THE EFFECT OF CHEMICAL STERILIZATION ON THE FUNGUS POPULATION OF SOIL IN RELATION TO ROOT DISEASE OF DOUGLAS-FIR SEEDLINGS[1]

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ABSTRACT

Nursery beds were treated with methyl bromide, Mylone, Vapam, CIPC, and Simazine before sowing with Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seed. In beds treated by the first three chemicals, the incidence of seedling root disease was 15, 45, and 75% of that in the untreated bed. There was little or no reduction of disease in the Simazine- and CIPC-treated beds. Counts of fungi in soil dilution plates showed that methyl bromide and Mylone caused the greatest reduction in the total fungus population of the soil; the proportion of Trichoderma viride Pers. ex Fr. was larger and that of Penicillium spp. was smaller in soil treated by these chemicals than in soil from other treatments. Vapam caused a moderate reduction in the total population. All treatments reduced the proportion of Fusarium oxysporum Schlect. ex. Fr. in the soil, the fungus principally found to be associated with root disease; however, this proportion did not appear to be correlated with disease incidence.

INTRODUCTION

The changes in the microbiological populations of soil following partial sterilization have been a major subject of research in chemical control of plant diseases. After treatment there is often a drastic reduction in fungus populations followed by a gradual recolonization. One of the earliest colonizers of partially sterilized soil is *Trichoderma viride* Pers. ex Fr. (Bliss 1951, Martin *et al.* 1957, Saksena 1960, Warcup 1952). The antagonism shown by this fungus to many pathogenic species is believed to be an important factor in the control of soil-borne diseases (Wood and Tveit 1955).

In the present study, comparisons were made between several chemical soil sterilants with respect to the reduction of the fungus population, recolonization, and root disease in 1-0 Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings in nursery beds at Duncan, B.C. From these comparisons, it was hoped to obtain a better understanding of the action of chemical control in forest nurseries so that more effective use could be made of it. Previous tests at this nursery (van den Driessche 1963) showed that a good growth response of Douglas fir seedlings occurred after partial soil sterilization.

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Materials and Methods

Six nursery beds 3.6 feet wide x 50 feet long (183 sq. ft.) were prepared in September as for sowing. One bed was designated an untreated control and each of the remaining 5 beds was treated with one of the following sterilants:[3]

Methyl bromide, 27 oz./183 sq. ft., vaporized under a plastic sheet.
Mylone, 64 oz./813 sq. ft., broadcast dry and watered in.
Simazine, 2 oz./183 sq. ft., sprayed in water.
CIPC (Isopropyl=N=(3 chlorophenyl) carbamate) 10 oz./183 sq. ft. broadcast dry and watered in.
Vapam, 1.4 pints/183 sq. ft. sprayed in water and watered in.

Precautions against drift were taken when applying the spray treatments. The following April, the beds were sown with Douglas-fir seed to give a density of approximately 40 seedlings/sq. ft.

Soil samples were collected from the beds immediately before treatment, and 6 weeks, 13 months, and 20 months after treatment. The sampling dates were September 1962, October 1962, October 1963, and June 1964, respectively. At each sampling time, 20 randomly located samples of about 200 g each were taken from the top 2 inches of the soil and were mixed to form a composite sample. After passing the soil through a soil shredder, 16 aliquots of 10 g were taken, the lumps crushed, and soil dilutions prepared from each aliquot using the procedure described by Johnson *et al.* (1959) with modifications; a syringe (Fig. 1) was used to transfer the soil solution from one dilution to the next higher one; and a 1 ml dipper (Menzies 1957) was used to transfer the final dilution to the petri dish. The purpose of these modifications was to include the larger soil particles in the sample which is not possible when using a pipette.

Soil dilutions of 1:100 up to 1:100,000 were tested for suitability. A dilution of 1:10,000 was selected as the optimum for accurate estimates of the fungus population. The culture medium was soil extract agar (Lochhead 1940) adjusted to the pH of the nursery soil (5.7). After incubation at 22-25° C for 5 days, the fungus colonies in each dish were counted and identified. A further incubation period up to 6 days under white light was used to stimulate sporulation by *Trichoderma* (Miller and Reid 1961).

