NURSERY DISEASE NOTES USDA FOREST SERVICE

NORTHERN REGION
FOREST HEALTH PROTECTION

No. 140 June 1998

EFFECTS OF BENOMYL SOIL DRENCH TREATMENT ON SOIL POPULATIONS OF *FUSARIUM, TRICHODERMA* AND *PYTHIUM* - USDA FOREST SERVICE NURSERY, COEUR D'ALENE, IDAHO

R.L. JAMES
Plant pathologist*

ABSTRACT

A section within field 10 at the USDA Forest Service Nursery in Coeur d'Alene, Idaho was treated with benomyl applied as a soil drench as a possible alternative to fumigating soil with dazomet. Dazomet often causes toxicity to western white pine located near fumigated fields. Benomyl reduced soil populations of *Fusarium* by about 48%; the fungicide also reduced populations of *Trichoderma* by 36%. *Pythium* populations were reduced by only about 28%. *Fusarium oxysporum*, the most important potentially-pathogenic *Fusarium* species in the soil was more susceptible to benomyl than the next most common species, *F. solani.* Treating soil with benomyl is not as effective as fumigation with dazomet in reducing populations of potentially-pathogenic fungi. However, populations are significantly reduced to levels where conifer seedling diseases would probably not occur at high levels.

INTRODUCTION

Western white pine (*Pinus monticola* Dougl.) is one of the most important conifer species grown at the USDA Forest Service Nursery in Coeur d'Alene, Idaho. An aggressive breeding program for resistance to blister rust caused by *Cronartium ribicola* J.C. Fisch. has been ongoing at the nursery for several years. Tested seedling stock that has been inoculated with the blister rust fungus is usually outplanted somewhere in the nursery and evaluated for resistance; these plantings are maintained for several years.

Pre-plant soil fumigation with dazomet has been standard operating procedure at the nursery for several years; dazomet replaced methyl bromide/chloropicrin as the general-use fumigant. Although dazomet is efficaceous in controlling soil-borne pathogens at the nursery (James et al. 1990, 1996), some toxic effects

*Stationed in Coeur d'Alene, Idaho

have occurred on western white pine located near fumigated fields. Affected seedlings turn yellow and prematurely cast their foliage; in extreme cases, plants can be killed by the fumigant.

Plantings of western white pine have been maintained in the southern portion of field 10 at the nursery for several years. In order to use other parts of this and nearby fields for operational bareroot seedling production, a procedure of pre-plant soil treatment was needed that would not potentially damage white pine plantings. Therefore, a test was conducted to evaluate non-dazomet soil treatment on populations of potential soil-borne pathogens.

MATERIALS AND METHODS

The test was conducted in section 36, just north of tree improvement plantings of western white pine in the southern portion of field 10. Ten sampling plots were randomly located in four seedbed areas to represent the field. Each soil sample consisted of a composite of three soil cores taken to a depth of 15 cm (6 inches). The first sample was collected on April 15. Section 36 was treated with benomyl applied as a soil drench on May 14; the fungicide was applied at the rate of 120 kg/ha (107 lbs/acre). A second soil sample was collected on May 28 in approximately the same locations as the first sample.

All soil samples were processed for determining populations of potentially-pathogenic *Fusarium* and *Pythium* spp. using standard soil dilution procedures (James and Gilligan 1986, 1990; James et al. 1990, 1996). Soil was initially sieved (2 mm sieve) to remove rocks, pieces of undecomposed organic matter, and soil aggregations. From each sample, a 5 g subsample was dried at about 100^{o}C for at least 24 h or until sample weight had stabilized (all excess moisture removed). Oven-dry weight was then calculated to provide a standard for comparison. For assay of *Fusarium* and *Trichoderma* populations, 0.5 g of field-moist soil was combined with 100 ml of 0.3% water agar and thoroughly mixed. One ml of solution was placed on each of three plates of selective agar medium (Komada 1975) and spread uniformly. Plates were incubated seven days at about 24^{o}C under diurnal cycles of cool, fluorescent light. *Fusarium* and *Trichoderma* colonies were identified by their morphology on the selective medium and populations determined. Ratios of *Trichoderma* to *Fusarium* populations were calculated for a rough estimate of potential soil suppressiveness to pathogens (James et al. 1996). For assay of *Pythium* populations, 5.0 g of soil was combined with 100 ml of 0.3% water agar. One ml of solution was placed on each of three plates of another selective medium consisting of V-8 juice agar amended with pimaricin, rifamycin, ampicillin, and pentachloronitrobenzene (James et al. 1990, 1996). Plates were incubated three days in the dark at about 24^{o}C. *Pythium* colonies were identified based on their diameter after 3 days (15-20 mm), their feathery margin, and whether they grew within rather than superficially on the agar surface (Hendrix and Kuhlman 1965; Vaartaja and Bumbieris 1964). All fungal populations were expressed as colony-forming units per g (cfu/g) of oven-dry soil.

