Bull. Environ. Contam. Toxicol. (1985) 34:533–540

Bulletin of Environmental Contamination and Toxicology

Persistence of Benomyl and Captan and Their Effects on Microbial Activity in Field Soils

Ching Y. Li and Earl E. Nelson

U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station, Forestry Sciences Laboratory, Corvallis, OR 97331

The J. Herbert Stone Forest Nursery was established by the Forest Service, United States Department of Agriculture, in 1976 on 104 ha of former orchard, pasture, and grain-producing land near Medford, Oregon.

Fusarium root rot, caused by Fusarium oxysporum schl. f. sp. pini (Hartig) Snyder & Hansen, causes severe losses to some species of conifers even though beds are fumigated with methyl bromide-chloropicrin (3:2) the autumn before spring seeding. Benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate) at 11.2 kg a.i./ha or captan (N-trichloromethylthio-4-cyclohexene-1,2-dicaboximide) at 7.3 kg a.i./ha were applied to beds of sugar pine (Pinus lambertiana Dougl.) on Central Point sandy loam to control seedling mortality (Cooley 1983), but no measurement was made of its persistence or of its effect on soil microbial activity.

Benomyl in soil is easily hydrolyzed into the relatively stable carbendazim (methylbenzimidazole-2-ylcarbamate) that is the major fungitoxic principle of benomyl. The carbendazim residues may remain in soil from less than 3 months to more than 2 years. Captan residues may remain for one day to several months depending on soil type, temperature or moisture content (Agnihotri 1970; Austin & Briggs, 1976; Griffith & Mathews, 1969; Edwards & Thompson, 1973).

Our study was designed to measure the persistence of benomyl and captan and their effects on microbial activity in the upper 30 cm of soil in each of the two major soil types at the nursery.

This publication reports research involving pesticides. It does not contain recommendations for their use nor does it imply that the uses discussed here have been registered. All pesticides must be registered by appropriate State and/or Federal agencies before they can be used.

MATERIALS AND METHODS

There are six soil types at the nursery; two of which cover large areas now in seedling production and are sufficiently different from one another to suspect differences in the persistence of fungicides over time: (1) Central Point sandy loam consists of excessively drained sandy loam formed in alluvium; mainly of granite and metamorphic origin. The permeability is moderately rapid. The available water holding capacity is 14 to 19 cm. Soil pH in the top 30 cm varies between 6 and 6.9. Organic matter content in the top 30 cm is under 2%. (2) Kubli loam consists of somewhat poorly drained loam over clay or heavy clay loam soils formed in alluvium from granite rocks abruptly over older valley clay fill. The permeability is slow. The available water holding capacity is 14 to 25 cm (up to 14 cm over the clay). Soil pH in the top 30 cm varies between 5.8 and 6. Organic matter content in the top 30 cm is just above 3%.

Benomyl[a] was applied as a drench at rates of 0, 14, 28, and 42 kg a.i./ha, and captan as a drench at rates of 0, 7, 14, and 21 kg a.i./ha, on April 18, 1981, immediately following seeding of sugar pine. Drenches of 8 liters and 1 liter/plot were used for benomyl and captan respectively. Registered dosage for benomyl is 61-122 kg/ha and for captan is 15 kg/ha. Each treatment was applied at random over the surface of three replicated 1.22- x 1.22-m plots separated by a 1.22-m buffer zone. For each of the two soil types, plots were situated on two adjacent beds.

Soil samples, from depths of 0-15 cm and 15-30 cm, were collected with a 20-mm diameter soil sampling tube weekly during the first month and bimonthly thereafter for 1 year following application of the fungicides. Five subsamples were taken at random from each plot for each depth at each sampling time. For each plot subsamples were combined, sieved through a 2-mm screen and assayed.

Fifty grams of screened soil were extracted by shaking for 2 h at room temperature with 100 ml acetone-1 M aqueous ammonium chloride (1:1), (Austin and Briggs 1976). The soil was separated by filtration. The acetone in the clear filtrate was removed by flash evaporation at 50° C. After adding 10 drops of concentrated HCl, the filtrate was extracted twice with 25 ml of ethyl acetate. The aqueous phase was retained, 0.7 ml of 6.5 M NaOH added to it, and then extracted two times with ethyl acetate. The combined ethyl acetate was extracted twice with 5 ml of 0.1 M HCl. The carbendazim in HCl was determined with an

---

[a]Trade names are included for information only and do not imply endorsement by the Forest Service, United States Department of Agriculture.

ultraviolet spectrum at 282 nm using processed control soils as blanks (Austin and Briggs 1976). Residue data were regressed over time for linear, quadratic, cubic, or quartic fit in attempts to more precisely determine the probable end point for fungicide detection in soil.

