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# Toxicity of Pesticides to Earthworms in Kentucky Bluegrass Turf

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Earthworms (Oligochaeta: Lumbricidae) are often abundant in turfgrass soils, where their burrowing and feeding activity enhances soil fertility, increases aeration and rates of water infiltration (Randell et al. 1972, Turgeon et al. 1975, Lee 1985), and aids in the physical and microbial degradation of plant litter (Potter et al. 1990a). Destruction of earthworms can result in chronic cultural problems, including soil compaction and excessive accumulation of thatch (Randell et al. 1972, Potter et al. 1990a,b). Evaluation of 17 pesticides commonly used on turfgrass showed that some formulations are highly toxic to earthworms (Potter et al. 1990b). For example, one application of the fungicide benomyl, or of the insecticides ethoprop, carbaryl, or bendiocarb at labeled rates reduced numbers and biomass of earthworms in Kentucky bluegrass by 60 to 99%, with significant effects lasting for at least 20 wk. Other pesticides, including diazinon, isofenphos, trichlorfon, chlorpyrifos, and isazofos, caused less severe, but significant mortality in some trials (Potter et al. 1990b).

This study continues our work (review, Potter 1993) on the effects of pesticides on non-target and beneficial invertebrates in turf. We evaluated the compatibility of 20 additional pesticides and plant growth regulators with earthworm populations in Kentucky bluegrass (*Poa pratensis* L.). Most of these products have been recently registered for use on turf (since 1988); several others are presently being developed for the turfgrass market.

## MATERIALS AND METHODS

Two evaluations were conducted in spring and fall of 1992 on turf research plots at the University of Kentucky's Spindletop Research Farm, near Lexington. Soil type was a Maury silt loam (fine-silty, mixed, mesic Typic Paleudalf) with pH of 6.4 and < 5 mm of thatch. The earthworm complex at this site consists mainly of *Aporrectodea turgida* (Eisen) [= *Allolobophora caliginosa* F. *typica*], with smaller numbers of *Aporrectodea trapezoides* (Duges) [=*Allolobophora caliginosa* (Savigny) F. *trapezoides* (Duges)], *Lumbricus terrestris* L., and *Eisenia* (Malm) sp. (Potter et al. 1990a,b).

The spring test was conducted on a 9-yr old turf that had been seeded with a blend (1:1) of 'Kenblue' and 'Adelphi' Kentucky bluegrass. The turf was fertilized each fall with 37 kg nitrogen/ha from urea. No pesticides were used for 4 yr before our study. The turf was mown weekly to 5 cm height during the growing season, with clippings left on the surface. The fall test site was a 20-yr old turf of 'Kenblue' Kentucky bluegrass located < 100 m from the spring test site. Management parameters were as described above. Plots within each test were arranged in a randomized complete block with six replications of each formulation plus untreated control. Plot size was 2.1 x 3.1 m, with 0.6 m untreated borders.

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We evaluated 23 pesticides and plant growth regulators in each test (Table 1). Prodiamine and dithiopyr are used mainly for preemergence control of crabgrass; isoxaben and 2,4-D are used respectively for preemergence and postemergence control of broad-leaved weeds. Cyproconazole, tebuconazole, and myclobutanil are broad-spectrum, systemic, ergosterol biosynthesis-inhibiting fungicides. Bendiocarb and thiophanate-methyl are systemic, benzimidazole fungicides used for a variety of foliar diseases. Iprodione is a broad-spectrum dicarboximide, and fosetyl-Al is a systemic, phosphonate fungicide specifically active against oomycete fungi (e.g., pythium blight). Pace WP contains 7% metalaxyl, used for systemic protection from oomycete fungi, plus 70% mancozeb, a broad-spectrum, EDBC fungicide.

Table 1. Formulations and rates of pesticides and plant growth regulators evaluated for toxicity to earthworms.

