromide; MB-67 = 67% methyl bromide and 33% chloropicrin; MB-33 = 33% methyl bromide and 67% chloropicrin; CP-100 = 100% chloropicrin.

[x] Data are averages of 24 subplots for each treatment. Means followed by the same letter do not differ significantly ($P = 0.05$) according to the Student-Newman-Keuls mean separation test.

[y] Per 0.5-m^2 plot.

[z] Percentage data were analyzed by analysis of variance after square-root arcsine transformation.

seedlings were germinating (4 April 1987). These samples consisted of 10 soil cores collected from within the four permanent subplots. Each composite soil sample was mixed thoroughly before subsamples were selected for quantifying populations of soilborne fungi.

Relative numbers of soilborne fungi were estimated by soil dilution and use of selective media for *Fusarium* spp. (17), *Pythium* spp. (15), *Rhizoctonia* spp. (8,9), and total soil fungi (12,25). Identification of *Fusarium, Pythium,* and *Rhizoctonia* spp. was based on the descriptions of Toussoun and Nelson (22), Middleton (14), and Parmeter and Whitney (19), respectively. Relative fungal populations were determined from all six replicates of each treatment and were recorded as colony-forming units (cfu) per gram of oven-dry soil.

Seedling characteristics and stand densities. A germination capacity of 90% was determined for the white pine seed by in vitro and greenhouse tests. With a sowing rate of 14 g/m^2, approximately 360 seedlings were expected to emerge per 0.5-m^2 permanent subplot. The difference between the expected number of seedlings and the actual number of seedlings that emerged was considered preemergence mortality. Seedlings that died in the cotyledon or primary needle stage were classified as having postemergence damping-off. Preemergence mortality and postemergence damping-off were determined on 21 May 1987. Every 4 wk from 5 May to 23 September 1987, the number of seedlings in each permanent subplot was counted to evaluate seedling stand densities.

To determine the pathogens associated with seedling mortality, 10 seedlings with symptoms of damping-off were collected from each plot. Their roots were surface-disinfected for 6 min in a 0.5% sodium hypochlorite solution and then placed on malt-yeast agar (MYA). Plates were incubated at 21 C for 2 days, and hyphae growing from seedlings were transferred to another plate of MYA for identification.

Seedling root growth was evaluated four times during the growing season (on 14 June, 17 July, 13 August, and 14 September 1987) on 10 seedlings per plot selected arbitrarily and removed from the soil. Root length was measured, the percentage of mycorrhizal root tips was determined by methods of Daughtridge et al (5), and the numbers of healthy (white root cap) and diseased (brown and necrotic root cap) root tips were recorded.

Data were analyzed by an analysis of variance. All data recorded as percentages were subjected to square-root arcsine transformations before analysis. Statistically significant differences among means were determined with the Student-Newman-Keuls test (6).

RESULTS

Quantification of soilborne fungi. After fumigation, populations of *Fusarium* spp., *Pythium* spp., and total soil fungi fell by 40–100%, regardless of treatment (Table 1). Populations of *Fusarium* spp., however, were lower ($P = 0.05$) in plots treated with MB and/or CP (0–33 cfu/g) than in all other treatments (220–590 cfu/g). Populations of *Fusarium* spp. and total soil fungi were suppressed throughout the sampling period in plots treated with MB and/or CP but rose significantly in all other treatments. Populations of *Pythium* spp. were suppressed in all treatments throughout the sampling period. MB and/or CP reduced populations of *Rhizoctonia* spp. from 10–12 cfu/g to 3–7 cfu/g. Dazomet, captan, thiram, captan-thiram, and the silica sand covering were ineffective against *Rhizoctonia* spp. (Table 1).

Seedling characteristics and stand densities. MB, CP, and dazomet significantly reduced preemergence mortality and postemergence damping-off compared to controls (Table 2). Preemergence mortality ranged from 49% in plots treated with silica sand to 21% in plots treated with MB-100 or MB-67. Incidence of postemergence damping-off ranged from 19% in plots treated with captan to 1% in plots treated with MB-67. *Rhizoctonia, Fusarium,* and *Pythium* spp. were isolated from 72, 16, and 12%, respectively, of the 600 damped-off seedlings cultured.

Seedlings continued to emerge through June 1987 and as a result, seedling stand densities increased in the MB and/or CP treatments (Fig. 1). Seedling stand densities in these treatments did not change significantly after June, whereas stand densities in all other treatments fell markedly (Fig. 1). At the end of the first growing season, seedling stand densities averaged 232 seedlings per 0.5 m^2 in plots treated with MB and/or CP and 125 seedlings per 0.5 m^2 in plots treated with dazomet. Other treatments resulted in an average of 53 seedlings per 0.5 m^2.