The colorimetric method developed by Simon and Kulik (1971) was used for estimation of the amount of captan remaining in soil. The technique involved the extraction of 50 grams of soil with 100 ml of benzene, removal of interferring dyes with Florisil[a], reaction with a resorcinol solution, and determination of color intensity at 428 nm with a colorimeter.

Microbial activity in the fungicide-treated soil was determined using the soil dehydrogenase assay, which has been used by a number of workers to determine the activity of the soil microflora after exposure to agricultural pesticides (Smith & Pugh, 1979). Twenty g of sieved soil were mixed with 0.2 g of $CaCO_3$. Six g of soil were then dispensed into a test tube. One ml of 3% 2, 3, 5-triphenyltetrazolium chloride and 2.5 ml of H_2O were added to the test tube. The tube was then incubated at 37° C for 24 h. The triphenylformazan formed was extracted with methanol and its color intensity was determined in a spectrophotometer at 485 nm with methanol as a blank. Concentration of formazan was determined by reference to a standard curve of formazan in methanol. Data were subjected to analysis of variance to detect significant differences (.05) in activity at each of the sampled soil depths.

RESULTS AND DISCUSSION

Captan was not detected at any rate of application in either soil type at depths of 0-15 cm or 15-30 cm one week after application or during any of the sampling periods thereafter. Captan, at rates equivalent to 7 kg a.i./ha mixed with either soil just prior to analysis, however, was readily detectable using the same analysis procedures.

In Kubli loam, benomyl could be detected in all treated plot samples 13 weeks after application. At 21 weeks, benomyl was detected in the 0-15-cm samples of all treatment levels, and at 30 weeks and thereafter could not be detected in any of the soil samples (Table 1).

In Central Point sandy loam, benomyl could not be detected at 13 weeks or thereafter in 14 kg a.i./ha plots, at 21 weeks or thereafter in 28 kg a.i./ha plots, nor at 30 weeks or thereafter in 42 kg a.i./ha plots. At the highest rate, benomyl was detected at 21 weeks but only in small amounts (.03 ug/g soil) and then only in the 0-15 cm soil level (Table 1).

Table 1. Concentration of benomyl residues determined as carbendazim in two soil types.

Time interval (weeks)	Central Point sandy loam Rate of application (kg a.i./ha) 14	28	42	Kubli loam Rate of application (kg a.i./ha) 14	28	42
1	3.33[a]	7.21	10.41	5.14	12.09	21.90
	0.50	1.24	3.29	--[b]	--	--
2	2.48	4.33	9.60	4.50	8.83	18.66
	0.07	0.12	0.14	0.49	1.10	1.31
3	1.98	2.73	7.78	2.69	5.13	11.15
	0.41	0.24	0.26	0.86	0.92	1.00
4	0.99	2.38	6.08	1.69	2.62	5.20
	0.46	0.55	0.99	0.62	0.81	0.99
13	-0-	0.02	0.07	0.44	1.63	2.59
	-0-	0.14	0.10	0.03	0.09	0.20
21	-0-	-0-	0.03	0.17	0.23	0.70
	-0-	-0-	-0-	--	--	--
30	-0-	-0-	-0-	-0-	-0-	-0-
	-0-	-0-	-0-	-0-	-0-	-0-
39	-0-	-0-	-0-	-0-	-0-	-0-
	-0-	-0-	-0-	-0-	-0-	-0-
48	-0-	-0-	-0-	-0-	-0-	-0-
	-0-	-0-	-0-	-0-	-0-	-0-

[a]Upper row represents carbendazim (mg/kg soil) in 0-15 cm soil layer; lower row represents carbendazim (mg/kg soil) in 15-30 cm soil layer. Data are means of three replicates.

[b]Soil not available for determination.

Regression analysis of benomyl residue data did not result in improved prediction of times when residues would no longer be detectable, probably because temperature and precipitation were greater factors than time itself. Zero levels shown in Table 1 can be used as conservative estimates of time when benomyl residues drop below the level of detection in these two soils.

In Central Point sandy loam, dehydrogenase activity peaked higher and earlier in captan- and benomyl-treated soils than in controls at the 0-15-cm depth (Fig. 1A). Peaks were earlier but lower than controls at the 15-30-cm depth (Fig. 1B). At either depth, dehydrogenase activity peaked 1-2 weeks earlier in captan than in benomyl-amended soils. In 13-week samples and thereafter there appeared to be little difference among captan-amended, benomyl-amended, and untreated soil.

Dehydrogenase activity was more erratic in the Kubli loam soil. In the 0-15-cm soil layer, dehydrogenase activity in benomyl-amended soil did not peak until 21 weeks (Fig. 1C). In captan-amended soil the peak was reached 2 weeks after application. At the 15-30-cm depth, dehydrogenase activity peaked at 4 weeks in both benomyl- and captan-amended soil, well above that of the control soil (Fig. 1D). Differences between control and treated plots were generally smaller at 13 weeks and thereafter at the 15-30-cm depth. At the 0-15 cm depth differences became rather small at 30 weeks and thereafter.