| Compound | Common name | Formulation | Rate, Kg[1] (AI)/ha | Category[2] |
|---|---|---|---|---|
| Prodiamine | Barricade | 65 DG | 0.73 | H |
| Dithiopyr | Dimension | 1 EC | 0.56 | H |
| Isoxaben | Gallery | 75 DF | 0.84 | H |
| 2,4-D | Dacamine | 4 D | 0.56 | H |
| Cyproconazole | Sentinel | 40 WG | 0.41 | F |
| Tebuconazole | Lynx | 25 DF | 0.38 | F |
| Myclobutanil | Eagle (RH3866) | 40 WP | 0.76 | F |
| Benomyl | Tersan 1991 | 50 DF | 12.21 | F |
| Thiophanate-methyl | Cleary's 3336 | 50 WP | 12.21 | F |
| Iprodione | Chipco 26019 | 50 WP | 6.1 | F |
| Fosetyl-Al | Alliete | 80 WP | 19.6 | F |
| Metalaxyl/Mancozeb | Pace | 7/70 WP | 15.0 | F |
| Mefluidide | Embark | 2 S | 0.56 | PGR |
| Flurprimidol | Cutless | 50 WP | 3.36 | PGR |
| Cyfluthrin | Tempo 2 | 2 E | 0.16 | I |
| Fluvalinate | Mavrik | 2 F | 0.18 | I |
| Bifenthrin | Talstar | 10 WP | 0.11 | I |
| Ethoprop | Mocap | 10 G | 5.60 | I |
| Fonofos | Crusade | 5 G | 4.48 | I |
| *Steinernema carpocapsae* | Exhibit | - | $2.5 \times 10^9$ | EN |
| *Steinernema glaseri* | none | - | $2.5 \times 10^9$ | EN |
| Azadirachtin | Margosan-O | 0.3% | 0.0264 | IGR |
| 1,2-dibenzoyl-1-tert-butylhydrazine | RH 5849 | 2F | 2.24 | IGR |

[1]Rates for nematodes are no. of infective juveniles/ha [2]H= herbicide, F= fungicide, PGR= plant growth regulator, I= insecticide, EN= entomopathogenic nematode, IGR= insect growth regulator.

Mefluidide and flurprimidol are plant growth regulators used to suppress formation of seed heads and foliar growth. Cyfluthrin, fluvalinate, and bifenthrin are synthetic pyrethroids, and ethoprop and fonofos are organophosphate insecticides. *Steinernema carpocapsae* and *S. glaseri* are entomopathogenic nematodes that are being developed for use against soil insects in turf and other crops. Azadirachtin (neem seed extract) acts as an insect growth regulator

(IGR) and a feeding deterrent. RH 5849 is a nonsteroidal ecdysone agonist that is representative of a novel class of IGR's that has shown promise against insect pests of turf (Monthéan and Potter 1992). Benomyl, ethoprop, and 2,4-D were evaluated previously (Potter et al. 1990b) and were included here as standards. All products were applied at labeled rates (Table 1). Our objective was not to compare the relation between active ingredient and toxicity. Rather, we sought to evaluate the effects of the formulated products on earthworms under field conditions, at rates representative of those used by the turfgrass industry.

Plots used in the spring test were treated on 15 April. Air and soil (5 cm depth) temperatures were 11° C and 13° C, respectively. Granular formulations were preweighed, mixed with dry sand as a carrier, and spread evenly by gloved hand. Liquid formulations were applied at a volume of 4 68 L/ha using a low volume $CO_2$ sprayer equipped with four Spraying System 8004 Tee Jet nozzles that delivered 2109 g pressure per $cm^2$. Entomopathogenic nematodes were obtained from Biosys Inc., Palo Alto, CA. Viability of nematodes was confirmed in the field with a binocular microscope and averaged 95% for *S. carpocapsae* and 85% for *S. glaseri*. Nematodes were suspended in water and applied with a sprinkling can (3.79 L solution/plot) at a rate of 2.5 x $10^9$ infective juveniles/ha. There was steady rain during the applications, with accumulation of 1 cm on 15 April and 1.2 cm additional rain during the next 48 h. There was no supplemental irrigation.

Earthworm populations were sampled at 1 and 3 wk after treatment (22 April and 6 May) using a formalin drench method (Potter et al. 1990b). On each sample date, we placed two galvanized steel hoops (0.1 $m^2$, 15 cm high) over scalped areas in each plot and drove them 2 cm into the soil. Sampled areas were at least 0.7 m from the plot edge. Two liters of a dilute (0.25%) formalin drench were poured into each hoop and all earthworms that surfaced within 15 min were collected into plastic containers containing moist paper towels. Number of earthworms and total fresh weight were determined for each sample.

The fall test was applied on 30 September and earthworms were sampled on 7 and 21 October. Air and soil (5 cm depth) temperatures at the time of treatment were 15.5° C and 18° C, respectively. Rates, application methods, and sampling procedure were as described for the spring test, except that the entire site was irrigated with 2 cm of water immediately after treatment. There was no rain for 5 d after the treatment date.

