Fumigation Effect on Soilborne Pathogens, Mycorrhizae, and Growth of Douglas-fir Seedlings[1]

Yasuomi Tanaka[2], K.W. Russe11[3] and R.G. Linderman[4]

Abstract.--Soils were treated with methyl bromide/ chloropicrin (MBC) at 360 lbs/A and 720 lbs/A or with Basamid at 350 lbs/A in a field trial with a randomized block design at two bare-root Douglas-fir nurseries near Olympia, Washington in 1984-85. The results showed that fumigation (1) increased fall 1+0 seedling count, (2) caused no 1+0 stunting or growth loss, (3) did not hinder formation of mycorrhizae, (4) suppressed and maintained low soilborne pathogen populations and (5) suppressed root infections by Fusarium spp. but not Pythium spp.

INTRODUCTION

The stunting of 1+0 Douglas-fir seedlings is frequently observed in the bare-root nurseries in the Pacific Northwest. It is characterized by a short stem (usually less than three inches long), short needles and a well defined terminal bud resulting from an early cessation of seedling growth. The distribution of stunted seedlings is random often occurring in patches. To determine the cause of stunting we conducted a number of studies the past several years. Although the stunt syndrome is not fully understood it appears that first-year Douglas-fir seedlings develop these symptoms under various stressful conditions. Based on our observations and those of other researchers, a number of factors appear to contribute to stunting, both singularly and more possibly in combination. They include an insufficient level of soil nutrients -- mainly phosphorus and to some extent nitrogen, an excessively high pH resulting from liming, a short growing season resulting from late sowing, an excessive buildup of soil pathogens or undesirable substances in the soil, and a deficiency or delay of mycorrhization of the root system. The involvement of some of these factors is supported by experimental evidence, while the involvement of others is still hypothetical at this time.

The effect of soil fumigation on mycorrhizal infection and/or microbial recolonization has been investigated in bare-root nurseries (Carpenter and Boyd 1980, Ridge and Theodorou 1972), but its effect on stunting is not well understood. An excessively high rate of application may delay recolonization and thus hinder normal formation of mycorrhizae which may, in turn, contribute to stunting due to decreased capacity for nutrient uptake. On the other hand, an excessive buildup of soilborne pathogens resulting from an insufficient rate or skips of fumigation may result in increased root disease causing stunted trees. To investigate these relationships and as a part of the effort to determine the cause of stunting, we conducted a study on the effect of fumigation on seedling establishment, 1+0 stunting, seedling growth, mycorrhizae development, and incidence of root disease of Douglas-fir as well as changes in population of soilborne pathogens.

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[2]Weyerhaeuser Company, Western Forestry Research Center, Centralia, Washington 98531.

[3]Washington State Department of Natural Resources, Division of Forest Pest Management, Olympia, Washington 98504.

[4]U.S. Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Laboratory, Corvallis, Oregon 97330.

For comparison, the study was carried out at two bare-root nurseries near Olympia, Washington; the Mima Nursery of Weyerhaeuser Company and the L.T. Webster Nursery of the Washington State Department of Natural Resources. The results reported here are only for Mima except where mention of the Webster data illustrates important points, but the trends were the same for both nurseries. A full report of data from all treatments at both nurseries is in preparation for publication elsewhere.

MATERIALS AND METHODS

A total of four fumigation treatments were applied at both the Mima and Webster nurseries in a randomized block design with three replications. The fumigation treatments were established in September 1984 as follows:

Treatment 1: Fumigation with methyl bromide/ chloropicrin (MBC) (2:1) at 360 lbs/A rate (lx); tarp removed after 1 month.

Treatment 2: Fumigation with MBC at 720 lbs/A rate (2x); tarp removed after 1 month.

Treatment 3: Fumigation with Basamid at 350 lbs/A; no tarp.

Treatment 4: Unfumigated control; no tarp.

MBC was applied in the standard manner by injection at the 6" depth followed immediately by tarping. Basamid granules were applied to the surface then rototilled into the top 6" of soil.

Soil samples were collected from the three replicate plots for each treatment at 1, 2, 4, 6, 8, and 10 months after the September, 1984 fumigation. Three 1" diameter core samples from each plot were separately pooled from two depths, 0-6 inches and 6-12 inches. The core sampler was flamed between plot samples to prevent cross contamination. Combined samples weighing approximately 500 g were screened to eliminate large particles and debris, and refrigerated until soilborne diseases were assayed, usually within one week.

