Journal of the Arkansas Academy of Science

Volume 33 Article 25

1979

Effect of Turf Fungicides on Earthworms

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The Effect of Turf Fungicides on Earthworms[1]

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ABSTRACT

Numerous turf fungicides were tested under various conditions for possible deleterious effects upon the earthworm *Eisenia foetida*. Earthworms treated by immersion for one minute in 0.1% solutions of 10 different fungicides died in significant numbers after benomyl and thiophanate methyl treatments. After 1% fungicide treatments, there was significant mortality from benomyl, ethazole, Kromad, and thiophanate methyl fungicides. With 2% fungicide solutions, significant numbers died after benomyl, cadmium succinate, ethazole, thiophanate methyl, and thiram treatments.

Earthworms fed bermudagrass clippings treated with 10 different fungicides showed a significant decrease in longevity from clippings treated with benomyl, dinocap, ethazole, and thiophanate methyl.

Earthworms reared for 84 days in soil treated with 15 different turf fungicides showed a significant decrease in longevity from soil treated with aniyaline, benomyl, chlorothalonil, Duoson, ethazole, fenaminosulf, Kromad, mancozeb, PCNB, thiabendazole, thiophanate methyl, and thiram. Cadmium succinate, dinocap, and RP 26019 did not cause a decrease in longevity. There was no reproduction by worms in soil treated with Duosan, PCNB, thiophanate methyl, and thiram, and only trace amounts in soil treated with chlorothalonil, ethazole, and Kromad.

The toxicity of benomyl, thiabendazole, and thiophanate methyl to earthworms was confirmed in the present study, and additional fungicides used for turf disease control were also found to cause significant amounts of mortality.

INTRODUCTION

Certain pesticides are known to be toxic to earthworms, and the toxicity of some insecticides and herbicides is well-documented (Edwards and Lofty, 1972). Information on fungicides is more limited, however, and the relative toxicities of many fungicides to earthworms are not fully known.

Copper fungicides were among the first materials reported as being toxic to earthworms (Raw and Lofty, 1960, 1962). Dinocap and thiram were reported as causing 0 to 48% and 0 to 35% reductions in earthworm populations, respectively, depending upon time of year and soil moisture content in field tests (van der Drift, 1963). Captan has been reported as being non-toxic (Edwards and Lofty, 1972, 1973; van der Drift, 1963; Kennel, 1972), and earthworm populations in Kentucky bluegrass (*Poa pratensis* L.) turf treated with phenyl mercury acetate were not significantly different from those in untreated plots (Randell, et al., 1972).

In tests which included systemic fungicides, Kennel (1972) reported that very little grass mulch was eaten by earthworms in plots treated with benomyl, thiophanate methyl, or thiabendazole. Under unspecified conditions he reported that dodine and steeped sulfur apparently had no ill effect on earthworm activity. Edwards and Lofty (1973) reported no significant effect upon earthworms in field tests in which formaldehyde, captan, quintozene, thiram, or dicloran were cultivated into soil; benomyl, however, was very toxic. Stringer and Wright (1973) and Wright and Stringer (1973) conducted extensive tests upon the toxic effects of benomyl, methyl benzimidazole-2-yl carbamate (MBC), thiophanate methyl, and thiabendazole upon earthworms under various conditions. They recently reported that benzimidazole and its 2-amino analogue were nontoxic to *Lumbricus terrestris* when administered orally (Stringer and Wright, 1976). The fungicidal 2-substituted benzimidazoles (benomyl, carbendazim, fuberidazole, and thiabendazole) and 1-(benzimidazol-2-yl)-3 butylurea, however, were highly and equally toxic.

Stringer and Lyons (1974) reported a drastic reduction in ten resident earthworm species in orchard plots sprayed with benomyl and thiophanate methyl. Only *L. terrestris* and *Allolobophora chlorotica* failed to recover to normal levels after a two-year non-treatment period following a single year's treatment with benomyl. Black and Neely (1975) similarly observed that soon after injection of soils with benomyl there was a significant reduction in earthworm populations. Within months the populations increased substantially and in plots treated with benomyl one or more years previously the earthworm populations did not differ significantly from untreated plots.