Because of the difficulty of separating species of lumbricids, especially immature specimens, species were combined for analysis. Large species (e.g., *L. terrestris*) were numerically less abundant but contributed disproportionately to sample weights, so both number of worms and total live weight per sample are reported. Data were transformed by log (x + 1) because the sample variances were positively correlated with the means. Within each test, data were subjected to analysis of variance followed by Dunnett's multiple comparison procedure for treatments versus control at $\alpha$ = 0.05 (one-sided test; Steel & Torrie 1980). Summary data are presented as untransformed means $\pm$ SE.

## RESULTS AND DISCUSSION

Only four of the 23 pesticides and growth regulators evaluated in the spring test had significant negative effects on earthworms (Table 2). Two of these, benomyl and ethoprop, had been included as standards because they were known to be toxic to earthworms in turf (Potter et al. 1990b). These compounds, together with thiophanate-methyl and fonofos, had the greatest adverse effect, reducing abundance of earthworms by 88 to 95% and biomass by 83 to 96% after 3 wk. None of the other pesticides or growth regulators caused significant mortality of earthworms.

Table 2. Effect of pesticides and plant growth regulators on earthworm populations in Kentucky bluegrass turf (plots were treated 15 April 1992).

| Treatment | 1 wk after treatment | | 3 wks after treatment | | |
|---|---|---|---|---|---|
| | no./ sample[1] | Biomass (g/sample) | no./ sample | % reduct-ion | Biomass (g/sample) |
| Isoxaben | 73.8 ± 4.6 | 19.6 ± 1.4 | 53.8 ± 8.9 | 0 | 16.5 ± 3.3 |
| Prodiamine | 67.8 ± 5.8 | 21.4 ± 2.0 | 44.0 ± 4.7 | 0 | 16.5 ± 4.9 |
| *S. carpocapsae* | 89.2 ± 6.2 | 27.0 ± 3.1 | 43.8 ± 8.0 | 0 | 14.7 ± 2.7 |
| Dithiopyr | 70.7 ± 6.8 | 21.9 ± 2.3 | 42.3 ± 4.5 | 0 | 15.1 ± 1.5 |
| 2,4-D | 78.7 ± 8.7 | 22.9 ± 3.8 | 40.7 ± 10.0 | 0 | 12.2 ± 2.7 |
| Myclobutanil | 87.2 ± 9.4 | 23.8 ± 1.6 | 40.0 ± 3.7 | 0 | 13.2 ± 2.8 |
| Bifenthrin | 83.5 ± 9.7 | 21.9 ± 1.2 | 39.8 ± 4.8 | 0 | 14.7 ± 2.7 |
| Flurprimidol | 69.8 ± 6.4 | 20.6 ± 2.3 | 39.0 ± 4.6 | 0 | 14.6 ± 3.3 |
| Mefluidide | 72.0 ± 8.2 | 25.1 ± 3.2 | 37.5 ± 3.3 | 0 | 14.8 ± 1.3 |
| Azadirachtin | 71.0 ± 10.2 | 21.9 ± 2.3 | 37.0 ± 6.3 | 0 | 15.3 ± 3.6 |
| Fluvalinate | 75.2 ± 8.4 | 21.4 ± 2.6 | 36.5 ± 2.1 | 0 | 13.3 ± 1.2 |
| Metalaxyl/ mancozeb | 65.8 ± 3.5 | 19.1 ± 2.1 | 36.0 ± 2.8 | 0 | 13.2 ± 1.3 |
| Fosetyl-al | 81.3 ± 5.6 | 24.6 ± 1.8 | 35.8 ± 6.2 | 0 | 15.6 ± 3.0 |
| Cyfluthrin | 74.5 ± 9.3 | 19.6 ± 1.3 | 34.5 ± 4.2 | 0 | 12.8 ± 1.3 |
| Cyproconazole | 65.8 ± 7.9 | 20.8 ± 3.4 | 34.0 ± 7.2 | 0 | 10.3 ± 2.9 |
| Iprodione | 64.3 ± 4.3 | 19.0 ± 2.0 | 33.5 ± 4.2 | 0 | 16.1 ± 4.0 |
| Untreated | 72.2 ± 9.3 | 22.9 ± 5.1 | 32.8 ± 4.1 | - | 13.6 ± 2.7 |
| *S. glaseri* | 83.2 ± 9.9 | 22.0 ± 2.7 | 32.7 ± 8.4 | 0 | 13.2 ± 2.1 |
| Tebuconazole | 71.7 ± 9.1 | 20.9 ± 1.8 | 27.5 ± 4.9 | 16 | 10.1 ± 2.6 |
| RH 5849 | 79.7 ± 8.8 | 23.1 ± 2.3 | 26.2 ± 4.8 | 20 | 11.9 ± 2.8 |
| Thiophanate-methyl | 21.3 ± 2.5* | 6.7 ± 1.0* | 4.0 ± 1.0* | 88 | 2.3 ± 1.2* |
| Ethoprop | 1.7 ± 0.4* | 0.5 ± 0.1* | 3.8 ± 1.5* | 88 | 1.3 ± 0.8* |
| Benomyl | 17.8 ± 2.5* | 3.9 ± 0.8* | 1.8 ± 0.6* | 95 | 0.3 ± 0.1* |
| Fonofos | 9.3 ± 1.6* | 2.9 ± 0.7* | 1.5 ± 0.6* | 95 | 0.5 ± 0.2* |