Standard soil dilution plating techniques were employed to determine populations of species of Pythium and Fusarium on selective media for each, namely Rose bengal (Russell 1986a) and Komada's medium (Komada 1975) respectively.

Inoculated Rose bengal plates were incubated in the dark at 20°C for 60-72 hrs, then washed with running tap water to remove the soil particles and thereby facilitate counting. Pythium species were not differentiated, and populations were expressed as propagules/g moist soil.

Fusarium populations were assayed as in the Pythium assays except that the soil was suspended in 0.3% water agar and plates were incubated for 5 days at 22-24°C in natural light and colonies were counted as described by Komada (1975).

Seeds were sown in early May 1985. Seedling emergence counts were made in June based on the number of seedlings present in 6 square feet of bed (1.5 linear feet of bed) determined at 2 locations within a plot. The 1+0 stand counts were made in October. The incidence of 1+0 stunting was also determined within the same 6 square feet areas used for the seedling counts. A seedling was considered stunted if it was less than 3 inches tall from ground to apical bud. Actual stunting incidence was based on linear feet of bed rows within the measured area.

Seedlings used for determination of mycorrhizae colonization were collected from each plot in October 1985 and again in May 1986. A total of 10 seedlings per replicate plot were collected in fall and 15 seedlings in the spring. They were carefully washed to remove adherent soil, bagged and shipped on ice to Corvallis, Oregon where each was examined for presence of mycorrhizae. The percent seedlings with mycorrhizal roots were compared among treatments. Root collar diameter and dry weight of shoots and roots (oven dried at 70°C) were determined for seed-lings harvested in October 1985.

The incidence of Pythium and Fusarium root rot was determined on 1-year-old seedlings collected in May, 1986. Root systems were washed thoroughly and cut into approximately 1 cm lengths. Root pieces from 5 seedlings per replicate plot were pooled, surface sterilized with 1.0% sodium hypochlorite for 3 min. and rinsed in sterile distilled water. Twenty-five root pieces from each replicate plot were plated on Pythium or Fusarium selective medium, 5 pieces per plate. Recovery of Pythium was determined after 3-day dark incubation at 20°C; Fusarium plates were incubated in natural light for 10 days at 22-24°C. Recovery was considered positive if one or more colonies emerged from the root piece. The number of positive recoveries became % recovery as an index of root rot incidence.

All data were analyzed using analyses of variance. The treatment differences were tested using the Duncan's new multiple range test at a 5% level of probability (Steel and Torrie 1960). Percentages were analyzed after arcs in transformation.

RESULTS AND DISCUSSION

The results from treatments at the two nurseries were comparable in most regards. No striking treatment effects on seedling emergence were observed. There was, however, a trend toward higher emergence in all fumigation treatments compared to the untreated control and Basamid did significantly increase the emergence by 11% (Fig. 1). The 1+0 seedling count was significantly greater in MBC (lx) and Basamid treatments (by 12%) than in the control. The reduced 1+0 count in the control is probably due to the higher levels of pathogens in soils at the time of sowing as reported below.

The incidence of 1+0 stunting was very low at both nurseries in the blocks used for this study, the highest level being 0.1% at Mima and 2.5% at Webster. The bulk of stunting was found in the control treatments at both nurseries.

In general, fumigation treatments tended to produce larger seedlings and MBC (lx) significantly increased root collar diameter and dry weights of shoots and roots compared to the control (Fig. 2). Root collar diameter and root dry weight were significantly greater in MBC (lx) than in MBC (2x). It is not certain why the higher rate of MBC reduced seedling size. It is possible, however, that beneficial microorganisms, which were not measured in the study, could have been adversely affected at the 2x rate, which, in turn, contributed to size reduction. An adverse effect (on germination and survival) associated with an increased rate of MBC has been reported for white spruce and several other conifers (Hill 1965).