RESULTS AND DISCUSSION

Effects of a benomyl drench on soil populations of *Fusarium* and *Trichoderma* spp. are summarized in table 1. Average cfu/g were reduced by about half (from 1055 to 554) for *Fusarium* spp. Populations of *Fusarium* before benomyl treatment were similar to those previously assayed in an adjacent section in field 10 (James and Gilligan 1990). For *Trichoderma* spp., reductions were about 36% (from 773 to 492). Ratios of *Trichoderma* to *Fusarium* (T/F ratio) increased slightly from 0.732 before fungicide drench to 0.887 after soil treatment. In general, the higher the T/F ratio the greater the potential suppressiveness by antagonistic *Trichoderma* spp. to pathogenic *Fusarium* spp. (James et al. 1996). Of course, not all soil isolates of *Trichoderma* are disease suppressive (Papavizas 1985) nor are all *Fusarium* isolates in the soil plant pathogens (James et al. 1998).

Effects of drenches on *Pythium* populations are summarized in table 2. A much lower effect, only about a

Table 1. Effects of benomyl soil drench on soil populations of *Fusarium* and *Trichoderma* spp. at the USDA Forest Service Nursery, Coeur d'Alene, Idaho [1].

	Fusarium			*Trichoderma*		
Sample No.	Pre-Treat [2]	Post-Treat [3]	% Diff. [4]	Pre-Treat [2]	Post-Treat [3]	% Diff. [4]
1	1345	804	- 40.2	538	402	- 25.3
2	1345	402	- 70.1	807	67	- 91.7
3	2220	1340	- 39.6	134	737	+ 450.0
4	1951	603	- 69.1	404	293	- 27.7
5	471	670	+ 42.2	1076	268	- 75.1
6	404	469	+ 16.1	2287	938	- 59.0
7	1541	469	- 69.6	740	268	- 63.8
8	336	268	- 20.2	673	938	+ 39.4
9	673	385	- 50.2	269	201	- 25.3
10	269	134	- 50.2	807	804	- 0.4
Average	1055.5	554.4	- 48.0	773	492	- 36.3
Std. Dev.	679.0	319.4	------	570.2	311.2	------
Variance	460997	102046	------	325113	96856	------

[1] Soil populations in table expressed as colony-forming units per g of oven-dried soil.
[2] Soil populations one month prior to soil drench treatment.
[3] Soil populations 14 days after soil drench treatment.
[4] Percent difference between pre- and post-treatment populations.

27% reduction, was found for these water mold fungi. However, this would be expected since benomyl and other closely-related benzimidazole fungicides are generally not effective against water mold fungi (Thomson 1997). Other specific fungicides, such as metalaxyl selectively control *Pythium* spp. and other water molds (Thomson 1997). *Pythium* populations from previous samples in field 10 were higher than untreated populations in this evaluation (James 1986, 1990). However, *Pythium* populations and associated disease will often vary widely depending on level and persistence of soil moisture (James et al. 1990, 1996; Vaartaja and Bumbieries 1964).

Soil populations of microorganisms vary widely within fields at the nursery (James and Gilligan 1986, 1990; James et al. 1990, 1996). This results in high standard deviations when population counts are averaged (tables 1 and 2). *Fusarium, Trichoderma* and *Pythium* spp. tend to be aggregated within and around soil organic matter (James 1998). High populations are usually located on organic debris, such as roots of a previous conifer crop or cover crop material incorporated into soil (James, 1998; James et al. 1991, 1996).

The major *Fusarium* species isolated from soil samples was *F. oxysporum* Schlect. (table 3). This species comprised about 72% of the *Fusarium* population in untreated soil, which is similar to the proportion found in other parts of the nursery (James et al. 1996). Other *Fusarium* species found in soil included *F. solani* (Mart.) Appel & Wollenw., *F. scirpi* Lambotte & Fautr., *F. equiseti* (Corda) Sacc., and *F. acuminatum* Ell. & Ev. Apparently *F. oxysporum* was more susceptible to benomyl than the other common soil species, *F. solani*. Percent of the *Fusarium* population made up of *F. oxysporum* was reduced by

Table 2. Effects of benomyl soil drench treatment on soil populations of *Pythium* spp. at the USDA Forest Service Nursery, Coeur d'Alene, Idaho [1].