Analysis of variance showed the differences in dehydrogenase activity between soils and among sampling times to be significant at either depth. Interactions of control vs fungicides and sampling time; control vs fungicides, sampling time and soil type; benomyl vs captan and sampling time; and benomyl vs captan, sampling time and soil type were significant.

Our results show that captan disappeared quickly from Central Point sandy loam and Kubli loam. Captan was not detected 1 week or thereafter following application. The results support the findings of other workers that captan persists in natural soil for 1 to 2 weeks at most, and only 1 to 2 days in many instances (Agnihotri 1970; Simon & Kulik, 1971; Burchfield 1960). When applied to the soil at higher concentrations in specific areas (e.g., for use as a seed protectant), captan residues are detectable for longer periods in those specific locales. The degradation of captan in the soil is probably due to chemical hydrolysis and the actions of soil microorganisms.

In comparison to captan, benomyl (carbendazim) persisted longer in both soil types. Kubli loam retained more than did Central Point sandy loam and for longer periods. Solel et al. (1979) reported that the persistence of carbendazim in sandy loam and sandy clay was short. Degradation was nearly complete 10 weeks after application. Different factors may affect persistence in soil. High temperature accelerates the disappearance of

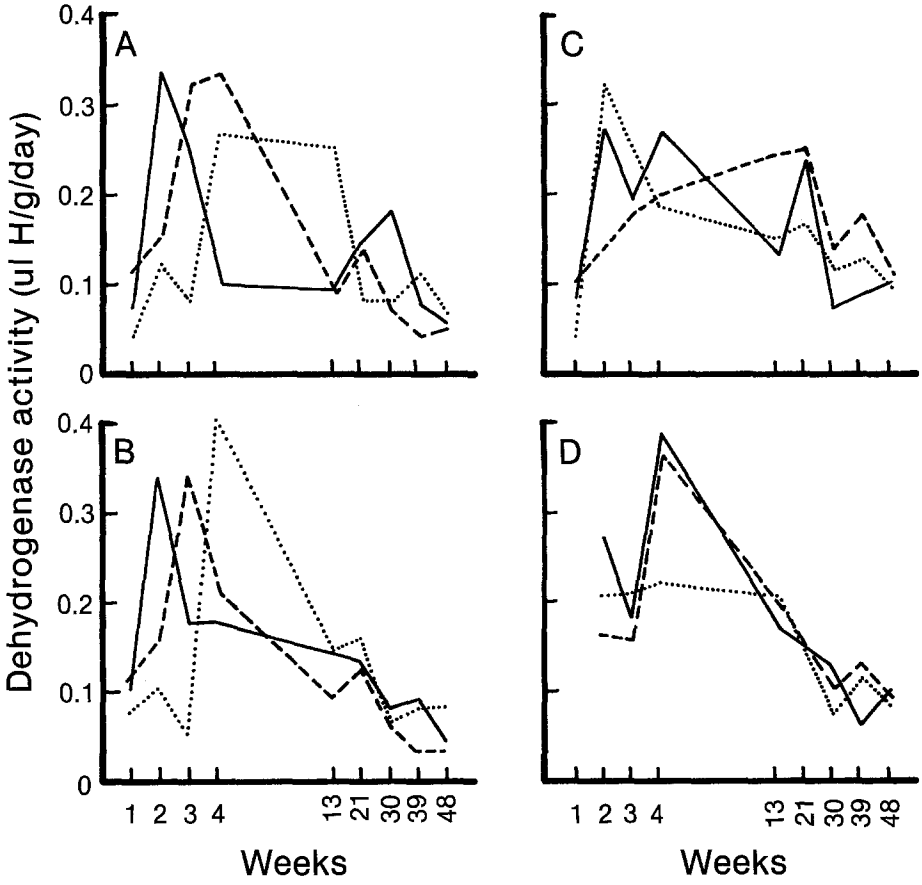

Figure 1. Dehydrogenase activity in two benomyl- and captan-amended soils: Central Point sandy loam--(A) 0-15 cm, (B) 15-30 cm; and Kubli loam--(C) 0-15 cm (D) 15-30 cm. (Control....., benomyl at 14-42 kg a.i./ha -----, captan at 7-21 kg a.i./ha _____).

benomyl, especially under conditions of higher moisture contents (Van Wambeke 1979). Nursery beds were watered daily during the summer to moderate high temperatures (32-46° C). Thus benomyl, applied in spring, would not be expected to persist long into summer, though we cannot be sure how much was lost to degradation and how much passed on to soil deeper than 30 cm. Moisture content and temperature also affect microbial activity that degrades benomyl, although the chemical or physico-chemical degradation mechanism may be no less important.