Length of seedling taproots increased from 7–9 cm in June to 13 cm in July (Table 3). Taproots continued to lengthen in plots treated with MB and/or CP to almost 15 cm. As seedling roots became more diseased in plots treated with captan, thiram, captan-thiram, silica sand, or dazomet and in control plots, root length decreased to an average of 10 cm (Table 3).

Ninety percent or more of all seedlings, regardless of treatment, had healthy root tips in June (Table 3). Seedlings from captan, thiram, captan-thiram, silica sand, dazomet, and control plots averaged only 53% healthy root tips by July and 22% healthy root tips in September (Table 3).

Treatments did not differ significantly in the percentage of mycorrhizal root tips on seedlings collected in June or July (Table 3). Although the percentage of mycorrhizal root tips of seedlings in plots treated with MB and/or CP increased somewhat in August, by September there were again no significant differences among treatments in this respect.

DISCUSSION

Fumigation of seedbeds with MB and/or CP is economically justifiable at this nursery because of the high potential for root disease on white pine seedlings. Only MB and/or CP treatments were effective in protecting seedlings from soilborne pathogens throughout the first growing season. Use of these fumigants resulted in the greatest numbers of plantable seedlings and the seedlings with the healthiest and longest roots. Furthermore, there was no evidence to suggest that the fumigants and fungicides adversely affected the colonization of seedling roots by mycorrhizae. Although the fumigants eliminated most of the residual inoculum, spores disseminated after fumigation apparently provided adequate mycorrhizal inoculum. Differences among treatments with respect to the percentage of mycorrhizal root tips observed in August were probably the result of the reduced number of healthy roots. Roots of seedlings growing in plots treated with captan, thiram, captan-thiram, silica sand, or dazomet and in control plots likely became diseased and decayed faster than they could be regenerated. Consequently, the percentages of mycorrhizal root tips of seedlings in these treatments were lower than in seedlings growing in soils treated with MB and/or CP.

Dazomet was initially as effective as MB and/or CP in suppressing soilborne pathogens; however, populations of soil fungi and disease incidence in dazomet plots began to increase in midsummer, and by the end of the test period, seedling stand densities were significantly lower with dazomet than with the other fumigants tested (Fig. 1). Higher application rates might have given better disease control; however, Hildebrand and Dinkel (10) also reported that dazomet did not provide satisfactory results.

Captan and thiram treatments and

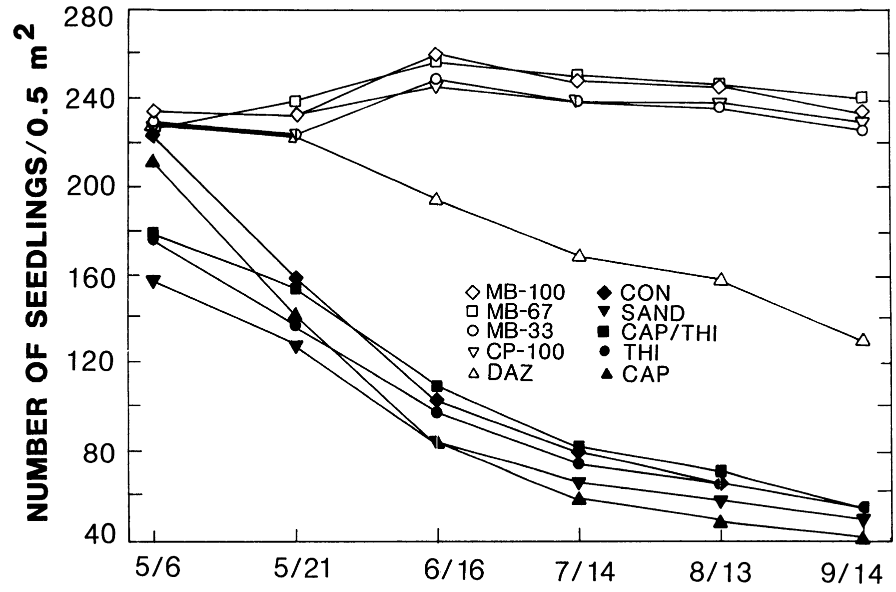

Fig. 1. Stand densities of white pine seedlings in plots treated with eight chemical treatments and one cultural disease management practice at the F. G. Wilson Nursery in Boscobel, WI, in 1987. Values are the means of six plots per treatment. Treatments were as follows: 100% methyl bromide (MB-100); 67% methyl bromide and 33% chloropicrin (MB-67); 33% methyl bromide and 67% chloropicrin (MB-33); 100% chloropicrin (CP-100); dazomet (DAZ); control plots (CON); silica sand (SAND); captan soil drench (CAP); thiram seed coat (THI); and captan and thiram treatments (CAP/THI).

