Microbial Activity in Benomyl-Treated Soils

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ABSTRACT

Benomyl did not alter the gross microbial populations of soils treated in the field at 2.2, 22.4, or 89.6 kg (active ingredient)/ hectare, and only an initial slight increase was observed in the respiration rates of soils treated in the laboratory at 10 μg/g of soil. Dilution plates on three selective media were made periodically from soils treated two or three times annually by incorporation into cropped area, or by surface application to turf or fallow areas in Delaware, Florida, and North Carolina. Respiration was measured by CO_2 evolution.

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Additional key words: fungicide, soil microflora, *Penicillium*, *Fusarium*, *Trichoderma*.

Significant changes in soil microbial populations due to commercial applications of herbicides, insecticides, or nematicides have rarely been observed (1, 2, 3, 8, 18, 19), but growth responses in pure culture may increase or decrease depending on the pesticide and the microorganism (4, 13, 14, 15). Very little information is available in the literature concerning the longer-term effects of fungicides on the soil microflora. While one can expect target pathogens and other sensitive soil microorganisms to be temporarily affected by fungicides, it is important to be aware of any more lasting effects on the common soil microflora which play an important role in soil fertility.

Benlate® Benomyl Fungicide [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate], E. I. du Pont de Nemours & Company, Wilmington, Delaware 19898, is a commercial fungicide which is particularly effective in controlling many fungal plant pathogens by foliar application (5). In addition, certain soil-borne pathogens, such as *Verticillium albo-atrum* Reinke & Berth., are controlled by direct soil application (5, 7, 9, 20). The studies reported in this paper were initiated to determine the effect of field applications of benomyl on the number of fungi and bacteria, and the evolution of carbon dioxide in soils.

MATERIALS AND METHODS.—*Microbial population studies.*—Benomyl was incorporated as a 50% wettable powder into a Keyport silt loam soil in Delaware in 1968 at rates of 2.2 and 89.6 kg (active ingredient)/hectare (ha). Plots were divided and one-half was planted to Kentucky bluegrass hereinafter referred to as "turf" which was retreated annually for 3 yr by surface applications at the above rates. The other half was planted each year to soybeans, sugar beets, and squash hereinafter referred to as "crop area" and retreated annually by soil incorporations at the above rates. In addition, benomyl was surface applied in 1970 and 1971 to a Keyport silt loam soil in Delaware, a Leon Immokalee fine sand soil in Florida, and a Cecil sandy loam soil in North Carolina at rates of 2.2 and 22.4 kg/ha. These treatments were applied to turf and fallow soil.

Soil samples were taken randomly in the plots to a depth of 15.2 cm (6 in) at prescribed intervals. Samples from each crop location were combined and mixed, moisture determined, and 10 g (oven-dry weight basis) of each diluted in sterile distilled water. The final dilution was made into peptone-dextrose rose bengal agar (12) for isolation of soil fungi and Thornton's medium (17) and arginine-glycerol salt agar (6) for isolation of soil bacteria. Three replicates of each medium were incubated 3 to 5 days at 27 C. The number of microorganisms per plate was averaged and reported as number of colonies

per gram of soil. Tentative identifications were made of predominant fungi.

CO_2 evolution studies. —Turf and fallow soil that had received two annual surface applications of benomyl at 2.2 and 22.4 kg/ha was randomly sampled to a depth of 15.2 cm (6 in). Untreated soil was similarly sampled to serve as control. The soil was air-dried and passed through a 2.3-mm (mesh size) screen. Percent moisture was determined and 100 g (oven-dry weight basis) of each sample was placed into each of four wide-mouth 500-ml Erlenmeyer flasks. Soil moisture in two flasks was adjusted to 65% of the waterholding capacity with distilled water, and soil moisture in the remaining two flasks was adjusted to 65% of the waterholding capacity with a benomyl distilled water suspension to provide 10 μg/g of soil. Thus, there were 12 treatments (See Table 4) and each treatment was replicated twice. A test tube containing 10 ml of 0.5 N NaOH trapping solution was placed in each flask and the flask sealed air tight with a rubber stopper. Evolution of CO_2 was monitored for 32 days at which time 2 ml of a nutrient solution containing 300 mg of glucose, 12 mg of K_2HPO_4, and 24 mg of KNO_3 was added to the soil in each flask. The experiment continued for 10 days. Trapping tubes were changed periodically over the 42-day incubation period. Production of CO_2 was determined according to the method of Stotzky (16).

RESULTS AND DISCUSSION.—*Microbial population studies.*—Results obtained from soil microbial population studies are presented in Tables 1, 2, and 3. Benomyl had no discernible effect on the fungal or bacterial populations as evidenced by the dilution plate counts. Even repeated annual applications at 89.6 kg benomyl/ha did not have an adverse effect on microbial populations. In a few instances, the numbers of fungi or bacteria for a benomyl treatment differed by as much as twofold from the control for that soil. Despite these unexplained variations and the expected variations due to differences in soil, season, and cropping practice, there was no evidence of a trend toward increased or decreased micropopulations due to benomyl treatments. These observations are in line with those made by Kaastra-Howeler and Gams (10) in a greenhouse soil treated with benomyl.

