# Effects of Nine Insecticides on the Numbers and Biomass of Earthworms in Pasture

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Earthworms (Lumbricidae) help to establish and maintain the structure and fertility of soil. They bury remains of plants, mix organic and inorganic particles in their excreta, and their burrows aerate and drain soils. Leaf litter is broken down by a succession of microflora and invertebrates and, in a review of the biology of earthworms, Satchell (1) concluded that they prepare organic debris for decomposition by bacteria, Protozoa and fungi. He considered this their most important function. Any chemical that disturbs the equilibrium between these organisms influences the structure of the soil. If organisms survive the dosage applied, they may accumulate residues in their tissues and, as carriers, they then contaminate food chains that lead to birds and other vertebrates.

The great majority of work on the effects of insecticides on invertebrates in soil has concerned changes in numbers, and those of earthworms were reviewed in 1963 by Davey (2). Kelsey and Arlidge (3) described the loss of structure and the development of a mat of undecomposed leaves on the surface of soil after it had been treated with telodrin, but there are very few reports of the loss of structure or fertility caused by lessened numbers of earthworms.

Chlorinated hydrocarbon and carbamate insecticides that have been studied with numbers of earthworms include chlordane and heptachlor (4, 5, 6, 7), BHC (8, 9, 10, 11, 12), aldrin and dieldrin (13, 14, 15), telodrin (3), DDT (14, 16, 17, 18, 19, 20), and carbaryl (21, 22, 23).

Less work has been done with organophosphorus insecticides. Hopkins and Kirk (24) included azinphosmethyl and malathion in studies on Eisenia sp., Edwards et al.

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(25) studied the effects of phorate, parathion, diazinon and disulfoton on field populations and Edwards et al. (26) sampled plots treated with chlorfenvinphos. Way and Scopes (27, 28) measured the activity of earthworms after soil had been treated with menazon and phorate, and Griffiths et al. (15) used Thionazin, BAYER 38156 and fenitrothion in a field experiment. Other insecticides that have been studied include Dursban (29) and Dyfonate and Zinophos (30).

Edwards and Thompson (31) reviewed the effects of pesticides on beneficial invertebrates in soil and included work on the changes in numbers and accumulation of residues of insecticides by earthworms in treated soil. In 1958 Barker (32) reported that residues of DDT were greater in tissues of earthworms than in the soil in which they were found under sprayed elm trees. Since then, there have been similar analyses of DDT (4, 33, 34, 35, 36), aldrin and dieldrin (37), heptachlor (38, 39) and  $\gamma$ -BHC (40). Recently, Gish (41) published data to show that earthworms from 67 agricultural fields in eight States of the U.S.A. had concentrated residues of six chlorinated hydrocarbons and their metabolites. Very few earthworms have been analysed for residues of organophosphorus insecticides. Edwards et al. (26) reported that individuals from plots treated with chlorfenvinphos at normal agricultural dosages had not accumulated residues of the insecticide or its metabolites.

Partly because of these and similar accumulated residues in individuals in food chains, and the persistence of some of the chlorinated hydrocarbons in soil, the use of several insecticides has been restricted in Canada and many other countries. As a result, new insecticides, which are often organophosphorus compounds or carbamates, have been registered for use in agriculture. Little is known of the ecological effects of some of these compounds in soil.

An experiment was therefore started at London, Ontario to determine the effects of nine insecticides which have been developed for control of soil insects on earthworms. BAYER 37289 is registered in Canada for use against the cabbage maggot, and Dasanit is recommended in Ontario to control root maggots on brassica crops. The use of DDT in Canada has been restricted but the insecticide is still used to control cutworms in tobacco and some vegetable crops and Dursban has been registered and recommended for control of cutworms in tobacco. Endrin is also registered in Ontario to control subterranean cutworms but is not recommended. Carbaryl is recommended in Ontario for use on climbing cutworms, and BUX is registered and recommended for control of corn rootworm. Carbofuran is registered for use against root

maggots in rutabagas and the ninth insecticide, Stauffer N-2596, is being actively developed as a soil insecticide. The experiment will study effects of the insecticides on earthworms immediately after the soil was treated and also the later recolonisation of treated plots. Chemical analyses are being done on all earthworms extracted from the plots. Data presented here are from the first samples. A complete account of the study, with all experimental details and chemical analyses, will be published elsewhere when the experiment is finished.