Counts of diseased seedlings were made in 16 plots of 1 sq. ft. located randomly in each nursery bed. Counts were made from germination in May 1963 until the onset of dormancy in October 1963 at intervals of 3-4 weeks. A final count of surviving seedlings was made and disease expressed as a percentage of the total number of seedlings which had emerged during the growing season. Diseased seedlings were cultured on malt agar to isolate the fungi present. Pathogenicity of the isolated fungi to Douglas-fir seedlings was tested in the greenhouse by sowing seed, surface-sterilized with 10% Javex solution, in soil which had been sterilized with propylene oxide (Martin *et al.* 1957) and inoculated with mycelium.

Replication of nursery beds was not possible under the circumstances, but variation was reduced as much as possible by locating the beds closely to-

[3] The treatments were carried out by the Reforestation Division, B.C. Forest Service. The author expresses his thanks for permission to use this information.

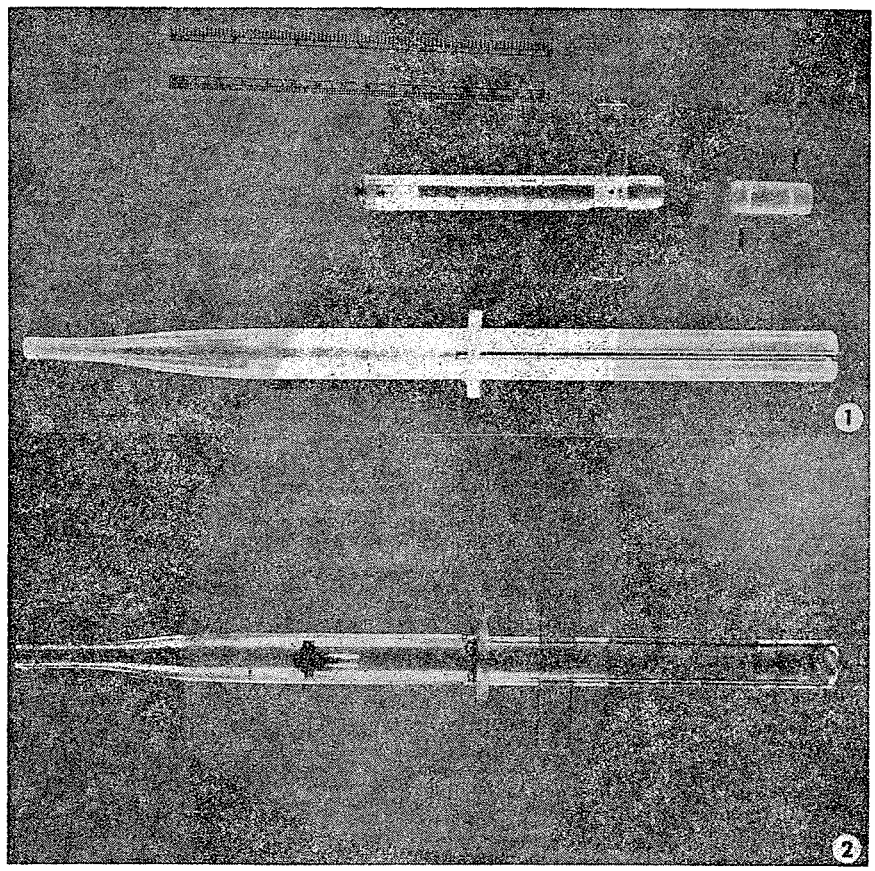

FIGURE 1 Soil syringe made from Perspex rod and tubing for purpose of including larger soil particles when sampling soil solutions. Plunger washer is made of Teflon. Length of syringe is 12 ins., internal diameter is ½ in. tapering to ⅛ in. Piston movement can be set to deliver a given volume of solution.

gether, in this case, parallel to each other and mostly within a one-bed width of another treatment. Samples from untreated beds showed the cross-bed variation to be relatively slight in its effect on disease and fungus populations within the distance involved.

Results

The counts of fungus colonies (Table 1) show that methyl bromide and Mylone treatments decimated the fungus population, Vapam reduced it by approximately 50%, but CIPC and Simazine treatments caused relatively small changes. After 13 months, the population levels were approximately equal to, or higher than, those before treatment in all except the Vapam and methyl bromide bed. After 20 months, only the population level in the methyl bromide-treated bed remained lower than the level before treatment.