Certain common soil fungi, *Penicillium*, *Aspergillus*, and *Trichoderma* were consistently isolated from benomyl-treated soil. These fungi are sensitive to benomyl in culture, but appear to grow in normal

TABLE 1. Effect of benomyl on soil microbial populations in a Keyport silt loam soil treated in 1968 and annually thereafter

Benomyl rate (kg active ingredient per hectare per year)	Organisms per gram of soil			
	Fungi × 10^4		Bacteria × 10^6	
	3 Dec 70[a]	14 June 71[b]	3 Dec 70[a]	14 June 71[b]
Turf[c]				
Control	3.2	6.5	12.2	23.3
2.2	5.3	5.0	15.5	26.3
89.6	3.9	5.4	8.8	26.3
Crop Area[d]				
Control	2.4	5.2	8.4	26.0
2.2	3.5	3.7	13.3	20.9
89.6	4.6	4.0	14.1	25.7

[a]Sample taken 7 mo after third soil incorporation treatment.
[b]Sample taken 1 mo after fourth soil incorporation treatment.
[c]Kentucky bluegrass.
[d]Soybeans, sugar beets, squash.

TABLE 2. Effect of benomyl on soil microbial populations in a Keyport silt loam soil treated 25 June 1970 and retreated 27 May 1971

Benomyl rate (kg active ingredient per hectare)	Organisms per gram of soil					
	Fungi × 10^4			Bacteria × 10^6		
	6 Aug 70	3 Dec 70	21 June 71	6 Aug 70	3 Dec 70	21 June 71
Turf[a]						
Control	2.4	4.5	9.6	8.0	17.4	28.9
2.2	6.4	4.3	5.0	9.1	16.4	38.1
22.4	8.5	4.5	7.1	11.5	8.6	32.5
Fallow						
Control	4.8	6.1	5.1	5.3	14.1	18.9
2.2	4.6	4.6	4.7	8.8	7.9	19.3
22.4	7.8	5.4	5.1	8.7	6.9	25.7

[a]Kentucky bluegrass.

TABLE 3. Effect of benomyl on soil microbial populations in a Leon Immokalee fine sand soil (Florida) and a Cecil sandy loam soil (North Carolina)

Benomyl rate (kg active ingredient per hectare)	Organisms per gram of soil			
	Fungi × 10^4		Bacteria × 10^6	
	Florida[a]	North Carolina[b]	Florida[a]	North Carolina[b]
Turf[c]				
Control	10.3	3.8	18.2	18.3
2.2	6.0	5.7	21.9	25.6
22.4	7.3	2.8	18.7	19.6
Fallow				
Control	8.3	2.3	6.9	3.9
2.2	4.0	1.9	4.7	4.7
22.4	5.2	1.3	3.8	6.6

[a]Sample taken 10 wk after second annual treatment.
[b]Sample taken 5 wk after second annual treatment.
[c]Kentucky bluegrass.

TABLE 4. Production of CO_2 by soil microorganisms in benomyl-treated soil

Benomyl field application [kg active ingredient per hectare per year (applied twice)]	Milligrams of CO_2 produced per 100 g soil					
	Incubation time (days)					
	8	32[a]	34	36	40	42
Turf[c]						
Control	85	244	431	479	544	570
Control[b]	84	243	431	478	542	567
2.2	93	284	475	522	587	613
2.2[b]	93	285	480	527	593	620
22.4	94	259	454	502	566	590
22.4[b]	91	258	453	503	568	593
Fallow						
Control	32	108	304	350	399	415
Control[b]	34	108	300	347	396	412
2.2	30	116	315	359	409	427
2.2[b]	28	98	301	347	393	410
22.4	26	102	293	338	384	398
22.4[b]	27	91	280	328	374	388

[a]Nutrients added after 32 days of incubation.
[b]Benomyl added in the laboratory at the rate of 10 μg benomyl/g soil.
[c]Kentucky bluegrass.

populations in benomyl-treated soil. Wensley and Huang (20) observed that *Penicillium*, *Fusarium*, and *Trichoderma* were recovered in substantial numbers from soil treated at rates of 40-160 μg benomyl/g of soil. J. W. Searcy from our laboratory has been able to grow *Trichoderma viride* on soil containing 5 μg benomyl/g of soil. This same culture was inhibited by 1 μg benomyl/ml of agar medium. Benomyl is adsorbed so tightly to soil that it is unavailable to affect these fungi.