#### Methods

The experiment is in a trefoil pasture that had not been treated with herbicides or insecticides for at least five years before the experiment started. It is well drained with a sandy loam soil. A Latin square of 100 plots was marked out on June 3rd, 1970 with ten replicates of the nine insecticides and the untreated control. Each plot is 10° square and there are untreated strips 6° wide between plots and around the experimental area. Plants were cut to 2-3° and cuttings were raked away before the insecticides were applied, but not incorporated into the soil, on June 4th. Table 1 gives the formulations and rates of the insecticides used.

Plots were sampled three weeks after they had been treated. Immediately before any sample was taken, a strip 2° wide was mowed close to the surface of the soil across the plot. Two two-feet square wooden quadrats were placed on the strip and 2 gallons of dilute formalin solution (25 ml of 40% formaldehyde in 1 gallon of water)(42) were poured on to the short grass. Worms brought to the surface of the soil were counted, weighed and then immediately frozen for later chemical analysis.

#### Results and discussion

Because the treatments in the Latin Square are inextricably associated with diagonals, there is no valid statistical estimate of error between plots. Differences between samples from some treated and the untreated control plots are, however, large enough to make conclusions valid.

TABLE 1 gives the arithmetic means and ranges of the numbers of earthworms from each set of 20 quadrats. Populations of earthworms are aggregated (1) and it is inevitable that numbers in samples, even from untreated plots, have a wide range. However, the large number of plots and samples helps to compensate for this variation. TABLE 2 gives the means for each treatment expressed as percentages of the untreated control, with the corresponding percentage reduction.

TABLE 1

Numbers and biomass of earthworms three weeks after plots in pasture were treated.

Treatment (1b a.i. insecticide/acre)	e/acre)	Arithmetic mean of numbers in	Range of numbers in quadrats	numbers irats	Biomass (gms fresh wt) obtained from 20 quadrats
		ans Tremb or	Maximum	Minimum	
Untreated control		17.9	32	6	9*40#
Dursban	2 lb. E.C.	. 14.1	53	7.	343.5
BAY 37289	3 1b. E.C.	. 13.6	%	н	253.2
DDT	5 lb. E.C.	. 11.4	25	М	271.4
Endrin	1 1b. E.C.	8.6	17	н	132.2
Bux	1 lb. gran.	ո. 7.9	<b>‡</b>	ы	241.8
Carbary1	2 lb. W.P.	7.2	15	rł	128.4
Dasanit	3 1b. E.C.	3.8	రు	Ħ	33.0
Carbofuran	4 1b. W.P.	. 3.1	2	0	160.3
N-2596	2 lb. E.C.	2.6	89	0	29.6

TABLE 2

Biomass and arithmetic means of numbers from treated plots as percentages of those from untreated

plots.

Insecticide	Arithmetic mean	ic mean	Bioness	S 85
	as % control % reduction	% reduction	as % control	as % control % reduction
Dursban	78.8	21.2	84.9	15.1
BAY 37289	76.0	24.0	62.6	37.4

Insecticide	Arithmetic mean	Le mean	Bioness	981
	as % control	% reduction	as % control	% reductio
Dursban	78.8	21.2	6*48	15.1
BAY 37289	76.0	0.4%	62.6	37.4
DOT	63.7	36.3	67.1	32.9
Bux	1.44	55.9	59.8	40.2
Endrin	0.84	52.0	32.7	67.3
Carbaryl	7.07	59.8	31.7	68.3
Dasanit	21.2	78.8	8.2	91.8
Garbofuran	17.3	82.7	39.6	4.09
N-2596	14.5	85.5	7.3	92.7

Numbers alone do not present a true record of the effects of the insecticides. The biomass is more important as it can be used to calculate the physiological requirements of the tissues and also the cycle of nutrients. The Lumbricidae can be separated into those individuals that live in surface organic layers of soil, and those that live mainly in the deeper mineral soil (1). Individuals in the surface soil tend to be small and active, and those in the mineral layers, larger forms which rarely emerge from the burrows which help to aerate and drain soil. Very many individuals of Lumbricus terrestris which belongs to the second category were found in this experiment. They feed on remains of plants which they pull into their burrows from the surface of the soil and also ingest mineral particles. Poor aeration and drainage of soil and slower decomposition of leaf litter may result when numbers of larger earthworms are lessened.