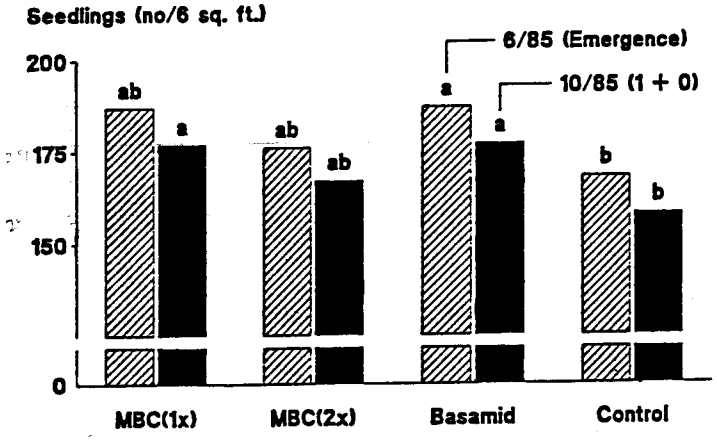

Figure 1.---Effects of soil fumigants on Douglas-fir seedling emergence and 1+0 stand count. The treatments followed by the same letters are not significantly ($p < 0.05$) different within each assessment time.

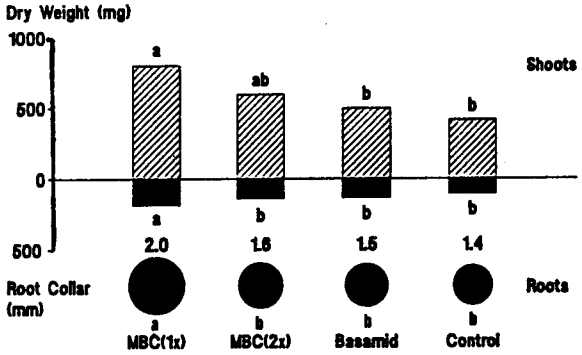

Figure 2.--Effects of soil fumigants on Douglas-fir seedling growth assessed in October 1985. The treatments followed by the same letters are not significantly ($p < 0.05$) different in each variable.

The percent seedlings with mycorrhizae ranged from 60% in the control to 80% in the Basamid treatment in October 1985 (Fig. 3). By May 1986, virtually all seedling roots in the four treatments were mycorrhizal (96%100%). The differences among treatments were not significant at either assessment time. Contrary to our hypothesis, MBC fumigation up to 720 lbs/A did not hinder mycorrhization of 1+0 Douglas-fir seedlings. Twenty to 40% of seedlings (depending on treatments) had no mycorrhizae, and yet there was virtually no 1+0 stunting in this block at the Mima nursery in 1985. These data suggest that lack of mycorrhization is not the cause of stunting in 1+0 Douglas-fir, although under certain circumstances its presence may prevent stunting.

Fusarium and Pythium populations were assayed at various times after fumigation at the 0-6" and 6-12" depths to determine the efficacy of the fumigation treatments and to determine when these pathogens reinvaded the fumigated treatment plots. The propagule count was usually greater at 0-6" depth than at 6-12" depth (especially with Fusarium), but the trend was the same with respect to seasonal changes and treatment differences. The means of propagule counts for two depths are summarized for Pythium and Fusarium in Figure 4. The data clearly show the effectiveness of MBC fumigation at the normal lx rate, and that the 2x rate was unnecessary. Basamid was nearly as effective as MBC fumigation in reducing propagule counts.

Pythium populations were effectively reduced by fumigation treatments and remained low throughout the study. In the untreated control, the populations increased in March with the onset of warmer weather, peaked in the May sampling and then declined rapidly by the *July* sample. Although the magnitude was ten-fold greater, Fusarium population in the unfumigated control fluctuated with a similar trend as those of Pythium. The main difference was that it peaked in March and began to decline thereafter reaching the lowest level in July.

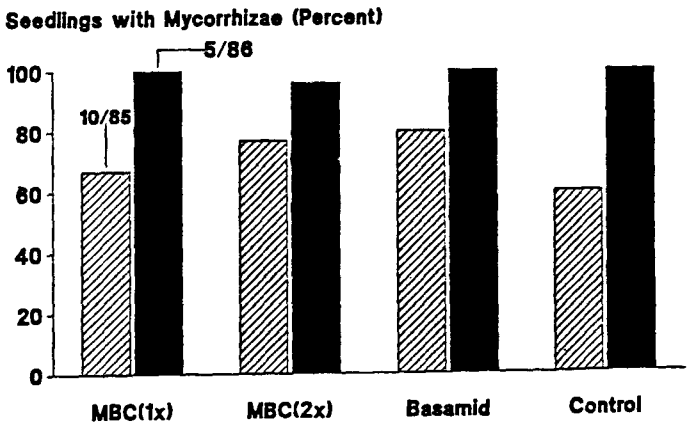

Figure 3.--Effects of soil fumigants on development of mycorrhizae on Douglas-fir seedlings. The treatments followed by the same letters are not significantly ($p < 0.05$) different within each assessment time.