CO_2 evolution studies.—Data from CO_2-evolution studies are presented in Table 4. No differences were observed in CO_2 evolved from untreated turf soil and turf soil treated in the laboratory at 10 μg benomyl/g soil. However, the amount of CO_2 evolved from turf soil treated in the field at 2.2 and 22.4 kg benomyl/ha was slightly greater than the amount of CO_2 evolved from the untreated controls. This increased respiration associated with field applications of benomyl plus additional applications in the laboratory might result from the degradation of benomyl by soil microorganisms. No differences in evolved CO_2 were evident from fallow soil. In view of the demonstrated lack of activity of benomyl against fungal and bacterial populations in the soil, the absence of an influence of benomyl on a biological process such as carbon dioxide evolution is not surprising. Kouba (11) has observed that the addition of benomyl to sewage increases the rate of decomposition of organic wastes. He has also demonstrated a more rapid and complete conversion of fertilizer nitrogen into nitrates when benomyl is added to soil. These findings concur with those reported in this paper and also point out a beneficial aspect of benomyl apart from plant disease control.

This investigation has revealed that the commonly observed soil fungi and bacteria can tolerate field applications of benomyl. While it is impossible to

conclude that all the life processes of all microorganisms in soil are in no way affected by benomyl, it seems probable that for most soil microorganisms there is nothing more than a slight or short-term influence.

LITERATURE CITED

1. AUDUS, L. J. 1964. Herbicide behavior in soil. II. Interactions with soil microorganisms. Pages 163-206. *in* L. J. Audus, ed. The physiology and biochemistry of herbicides. Academic Press, New York.
2. BARTHA, R., R. P. LANZILOTTA, and DAVID PRAMER. 1967. Stability and effects of some pesticides in soil. Appl. Microbiol. 15:67-75.
3. BOLLEN, W. B. 1961. Interactions between pesticides and soil microorganisms. Annu. Rev. Microbiol. 15:69-92.
4. CURL, E. A., R. RODRIGUEZ-KABANA, and H. H. FUNDERBURK, JR. 1968. Influence of atrazine and varied carbon and nitrogen amendments on growth of Sclerotium rolfsii and Trichoderma viride in soil. Phytopathology 58:323-328.
5. DELP, C. J., and H. L. KLOPPING. 1968. Performance attributes of a new fungicide and mite ovicide candidate. Plant Dis. Rep. 52:95-99.
6. EL-NAKEEB, M. A., and H. A. LECHEVALIER. 1963. Selective isolation of aerobic actinomycetes. Appl. Microbiol. 11:75-77.
7. ERWIN, D. C., H. MEE, and J. J. SIMS. 1968. The systemic effect of 1-(butylcarbamoyl)-2-benzimidazolecarbamic acid, methyl ester, on Verticillium wilt of cotton. Phytopathology 58:528-529.
8. FLETCHER, W. W. 1960. The effect of herbicides on soil microorganisms. Pages 20-62. *in* E. K. Woodford, ed. Herbicides and the soil. Blackwell Scientific Publications. Oxford, England.
9. HINE, R. B., D. L. JOHNSON, C. J. WENGER, and J. E. ENDRIZZI. 1968. A systemic fungicide controls Verticillium wilt. Progressive Agriculture in Arizona 20(4):6-7.
10. KAASTRA-HOWELER, L. H., and W. GAMS. 1973. Preliminary study on the effect of benomyl on the fungal flora in a greenhouse soil. Neth. J. Plant Pathol. 79:156-158.
11. KOUBA, N. R. 1972. Method of increasing the beneficial oxidation of a biological substrate with 2-aminobenzimidazole derivatives. United States Patent 3,649,530.
12. MARTIN, J. P. 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69:215-233.
13. PEEPLES, J. L., and E. A. CURL. 1969. Effect of paraquat, EPTC, and trifluralin on growth of Fusarium oxysporum f. sp. vasinfectum in liquid culture. Phytopathology 59:117 (Abstr.).
14. RODRIGUEZ-KABANA, R., E. A. CURL, and H. H. FUNDERBURK, JR. 1966. Effect of four herbicides on growth of Rhizoctonia solani. Phytopathology 56:1332-1333.
15. RODRIGUEZ-KABANA, R., E. A. CURL, and J. L. PEEPLES. 1970. Growth response of Sclerotium rolfsii to the herbicide EPTC in liquid culture and soil. Phytopathology 60:431-436.
16. STOTZKY, G. 1965. Microbial respiration. Pages 1550-1572. *in* C. A. Black, ed. Methods of soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy, Inc., Madison, Wisconsin.
17. THORNTON, H. G. 1922. On the development of a standardized agar medium for counting soil bacteria, with special regard to the repression of spreading colonies. Ann. Appl. Biol. 9:241-274.
18. TU, C. M. 1970. Effect of four organophosphorous insecticides on microbial activities in soil. Appl. Microbiol. 19:479-484.
19. TU, C. M. 1972. Effect of four nematicides on activities of microorganisms in soil. Appl. Microbiol. 23:398-401.
20. WENSLEY, R. N., and C. M. HUANG. 1970. Control of Fusarium wilt of muskmelon and other effects of benomyl soil drenches. Can. J. Microbiol. 16:615-620.