Earthworms respire carbon in leaf litter and release energy for metabolic processes in soil. Satchell (1) notes that individuals of L. terrestris may respire 12% of the carbon in litter and, although this may be less than that by other groups such as nematodes which have a smaller biomass but a larger metabolic rate, it is appreciable. Individuals of this and other species help to lower the carbon:nitrogen ratio of remains of plants and when this ratio is less than 20:1. mineral nitrogen usually becomes available (44). Estimates of biomass can be used to calculate the amount of carbon used by earthworms and thus show the amount of oxygen consumed and energy released. As well as making mineral nitrogen in organic debris available, earthworms contain nitrogen combined in protein which comprises up to 72% of the dry weight of the tissues (43). This nitrogen is released when earthworms die and decompose, and, with that excreted by live individuals in mucoproteins and fluid containing ammonia, urea and uric acid, is readily available to roots of plants. Thus, although the numbers of earthworms in soil can themselves be of interest, their value is increased when associated with the biomass. Minerals and energy normally available to plants and other animals may be cycled less when the biomass of earthworms is lessened.

TABLE 2 shows that the biomass in plots treated with five insecticides (endrin, carbaryl, Dasanit, carbofuran and N-2596) was less than 50% that in untreated plots, and two insecticides, Dasanit and N-2596 lessened the biomass by more than 90%.

The percentage reductions of numbers obtained from plots treated with BUX and carbofuran were 15.7% and 22.3% more than the reductions of biomass (TABLE 2). This suggests that these two insecticides did not affect the larger earthworms as much as the small, shallow-living species in the surface soil. Individuals of L. terrestris contact insecticide when they come to the surface to feed and when residues are leached down through the soil, but other species that live in the deeper layers of soil rarely surface. BUX was applied in granules and the active ingredient may not have been released fast enough for these deep earthworms to contact or accumulate an effective dosage in the three weeks before samples were taken. In an experiment to study control of root maggots in tobacco. Kring (45) observed many dead and dying individuals of L. terrestris on the surface of plots six days after they had been treated with BUX and carbofuran at 0.5-4 1b/acre. No dead specimens of this species were seen on the surface of soil three weeks after plots in London were treated. Kring (45) did not mention effects on other species.

Dursban, BAYER 37289 and DDT had no effects on numbers or biomass that could be assessed in this experiment. Earthworms were also apparently unaffected by Dursban applied to soil at 20 to 50 lb/acre (29) and, although results vary with species, DDT often does not affect numbers of earthworms (12, 14, 17, 18, 19, 20, 46).

Endrin and carbaryl had moderate effects and lessened numbers in treated plots by 52.0% and 59.8% respectively. (TABLE 2). An de Lan and Aspock (21) found that even the smallest amounts of powder or suspension of carbaryl caused severe paralysis and irreversible histopathological changes in earthworms, and Heungens (23) showed that carbaryl was the most efficient of 10 insecticides tested to control L. terrestris in spruce litter but TABLES 1 and 2 show that, at London, effects of this insecticide were not as drastic as those of Dasanit, carbofuran and N-2596.

Kelsey and Arlidge (3), Edwards et al. (25) and Way and Scopes (27, 28) showed that, at normal agricultural rates, phorate may almost eliminate earthworms and, until now, no other organophosphorus insecticide has been reported to have such a great effect on earthworms. This data indicates that the initial effects of Dasanit and N-2596 are at least as great as those of phorate. The action of carbofuran at the same time was similar, though it may have been more specific to individuals in the surface layers of soil than in the deeper mineral layers.

The precise effects of these insecticides on the structure and fertility of the soil, and the potential accumulation of their residues in food chains will be more certain when plots have been sampled again and chemical analyses are completed.

#### Chemical designations of experimental materials

BAY 37289 (0-ethyl 0-2,4,5-trichlorophenyl

ethylphosphonothicate)

BUX (m-(1-ethylpropyl) phenyl methyl-

carbamate mixture (1:4) with m-(1-methylbutyl) phenyl methyl

carbamate)

Dasanit (0,0-diethyl 0-[p-(methylsulfinyl)

phenyl] phosphorothicate)

Stauffer N-2596 (S-(p-chlorophenyl) O-ethyl ethyl-

phosphonodithicate)

carbofuran (2,3-dihydro-2, 2-dimethyl-7-benzo-

furanyl methyl carbamate)

## Summary

The effects of mine insecticides on the numbers and biomass of earthworms were studied three weeks after plots in pasture were treated. Dursban, BAYER 37289 and DDT had very little effect, but Dasanit, carbofuran and Stauffer N-2596 drastically lessened numbers and biomass of earthworms sampled with formalin solution. BUX, endrin and carbaryl did not lessen numbers by more than 60% or the biomass by more than 68.3%.

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