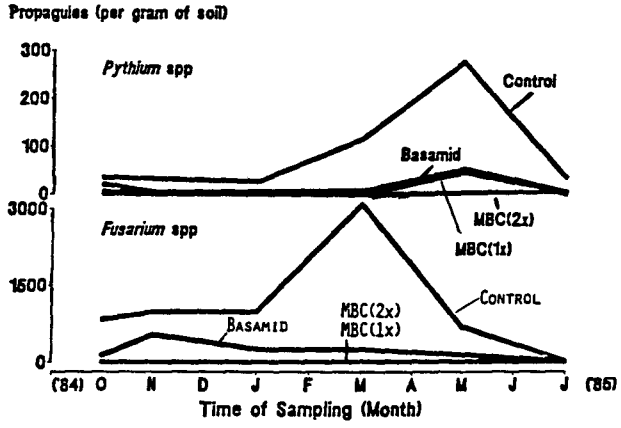

Figure 4.--Effects of soil fumigants on changes in populations of soilborne pathogens.

The reason for the decline in propagule counts of these pathogens in the unfumigated control in mid-summer (July) is not known; however, two possible reasons may be offered: (1) Application of fungicides (Benlate, Captan and Daconil) after sowing possibly reduced the level of fungi, and (2) Dry soil conditions created by the infrequent, deep watering may have contributed to the reduction (Russell 1986b).

The incidence of root rot by Fusarium in May 1986 was highest in the non-fumigated control (Fig. 5). It was not reduced by Basamid as effectively as by MBC at lx or 2x. Pythium root rot incidence, on the other hand, was nearly as high in all fumigation treatment plots as the non-fumigated control plots in the spring of the second year, presumably due to the aggressive recolonization of fumigated soil from below the fumigation layer. Although pathogenicity of these fungi was not tested in this study, it appeared that species of Pythium involved may not have been as pathogenic as the species of Fusarium based on seedling growth data and 1+0 seedling counts.

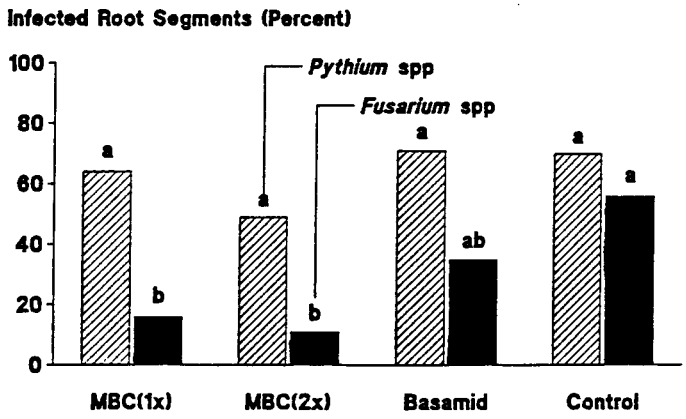

Figure 5.-- Effects of soil fumigants on the incidence of infections of Douglas-fir roots by species of Pythium and Fusarium. The treatments followed by the same letters are not significantly ($p < 0.05$) different in each fungus.

CONCLUSIONS

Based on the results of this fumigation study, the following conclusions were reached.

1. MBC (lx) and Basamid significantly increased 1+0 seedling count by 12% in both treatments.

2. None of the fumigation treatments [MBC (lx), MBC (2x) or Basamid) caused 1+0 stunting or growth loss in root collar diameter and shoot and root dry weights.

3. None of the fumigation treatments caused a reduction in mycorrhizal roots.

4. MBC (lx) and Basamid suppressed and maintained low levels of soilborne pathogens (Fusarium spp. and Pythium spp.) throughout the first full year of seedling growth. MBC at the 2x rate was not necessary for disease control.

5. MBC (lx) and MBC (2x) suppressed root infections by Fusarium spp. but not Pythium spp. in the spring of the second year.

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