A reduction in earthworm casts, an indirect measurement of decline in earthworm activity, has been observed locally in bentgrass plots treated with various turf fungicides (King and Dale, 1977). Some of these fungicides had not previously been tested or reported as being harmful to earthworms. Since a wide range of fungicides are used on turf, and since the activity of many of these materials against earthworms is unknown, research was conducted to determine whether they might show differential toxicity to earthworms when simultaneously tested under controlled conditions. The fungicides which were tested are used for turf disease control, but some of them are increasingly being utilized or being considered for use on field crops where their effects on earthworm populations could possibly be of ecological importance.

MATERIALS AND METHODS

The earthworms used in the study were commercially grown *Eisenia foetida* Savigny (kindly identified by Dr. Walter J. Harman, Department of Zoology and Physiology, Louisiana State University). In the tests the earthworms were housed in 946 ml plastic containers closed with lids containing a 33 mm hole covered with 50-mesh plastic screening for ventilation. The containers were half-filled with a steam-sterilized soil mixture consisting of 4 parts soil and 1 part sand, except where noted. Water was added periodically to maintain a moisture content of approximately 8.5%. The worms were normally fed a finely ground mixture of equal parts of corn and oats. Each 454 g of feed contained 11 g of bone meal. The surface feeding worms were usually fed every seven days by sprinkling a small amount of feed on the soil in each container. This feeding schedule adequately maintained untreated worms during the experiments. All worms were held in a temperature controlled room maintained at 24°C. All treatments consisted of four replications of ten worms each. Mortality of

[1]Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

the earthworms was determined from counts made at approximately seven day intervals. The data were statistically analyzed as randomized complete blocks.

The turf fungicides used in the tests, with designated common names and trade names, were: 50% 2,4-dichloro-6-(o-chloroanilino)-s-triazine (aniyaline) (Dyrene); 50% methyl 1-(butulcarbamoyl)-2-benzimidazolecarbamate (benomyl) (Tersan 1991); cadmium succinate containing 29% total cadmium (Cadminate 60W); 75% tetrachloroisophthalonitrile (chlorothalonil) (Daconil 2787); 18.25% 2,4-dinitro-6-octyl phenyl crotonate and 2,6-dinitro-4-octyl phenyl crotonate, 1.25% nitrooctyl phenols (dinocap) (Karathane); 75% Duosan, Mallinckrodt experimental turf fungicide MF-598; 35% 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole (ethazole) (Koban); 70% sodium /4-(dimethylamino) phenyl/ diazene-sulfonate (fenaminosulf) (Dexon); 5% cadmium sebacate, 5% potassium chromate, 1% malachite green, 16% thiram (Kromad); zinc ion and manganese, ethylenebisdithiocarbamate 80%, coordination product of 16% manganese, 2% zinc, 62% ethylene-bisdithiocarbamate (mancozeb) (Fore); 75% pentachloronitrobenzene (PCNB) (Terraclor); 50% 1-isopropylcarbamoyl-3 (3,5 dichlorophenyl) hydantoin, Rhodia, inc. experimental turf fungicide RP 26019; 25% 2-(4-thiazolyl) benzimidazole, 1.5% malachite green (thiabendazole) (Tobaz); 50% dimethyl 4,4-o-phenylenebis (3-thioallophanate) (thiophanate methyl) (Fungo); and 75% bis (dimethylthiocarbamoyl) disulfide (thiram) (Thiramad). All weights and concentrations in tests are expressed in terms of the commercial preparations listed above, and not on the basis of active ingredients.

Earthworm immersion in fungicide solutions.—For each treatment ten adult worms of similar size were placed into a cylindrical metal container (7.5 cm x 6.5 cm diameter) having a bottom covered with 16-mesh galvanized screening. The container with worms was dipped and gently agitated for 1 min in 500 ml of 0.1, 1.0, or 2.0% concentrations (w/v) of the aqueous fungicide solutions or suspensions held in a 600 ml glass beaker. After treatment, excess fungicide solution was removed from the screened bottom of the container by blotting on clean paper toweling and the worms were then placed into soil in individual containers.