[1]Sample = two $0.1m^2$ soil drenches per plot. Data are means ± SE. Within columns, means marked by an asterisk are significantly lower than mean for untreated control (ANOVA; Dunnett's test; $\alpha = 0.05$).

Results of the fall test were similar (Table 3). Thiophanate methyl, benomyl, ethoprop, and fonofos again had severe impact on earthworm populations. Plots treated with the nematode *S. carpocapsae* also had reduced biomass of earthworms after 3 wk, although this was not accompanied by a significant decrease in abundance. As before, none of the other pesticides or plant growth regulators caused a significant reduction in numbers or biomass of earthworms.

Recent reviews (Dean-Ross 1983, Roberts and Dorough 1984, Lee 1985) have stressed the importance of evaluating toxicity of pesticides under conditions characteristic of those under which actual exposure will occur. This study and our earlier tests (Potter et al. 1990b) show that pesticides used on turfgrass differ markedly in their toxicity to earthworms. Severe effects of some pesticides are noteworthy in that it has been shown that, even under conditions of low thatch and high soil moisture, there is generally little downward movement of pesticides into turfgrass soils (e.g., Sears and Chapman 1979).

Table 3. Effect of pesticides and plant growth regulators on earthworm populations in Kentucky bluegrass turf (plots were treated 30 September 1992).