Table 3. Percentage of mycorrhizae (MYC), seedling taproot length (STL), and percentage of healthy root tips (HRT) on white pine seedlings at the F. G. Wilson Nursery on four sample dates in 1987

	14 June[z]			**17 July**[z]			**13 August**[z]			**14 September**[z]		
Treatment[y]	**MYC (%)**	**STL (cm)**	**HRT (%)**	**MYC (%)**	**STL (cm)**	**HRT (%)**	**MYC (%)**	**STL (cm)**	**HRT (%)**	**MYC (%)**	**STL (cm)**	**HRT (%)**
MB-100	2.4	8.3	98	33.4	12.3 abc	88 ab	33.8 ab	13.5 abc	85 ab	24.8	14.7 a	68 a
MB-67	1.1	8.4	98	34.0	12.4 abc	98 a	40.1 ab	13.8 a	97 a	29.0	14.8 a	72 a
MB-33	2.3	7.7	98	29.6	12.9 ab	95 ab	42.9 a	13.8 a	95 a	33.8	14.2 abc	76 a
CP-100	1.7	7.9	96	30.0	13.1 a	98 a	39.9 ab	13.6 ab	95 a	24.8	14.6 ab	55 ab
Dazomet	3.5	8.0	98	23.3	11.9 bc	73 bc	31.1 ab	13.7 a	63 bc	41.6	12.2 bcd	31 bc
Captan	3.2	8.2	90	25.3	11.3 c	41 cd	17.7 bcd	11.8 cd	40 c	35.3	9.0 e	8 c
Thiram	5.7	7.2	93	26.1	11.4 bc	56 cd	22.8 d	11.0 d	41 c	37.3	10.8 de	18 c
Captan and thiram	6.6	7.7	95	24.8	11.1 c	38 d	22.7 cd	11.2 d	46 c	31.5	10.1 de	16 c
Silica sand	3.4	8.6	95	28.9	11.8 bc	56 cd	21.2 cd	11.8 cd	45 c	38.8	11.7 cde	38 bc
Control	4.0	8.0	98	28.4	11.8 bc	51 cd	20.8 cd	12.0 bcd	45 c	36.6	10.5 de	20 c

[y] MB-100 = 100% methyl bromide; MB-67 = 67% methyl bromide and 33% chloropicrin; MB-33 = 33% methyl bromide and 67% chloropicrin; CP-100 = 100% chloropicrin.

[z] Treatment means followed by the same letter within a column do not differ significantly ($P = 0.05$) according to the Student-Newman-Keuls mean separation test. No differences were detected for any variable on 14 June or for percentage of mycorrhizae on 17 July and 14 September.

covering the seed with silica sand were not effective in reducing disease. Preemergence mortality was greatest when seed was treated with thiram. Phytotoxic effects of thiram may have reduced germination, as has been previously reported (2).

Interest is increasing in reducing the uses of chemical pesticides, particularly fumigants, in forest tree nurseries. Many questions remain unanswered concerning the health risks to both humans and wildlife (1) and the long-term effects on soil microflora (23). Still, many nursery managers routinely fumigate seedbeds to avoid potential disease outbreaks. At the Wilson nursery, the value of the seedling crop (about $250,000/ha at rotation) compared to the cost of fumigation ($2,400/ha) justifies the use of MB and/or CP on soils that are "addicted" to fumigation. However, fumigation may not be needed in all areas of the nursery or in every nursery. A recent study we conducted at a northern Wisconsin nursery (7) demonstrated that fumigation with dazomet increased the incidence of stunted, phosphorus-deficient white spruce seedlings, whereas covering seed with sand produced the most normal-sized, healthy seedlings. Sutherland (21) reported that nurseries in British Columbia are seldom fumigated unless they are newly established in agricultural soils.

At present, there are no guidelines for predicting outbreaks of diseases caused by soil fungi in forest tree nurseries. Thus, it is difficult for nursery managers to determine the risk of specific diseases and adapt their treatment schedule accordingly. If forest tree nurseries are to move toward an integrated approach to disease management, a better understanding of pathogen biology and seed quality is needed as well as the development of effective, economical, and environmentally sound alternatives to fumigation.

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