Earthworms fed fungicide-treated grass clippings.—Since turf thatch is a component of the diet of some earthworms in nature, this test was conducted to determine whether the ingestion of fungicide-treated grass clippings was deleterious to earthworms. Clippings from a pesticide-free plot of Bermudagrass (*Cynodon dactylon* (L.) cv. 'Common') were air dried and finely ground in a Wiley mill with a sieve of 1 mm porosity (approximately 25 mesh). For fungicide treatments, 15 g samples of this bermudagrass feed were stirred into 100 ml of 0.1% concentration solutions of fungicides. Control bermudagrass was treated with water. After 16 hr saturation, excess fungicide solution or water was removed from the bermudagrass by vacuum filtration on Whatman No. 2 filter paper using a 9 cm diameter Buchner funnel. The worm's diet consisted of only the bermudagrass feed.

Earthworms in soil containing fungicides.—The fungicides tested were mixed with 4719[3] cm of soil to simulate the dosage applied to 929[2] cm with an assumed uniform distribution to a 5.08 cm depth. The amount of fungicide mixed in the soil was the cumulative amount which would be applied to 929[2] cm of turf from five applications at the recommended dosages, except for PCNB where the amount was comparable to the amount applied from three applications. The fungicide dosages normally recommended in g/92.9[2]m of turf, and the amounts mixed in the soil were: aniyaline, 170.10 g, 0.849 g; benomyl, 28.35 g, 0.142 g; cadmium succinate, 14.18 g, 0.071 g; chlorothalonil, 170.10 g, 0.849 g; dinocap, 7.09 g, 0.035 g; Duosan, 113.40 g, 0.566 g; ethazole, 113.40g, 0.566 g; fenaminosulf, 113.40 g, 0.566 g; Kromad, 113.40 g, 0.566 g; mancozeb, 170.10 g, 0.849 g; PCNB 226.80 g, 0.679 g; RP 26019, 28.35 g, 0.142 g; thiabendazole, 56.70 g, 0.283 g; thiophanate methyl, 28.35 g, 0.142 g; and thiram, 85.05 g, 0.426 g. The fungicide used in each treatment was mixed with 10 g of talc to aid in thorough dispersal in the soil mixture. Worms were added to the surface of the fungicide-treated soil; control treatment consisted of worms added to soil treated with 10 g of talc.

RESULTS

Earthworm immersion in fungicide solutions.—With earthworms dipped in fungicide solutions for 1 min, at the 0.1% concentration, only benomyl and thiophanate methyl caused a significant reduction in longevity (Table 1). Treatment with 1% fungicide solutions resulted in significant earthworm mortality from benomyl, ethazole, Kromad, and thiophanate methyl treatments. Ethazole was most toxic with all worms being killed within seven days. Treatment at the 2% level resulted in significant mortality from treatments with benomyl, cadmium succinate, ethazole, thiophanate methyl, and thiram. Ethazole again killed all worms within seven days, whereas 75 days elapsed before benomyl and thiophanate methyl caused total mortality. Cadmium succinate and thiram caused lesser but significant amounts of mortality after 101 days.

Table 1. Longevitv of earthworms as affected by 1 minute of immersion in different concentrations of aqueous solutions of turf fungicides.

Fungicide treatment[a]	Elapsed time, in days				
	7	28	50	75	101
0.1% conc.					
Benomyl	10.0[b]	8.5	7.8	6.5	6.2
Thiophanate methyl	10.0	9.0	5.5	4.5	4.0
LSD (.05)	0.2	1.2	2.5	2.7	2.7
1.0% conc.					
Benomyl	10.0	7.8	4.5	3.0	2.2
Ethazole	0.0	0.0	0.0	0.0	0.0
Kromad	9.8	6.0	6.0	6.0	5.5
Thiophanate methyl	9.8	6.2	2.2	1.5	1.0
LSD (.05)	0.4	1.8	1.6	2.1	1.9
2.0% conc.					
Benomyl	10.0	4.0	2.2	0.0	0.0
Cadmium succinate	9.0	8.0	7.8	7.0	6.0
Ethazole	0.0	0.0	0.0	0.0	0.0
Thiophanate methyl	10.0	3.0	1.0	0.0	0.0
Thiram	9.5	7.2	6.5	6.5	6.2
LSD (.05)	0.5	2.2	3.4	2.6	2.5
Control, no treatment	10.0	9.8	9.2	9.2	9.2

[a]Fungicides tested at the three concentrations included aniyaline, benomyl, cadmium succinate, chlorothalonil, ethazole, Kromad, mancozeb, PCNB, thiophanate methyl, and thiram; only those materials which caused a significant increase in mortality are shown in the table.