Sample Number	Pre-Treatment[2]	Post-Treatment[3]	Percent Difference[4]
1	67	108	+ 61.2
2	0	87	------
3	67	93	+ 38.8
4	471	54	- 88.5
5	67	74	+ 10.4
6	0	80	------
7	67	67	None
8	0	10	------
9	67	30	- 55.2
10	67	50	- 25.6
Averages	87.3	63.5	- 27.6
Std. Dev.	131.4	28.3	------
Variance	17256	802	------

1 Soil populations in table expressed as colony-forming units per g of oven-dried soil.
2 Soil populations one month prior to soil drench treatment.
3. Soil populations 14 days after soil drench treatment.
4. Percent differences between pre-and post treatment soil populations.

about 73% by benomyl treatment, whereas the percent comprised of *F. solani* increased by 83%. Since *F. oxysporum* is usually the most pathogenic *Fusarium* species in nursery soil (James et al. 1991, 1998), reducing its population is most important from the standpoint of influencing potential conifer seedling diseases.

In conclusion, this evaluation showed that benomyl can effectively reduce soil populations of potentially-pathogenic *Fusarium* spp. Although benomyl treatments are not nearly as effective as dazomet soil fumigation, application of the fungicide might be an effective alternative to the fumigant in portions of the nursery which cannot be fumigated because of close proximity to western white pine. It is possible that additional applications of the fungicide, especially following soil cultivation and mixing, might further reduce soil *Fusarium* levels. Although benomyl also reduced soil levels of *Trichoderma* spp., T/F ratios were not greatly changed. It is important that growers realize that fungicide drenches with benomyl will probably not greatly affect populations of some soil fungi, such as *Pythium* spp. and other water mold fungi. Therefore, the specific organisms to be reduced by fungicide applications should be identified prior to treatment.

LITERATURE CITED

Hendrix, F.F. and E.G. Kuhlman. 1965. Factors affecting direct recovery of *Phytophthora cinnamomi* from soil. Phytopathology 55:1183-1187.

James, R.L. 1998. Effects of incorporating corn green manure crops on soil populations of *Fusarium, Trichoderma* and *Pythium* - USDA Forest Service

Table 3. Effects of benomyl soil drench treatment on *Fusarium* species in soil at the USDA Forest Service Nursery, Coeur d'Alene, Idaho.

Fusarium species	Population Pre-Treatment [1]	Percent of *Fus.* species	Population Post Treatment [1]	Percent of *Fus.* species	Percent Difference [2]
F. oxysporum	760	72.0	208	37.5	- 72.6
F. solani	181	17.2	332	60.5	+ 83.4
F. scirpi	20	1.9	0	0	- 100.0
F. equiseti	94	8.9	7	1.3	- 92.5
F. acuminatum	0	0	7	1.3	+ 100.0
All *Fusarium*	1055	100.0	554	100.0	- 36.3

[1] Soil populations expressed as colony-forming units per g of oven-dried soil.
2. Percent differences between pre- and post treatment soil populations.

Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Nursery Disease Notes No. 137. 8p.

James, R.L. and C.J. Gilligan. 1986. Soil populations of *Fusarium* and *Pythium* in Field 10, USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 32. 3p.

James, R.L. and C.J. Gilligan. 1990. Soil populations of *Fusarium* and *Pythium* within block 35, field 10, USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Nursery Disease Notes No. 102. 3p.

James, R.L., R.K. Dumroese and D.L. Wenny. 1991. *Fusarium* diseases of conifer seedlings. *In*: Sutherland, J.R. and S.G. Glover (eds.). Proceedings of the first meeting of IUFRO Working Party S2.07-09 (Diseases and Insects in Forest Nurseries). Forestry Canada. Pacific and Yukon Region. Information Report BC-X-331. pp. 181-190.

James, R.L., S. Metzger and C.J. Gilligan. 1990. Effects of soil fumigation on conifer seedling production at the USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Pest Management. Report 90-11. 18p.

James, R.L., D.S. Page-Dumroese, S.K. Kimball and S. Omi. 1996. Effects of *Brassica* cover crop, organic amendment, fallowing, and soil fumigation on production of bareroot Douglas-fir seedlings - USDA Forest Service Nursery, Coeur d'Alene, Idaho. USDA Forest Service, Northern Region, Forest Health Protection. Report 96-5. 16p.

James, R.L., R. Perez, R.K. Dumroese and D.L. Wenny. 1998. Virulence of *Fusarium oxysporum* on Douglas-fir germinants: comparisons of isolates from forest nursery soil and roots of healthy and diseased seedlings (In preparation).

Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Review of Plant Protection Research (Japan) 8:114-125.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annual Review of Phytopathology 23:23-54.

Thomson, W.T. 1997. Agricultural chemicals. Book IV - Fungicides (12th edition). Thomson Publications, Fresno, CA. 236p.

Vaartaja, O. and M. Bumbieris. 1964. Abundance of *Pythium* species in nursery soils in South Australia. Australian Journal of Biological Science 17:436-445.