TABLE 1

RELATIONSHIP BETWEEN SOIL STERILIZING TREATMENT, FUNGUS POPULATION, AND ROOT DISEASE IN 1-0 DOUGLAS-FIR SEEDLINGS

Treatment	No. of fungus colonies (000's) per gram of soil.				Seedlings diseased[1] (%) (Oct. 1963)
	Before treatment (Sept. 1962)	6 wks. after treatment (Oct. 1962)	13 mths. after treatment (Oct. 1963)	20 mths. after treatment (June 1964)	
Methyl bromide	227	14	81	140	1.6
Mylone	222	24	345	286	4.6
Vapam	331	172	217	387	7.1
CIPC	—[2]	346	306	347	9.2
Simazine	—[2]	280	279	449	14.3
Control	227	203	391	513	10.3

[1] Necessary difference for significance by multiple range test = 1.5%.
[2] Not sampled before treatment. These beds were situated between the Mylone and the Vapam beds. The fungus count may therefore be assumed to lie in the neighborhood of 200,000-300,000 colonies per gram of soil.

Significant differences occurred in percentage of diseased seedlings between beds treated with methyl bromide, Mylone, Vapam and control. In the methyl bromide-treated bed, the disease incidence was approximately 15% of that in the control bed; in the Mylone-treated bed it was about 45%, and in the Vapam-treated about 75%. CIPC, Simazine, and the untreated control were not significantly different according to the multiple range test.

Over 90% of the diseased seedlings yielded *Fusarium oxysporum* Schlect. ex. Fr. when cultured on agar plates. Identifications were kindly provided by Dr. C. Booth, Commonwealth Mycological Institute, Kew. In the pathogenicity test, an average of 29% of the seedlings became diseased in soil inoculated with *F. oxysporum* compared with none in the uninoculated soil.

The composition of the fungus population following recolonization (Table 2) shows that on both sampling dates, the proportion of *Trichoderma viride* in the soil treated by methyl bromide was at least 4 times greater than that in soil from any other treatment; 13 months after treatment, the proportion of *T. viride* in the Mylone-treated beds was about twice that in the Vapam, CIPC, Simazine and the untreated beds. The differences between the latter were relatively small.

The proportion of *Penicillium* spp. in soil treated by methyl bromide and Mylone was about half as great as that from the other treatments which were approximately equal in this respect.

The proportion of Phycomycetes in soil treated by methyl bromide was approximately two-thirds to one-half of that in soils treated by the other sterilants.

There were no consistent differences between treatments in the proportion of *Fusarium oxysporum* present in the soil and these differences were relatively small.

TABLE 2

COMPOSITION OF FUNGUS POPULATION BY GROUPS AFTER TREATMENT

	Trichoderma		Penicillium		Phycomycetes		Fusarium	
	No. of months after treatment							
Treatment	13	20	13	20	13	20	13	20
	Percentage of total number of colonies							
Methyl bromide	13.8	7.1	19.8	24.9	9.9	6.2	9.9	6.3
Mylone	1.9	1.5	29.4	18.7	20.0	18.7	7.3	3.1
Vapam	0.6	1.4	45.4	39.5	16.6	13.5	7.7	10.4
CIPC	0.8	1.1	44.5	48.9	21.8	14.6	9.9	11.4
Simazine	0.1	1.3	47.0	50.9	17.7	9.4	12.5	7.3
Control	0.5	1.1	38.2	42.6	11.3	5.2	19.6	12.5

CONCLUSIONS

Despite the limited number of samples taken, the differences between some of the treatments were large enough to merit attention. In beds treated with methyl bromide and Mylone, the incidence of seedling root disease was considerably below the average level for the whole nursery (12.6%); in the beds treated with Vapam it was somewhat below. These three sterilants have been found to be among the most effective in reducing damping-off losses in conifer nurseries (Vaartaja 1964). The incidence of disease appeared to be correlated with the total fungus population; in addition, the beds in which disease incidence was lowest contained the largest proportion of *Trichoderma viride* and the smallest of *Penicillium* spp. On the other hand, there did not appear to be any correlation between incidence of root disease and the proportion of *Fusarium* in the soils. In view of the antagonism of *Trichoderma* to *Fusarium* spp. (Wood and Tveit 1955), the difference in effectiveness of various chemical treatments of soil in reducing seedling disease may be explicable in terms of microbiological interactions.

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