[b]Figures represent average earthworm survival in trial of four replications, ten worms per treatment.

Earthworms fed fungicide treated grass clippings.—Earthworm populations which were fed bermudagrass clippings treated with several fungicides showed a significant increase in mortality from benomyl, ethazole, and thiophanate methyl treatments (Table 2). Worms fed grass clippings treated with thiophanate methyl were most severely affected and died in significant numbers within nine days. Worms fed the benomyl and ethazole treated clippings died in significant amounts within 34 and 84 days, respectively. After 101 days the mortality from benomyl, ethazole, and thiophanate methyl treatments was similar. Worms fed grass clippings treated with cadmium succinate showed a significantly greater longevity than control earthworms fed untreated grass clippings. In a second test, which lasted only 59 days and the results of which are not shown, a significant amount of mortality also occurred when worms were fed dinocap-treated grass clippings.

Visual observations indicated that worms fed benomyl, dinocap, and thiophanate methyl treated grass clippings ate moderate to small amounts of the clippings at the beginning of the tests, and after 2-3 weeks consumed only trace amounts. Worms fed the clippings treated with the other fungicides ate amounts comparable to that eaten by control worms fed untreated grass clippings.

Earthworms in soil containing fungicides.—There was a significant increase in mortality from all fungicide treatments except cadmium succinate, dinocap, and RP 26019 (Table 3) when earthworms were reared in soil. Duosan and PCNB soil treatments caused total earth-

Table 2. Longevity of earthworms fed diets consistine of finely ground bermudagrass clippings treated with 0.1% aqueous solutions of different turf fungicides.

Fungicide	Elapsed time, in days 9	34	59	72	84	101
Aniyaline	10.0[a]	9.2	7.8	7.2	6.8	6.0
Benomyl	9.8	6.8	3.2	3.2	3.0	1.5
Cadmium succinate	9.5	9.0	9.0	9.0	9.0	8.8
Chlorothalonil	9.8	9.5	9.2	7.0	7.0	6.2
Ethazole	9.8	8.8	6.5	4.2	1.8	1.5
Kromad	9.5	8.2	7.0	6.8	6.2	6.0
Mancozeb	10.0	8.5	7.8	7.0	6.5	6.0
PCNB	10.0	9.8	9.5	8.2	7.2	6.8
Thiophanate methyl	8.5	5.8	4.5	4.0	2.0	1.0
Thiram	10.0	9.0	8.5	8.0	8.0	5.8
Control, no treatment	10.0	9.0	7.8	7.0	6.8	6.2
LSD (.05)	0.7	1.7	2.8	3.0	2.6	2.4

[a]Figures represent average earthworm survival in trial consisting of four replications, ten worms per treatment.

Table 3. Longevity of earthworms when reared in soil containing different turf fungicides.

Soil fungicide treatment[a]	Elapsed time, in days 10	29	52	64	84
Aniyaline	9.2[b]	7.5	6.1	5.0	4.9
Benomyl	9.5	1.8	1.1	1.0	0.9
Cadmium succinate	9.9	8.4	8.0	7.6	7.5
Chlorothalonil	9.9	8.4	5.8	5.4	4.9
Dinocap	10.0	10.0	9.8	9.8	9.0
Duosan	9.8	0.0	0.0	0.0	0.0
Ethazole	5.1	4.9	4.8	4.6	4.6
Fenaminosulf	9.8	8.4	4.9	4.8	0.9
Kromad	7.6	5.6	4.5	4.4	4.4
Mancozeb	9.5	7.9	5.9	4.9	4.6
PCNB	9.8	0.0	0.0	0.0	0.0
RP 26019	9.8	9.1	7.5	7.5	7.0
Thiabendazole	9.6	7.2	5.4	4.9	4.6
Thiophanate methyl	7.4	6.8	5.9	5.9	5.6
Thiram	7.9	1.9	0.1	0.0	0.0
Control, no treatment	9.8	9.2	8.8	8.6	8.2
LSD (.05)	1.0	1.4	1.4	1.2	2.1

[a]Amounts of fungicide mixed in the soil are given in the text.

[b]Figures represent average earthworm survival in two trials consisting of four replications per trial, ten worms per treatment.

worm mortality within 29 days, and thiram caused death of all worms within 64 days. The other fungicides which caused significant decreases in earthworm numbers did so after varying periods of time. Although not shown in the condensed data in Table 3, all fungicides which caused significant amounts of mortality did so within 37 days after initiation of the tests.