| | 1 wk after treatment | | 3 wks after treatment | | |
|---|---|---|---|---|---|
| Treatment | no./ sample[1] | Biomass (g/sample) | no./ sample | % reduct- ion | Biomass (g/sample) |
| Prodiamine | 67.8 ± 6.3 | 30.8 ± 3.8 | 75.2 ± 8.9 | 0 | 24.6 ± 2.9 |
| Untreated | 70.8 ± 11.1 | 19.0 ± 1.8 | 70.2 ± 13.8 | - | 24.8 ± 3.6 |
| Iprodione | 88.5 ± 9.6 | 25.3 ± 1.3 | 64.5 ± 11.0 | 8 | 23.1 ± 8.1 |
| Fluvalinate | 63.3 ± 10.1 | 21.9 ± 2.1 | 62.7 ± 7.6 | 11 | 24.1 ± 5.1 |
| Tebuconazole | 67.7 ± 9.4 | 24.3 ± 1.7 | 61.8 ± 4.1 | 12 | 20.5 ± 0.9 |
| Isoxaben | 66.3 ± 6.3 | 22.0 ± 1.5 | 61.5 ± 7.1 | 12 | 24.0 ± 2.0 |
| Azadirachtin | 81.3 ± 11.2 | 34.8 ± 7.2 | 61.5 ± 8.9 | 12 | 23.3 ± 4.2 |
| Myclobutanil | 74.7 ± 11.0 | 21.1 ± 2.1 | 60.3 ± 5.8 | 14 | 16.3 ± 3.9 |
| 2,4-D | 68.7 ± 4.8 | 24.1 ± 3.2 | 60.0 ± 9.1 | 15 | 21.6 ± 4.0 |
| Mefluidide | 69.8 ± 4.4 | 21.3 ± 5.2 | 58.2 ± 6.8 | 17 | 21.2 ± 1.1 |
| Dithiopyr | 70.2 ± 8.4 | 25.0 ± 3.5 | 58.0 ± 4.7 | 17 | 17.8 ± 2.0 |
| *S. glaseri* | 71.3 ± 9.0 | 23.4 ± 3.8 | 55.7 ± 10.4 | 21 | 18.3 ± 4.0 |
| Cyproconazole | 73.8 ± 9.4 | 26.9 ± 1.7 | 55.2 ± 4.4 | 21 | 22.5 ± 5.1 |
| Fosetyl-al | 68.7 ± 3.7 | 26.0 ± 4.6 | 55.2 ± 9.6 | 21 | 18.7 ± 4.1 |
| Cyfluthrin | 87.0 ± 11.4 | 30.4 ± 1.0 | 54.8 ± 6.3 | 22 | 24.6 ± 4.6 |
| Bifenthrin | 59.5 ± 15.5 | 23.7 ± 4.3 | 53.2 ± 10.3 | 24 | 17.0 ± 2.1 |
| Flurprimidol | 68.5 ± 9.1 | 23.4 ± 2.7 | 51.8 ± 3.9 | 26 | 17.8 ± 2.7 |
| *S. carpocapsae* | 72.2 ± 11.9 | 24.6 ± 4.5 | 49.2 ± 4.5 | 30 | 13.2 ± 1.6* |
| RH 5849 | 59.8 ± 6.5 | 21.6 ± 4.0 | 48.2 ± 7.8 | 31 | 17.5 ± 3.1 |
| Metalaxyl/ mancozeb | 62.8 ± 8.0 | 18.2 ± 3.9 | 46.2 ± 8.0 | 34 | 13.7 ± 2.3 |
| Thiophanate-methyl | 56.5 ± 9.1 | 14.6 ± 3.2 | 28.0 ± 7.5* | 60 | 7.1 ± 2.2* |
| Benomyl | 33.8 ± 5.3* | 10.3 ± 1.8 | 20.7 ± 10.2* | 71 | 6.7 ± 2.7* |
| Ethoprop | 14.5 ± 4.0* | 5.9 ± 1.0* | 8.0 ± 4.1* | 89 | 6.6 ± 2.4* |
| Fonofos | 11.7 ± 3.8* | 3.1 ± 1.2* | 2.8 ± 0.8* | 96 | 1.2 ± 0.6* |

[1]Sample = two $0.1 m^2$ soil drenches per plot. Data are means ± SE. Within columns, means marked by an asterisk are significantly lower than mean for untreated control (ANOVA; Dunnett's test; $\alpha = 0.05$).

Herbicides, including 2,4-D, dicamba, trichlopyr, pendimethalin, prodiamine, dithiopyr, and isoxaben, appear to pose little or no threat to earthworms in turf. As has been reported from other agricultural systems (e.g., Stringer and Lyons 1974), the benzimidazole fungicides benomyl and thiophanate methyl are both extremely toxic to earthworms in turf. Other fungicides, including chlorothalonil, fenarimol, triadimefon, propiconazole, cyproconazole, tebuconazole, myclobutanil, iprodione, fosetyl-Al, and metalaxyl/mancozeb, are more compatible with preservation of earthworm populations. The plant growth regulators mefluidide and flurprimidol appear not to be harmful to earthworms.

Fonofos, ethoprop, bendiocarb and carbaryl are the most toxic to earthworms of the insecticides presently registered for use on turf. Although the effects were less severe, diazinon, isofenphos, trichlorfon, chlorpyrifos, and isazofos also caused some mortality of earthworms in our earlier tests (Potter et al. 1990b). Synthetic pyrethroids, including bifenthrin, fluvalinate and cyfluthrin, appear to have relatively little toxicity to earthworms.

Field data on effects of entomopathogenic nematodes on nontarget species are sparse. Applications of *S. carpocapsae* or *Heterorhabditis bacteriophora* Poinar did not measurably reduce populations of predatory insects, Collembola, or soil-inhabiting mites in turfgrass, although larvae of holometabolous predators were susceptible to infection (Georgis et al. 1991). In laboratory tests, *S. carpocapsae* fed upon and developed on cadavers or damaged tissue of *Aporrectodea* sp. but did not infect intact earthworms (Capinera et al. 1982). Our study suggests that *S. carpocapsae* and *S. glaseri* are compatible with earthworms in the field. Similarly, use of the ecdysone agonist RH 5849 or azadirachtin on turfgrass does not appear to be harmful to earthworms.

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