Smith and Pugh (1979) reported that soil dehydrogenase activity is an index of the biological activities of microbial populations. Increase or reduction in dehydrogenase activity can also be associated with a concomitant increase or reduction in the number of viable microorganisms. In benomyl- and captan-treated Central Point sandy loam, dehydrogenase activity reached its highest point 1-4 weeks after application, presumably at those periods with the highest active microbial populations. Agnihotri (1970) reported an increase of aerobic spore-forming bacteria in captan-treated soils 7 days after application. On the 35th day, no significant differences were observed between the bacterial population of control and captan-treated soils. In our study, captan and benomyl appeared to accelerate soil biological activity in the sandy loam, but trends were similar to control soils where populations apparently increased rapidly in the moist, warming soil. There appeared to be little difference in activity after about 13 weeks; by that time microbial populations apparently had approached equilibrium. In Kubli loam, activity was increased above the levels in control groups by either fungicide, but only at the 15-30-cm depth.

Siegel (1975) showed that benomyl added to soil caused no changes in bacterial populations but did reduce numbers of fungi and actinomycetes. Kaastra-Howler & Gam (1973) and Peeples (1974) however, reported little effect of benomyl on soil microbial populations. The accelerated increase of dehydrogenase activity in benomyl-treated soils in the J. Herbert Stone Forest Nursery may be related to the degradation of benomyl by soil microorganisms. Positive correlations between dehydrogenase activity and rate of respiration (perhaps related to degradation) have been found in some soils (Casida et al. 1964; Rawald et al. 1968).

The differences we found in dehydrogenase activity, though significant between soils and sampling times, appear to be minor between treated and control soils over the course of the study. This and the relatively rapid disappearance of the two fungicides from the two soils types, lead us to believe that either captan or benomyl can be used, at least occasionally, in similar soils under similar conditions without significant damage to the soil ecosystem. The fate of the benomyl that may have passed through the upper 30 cm of soil, however, has not been determined.

Acknowledgements. The authors wish to thank Ms. Susan Schechter for her help in collecting, processing, and analyzing soil samples and Dr. Robert K. Campbell and Ms. Nancy Mandel for their help in data analysis. The work was funded in part by the National Agricultural Pesticide Impact Assessment Program (NAPIAP).

REFERENCES

Austin DJ, Briggs GG (1976) A new extraction method for benomyl residues in soil and its application in movement and persistence studies. Pesticide Sci 7:377-383

Agnihotri VP (1970) Persistence of captan and its effect on microflora, respiration, and nitrification of a forest nursery soil. Can J Microbiol 17:377-383

Burchfield HP (1960) Performance of fungicides on plant and in soil - physical, chemical and biological considerations. In: Horsfall JG, Dimond AE (ed) Plant Pathology. Academic Press, New York, 3:477-520

Casida LE Jr, Klein DA, Santoro T (1964) Soil dehydrogenase activity. Soil Sci 98:371-376

Cooley SJ (1983) Fungicidal trials on sugar pine at a southern Oregon nursery. Tree Planter's Notes 34:15-18

Edwards CA, Thompson AR (1973) Pesticides and soil fauna. Residue Rev 45:1-79

Griffith RL, Mathews S (1969) The persistence in soil of fungicidal seed dressings captan and thiram. Ann Appl Biol 64:113-118

Kaastra-Howeler LH, Gams W (1973) Preliminary study on the effect of benomyl on the fungal flora in a greenhouse soil. Neth J Plant Pathology 79:156-158

Peeples JL (1974) Microbiol activity in benomyl-treated soil. Phytophathology 64:857-860

Rawald W, Domke K, Stohr G (1968) Studies on the relations between humus quality and microflora of the soil. Pedobiologia 7:375-380

Siegel MR (1975) Benomyl-soil microbial interactions. Phytopathology 65:219-220

Simon PW, Kulik MM (1971) Routine estimation of captan on individual Sorghum seeds. J Assoc Official Agr Chemists 54:1110-1112

Smith SN, Pugh GJF (1979) Evaluation of dehytrogencil as a suitable indicator of soil microflora activity. Enzyme and Microbial Technology 1:279-281

Solel Z, Sandler D, Dinoor A (1979) Mobility and persistence of carbendazim and thiabendazole applied to soil via drip irrigation. Phytopathology 69:1273-1277

Van Wambeke E (1979) The fate of organic fungicides in soil In: Mulder D (ed) Soil Disintestation. Elsevier Scientific Publishing Co., New York, pp.349-358

Received May 15, 1984; accepted June 12, 1984.