Earthworms in soil treated with Duosan, PCNB, and Thiram showed little feeding activity in the tests. Worms in soil treated with benomyl and thiophanate methyl fed only slightly throughout the tests. Worms in soil containing fenaminosulf fed normally and then stopped feeding near the end of the tests. Worms in soil treated with the other fungicides consumed feed amounts similar to that eaten by control worms in untreated soil.

There was no reproduction by worms in soil treated with Duosan, PCNB, thiophanate methyl, and thiram, and only trace amounts by worms in soil containing benomyl, fenaminosulf, and thiabendazole. There was an intermediate amount of reproduction by earthworms subjected to chlorothalonil, ethazole, and Kromad treatments. Worms in soil treated with the other fungicides reproduced in numbers comparable to the worms in untreated soil.

DISCUSSION

Of various fungicides which have previously been tested for possible deleterious effects upon earthworms, benomyl, thiabendazole, and thiophanate methyl rather consistently have been demonstrated as being toxic. Other fungicides have been reported as being non-toxic or of variable toxicity, depending upon test conditions. In the present study using *E. foetida*, the earthworm toxicity of benomyl, thiabendazole, and thiophanate methyl was confirmed, and additional fungicides used for turf disease control were also found to cause significant amounts of mortality. With the two testing procedures most closely resembling conditions which could occur in nature, rearing of earthworms in fungicide-treated soil generally affected worms more adversely than when they were fed fungicide-treated feed.

Since the tests were conducted with only one earthworm species, it can only be speculated whether or not the results might be representative of the activity of the tested fungicides against other earthworms found in nature. For example, a comparison of the toxicity of benomyl mixed in soil in the present study and the toxicity observed by Stringer and Wright (1973) when benomyl was used as soil drench treatments indicates benomyl was more toxic to *L. terrestris* in their tests than it was to *E. foetida* in the present work. The amount of benomyl mixed in soil in the present study was comparable to the 7.75 kg ai/ha rate of benomyl they used as one of their soil drench treatments. At this concentration 100% mortality of *L. terrestris* occurred within 14 days, which was the length of their experiment. Although not shown in the condensed data in Table 3, only a 65% mortality of *E. foetida* was observed after 19 days in the present study; after 52 days and also at termination of the test after 84 days, approximately 10% of the worms still survived. The results from the soil treatments, however, indicate that accumulations of various turf fungicides in soil possibly could affect other earthworms by direct toxicity, by affecting their feeding habits, or by causing a decrease in their reproduction.

The toxicity of turf fungicides to earthworms creates an anomolous situation with regard to turf culture. On golf course greens and fine playing surfaces, earthworms are undesirable due to roughness caused by casts formed on the surface by some species. Since earthworms are usually eliminated from greens by applications of insecticides, the toxicity of fungicides to earthworms in greens is usually not noticeable. The general elimination of earthworms from grass-covered soil, however, results in the accumulation of a layer of undecomposed plant material known as thatch which is undesirable in turfgrass culture (Beard, 1973). Although thatch on putting greens is normally removed by mechanical means, this procedure is not practical on large areas of turf. It has been reported that applications of certain insecticides and herbicides to Kentucky bluegrass turf reduced or eliminated earthworms and resulted in thatch accumulation with accompanying deterioration of turfgrass quality (Randell, et al., 1972; Turgeon, et al., 1975). The presence of earthworms could be considered a desirable attribute in maintaining turf quality with turf areas that are not closely mowed and where earthworm casts and activity are not objectionable.

The results of this and other studies suggest that some fungicides used on turf cause a reduction in earthworm numbers, especially on putting greens where fungicides are applied repeatedly during a growing season. Fortunately, on an average golf course only about 4% of the grass area is composed of putting greens which receive regular and numerous applications of fungicides. On golf course fairways, home lawns, or areas that are not closely mowed, it is problematical whether occasional applications of turf fungicides have a long term effect on earthworm populations. Even though occasional applications of fungicides may not have a pronounced effect on earthworm numbers in turf or a crop area, if such areas are also subjected to treatment with insecticides and herbicides, the fungicides would constitute an additional factor of stress in the earthworm's environment. The ecological effects of different fungicides upon earthworms in various environments merit additional consideration.

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