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Impact of Herbicide Application Rates and Crop Residue Type on Earthworm Weights

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It has been demonstrated that zero-till management increases earthworm populations in agricultural soils, relative to conventional-tilled practices (Shipitalo and Protz 1988). Under zero-till there is little soil disturbance or habitat destruction to suppress earthworm activity, and crop residues left at the soil surface provide nutrients and frost protection. The consequence for zero-till soils is that a greater earthworm biomass is usually observed for all earthworm species. However, the difference in earthworm biomass is most pronounced for *Lumbricus terrestris* Linnanaeus.

Zero-till regimes require often more herbicide applications than other tillage practices because weeds are no longer controlled by ploughing. While it has been reported that various insecticides, fungicides, and nematocides, even when applied at recommended field rates, are toxic to various earthworm species (Edwards and Bohlen 1992), relatively few studies have documented the effects of herbicides (Freemark and Boutin 1994). It is generally understood that most herbicides have a low acute toxicity to earthworms when applied at recommended field rates, but the use of triazines at greater rates could result in decreasing earthworm numbers (Caseley and Eno 1966).

The measurement of herbicide toxicity to earthworms is confounded by food availability. Fox (1964) found that earthworm numbers decline after atrazine applications, not due to toxicity, but because the vegetation cover is reduced by herbicide use. In contrast, Edwards and Bohlen (1996) reported that earthworm numbers increase after herbicide application as the result of an increasing availability of dead plant material at the soil surface. Little attention has been focused on the interaction between food quality and herbicide toxicity effects on earthworms. Different crop residues can have different nutrient contents and associated microbial populations, influencing earthworm growth rates. In a number of studies, earthworm weight changes were dependent on the type of food supplied (Martin 1986, Shipitalo et al. 1988), and it is possible that the type of crop residues at the soil surface may also influence the effects of herbicides on earthworm populations.

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The herbicides atrazine and metolachlor are often combined for use in corn to control broadleaf weeds. Atrazine-metolachlor mixtures are more effective than either herbicide alone and require less total herbicide use (Solomon et al. 1996). The residual activity of metolachlor is commonly ten to fourteen weeks. Atrazine may persist for more than one planting season. The objectives of this study were to: (1) quantify the effect of atrazine-metolachlor mixtures on earthworm weights, and (2) measure how the quality of crop residues may alter the effects of these herbicides on earthworms.

MATERIALS AND METHODS

A loamy Ap horizon (0-15 cm) of a Gobles soil (Gleyed Brunisolic Gray Brown Luvisol) was collected from a zero-till corn field near Belmont (42°55' N, 81°10' W), ON, Canada. Earthworm populations in this field included juvenile and adult *L. terrestris* with about 1 10^6 of these earthworms/ha or a biomass of 125 10^3 kg/ha (Tomlin, unpublished data). Key physical and chemical soil properties of the soil include the following: 29 % sand, 46 % silt, 24 % clay, 1.63 % organic carbon, pH 6.35, CEC 12.38 cmol kg^{-1}, and an exchangeable K, Ca, Mg and Na of 0.47, 5.62, 1.56 and 0.07 cmol kg^{-1} respectively (Farenhorst and Bowman, 2000). Neither atrazine nor metolachlor residues were detected in a sample of this soil (as determined by High Performance Liquid Chromatography with a detection limit of 25 ng g^{-1} soil).

Corn and soybean leaves were obtained from a pesticide-free field plot at the Southern Crop Protection and Food Research Centre, London, ON, Canada. Wet leaves were held in plastic bags at room temperature for one month to decompose and stimulate food acceptance by earthworms (Edwards and Bohlen 1996). Further decomposition was delayed by storing leaves at 4 °C. One week before using leaves in the experiment, leaves were left to dry at 12 °C and weighed. Leaves were pulverized into small pieces (2.5 - 7.5 mm^2) using a blender to produce a material more palatable for earthworms.

Mature earthworms (*Lumbricus terrestris L.)* were purchased locally and stored at 12 °C. Worm digestive tracts were cleared of previously ingested contents by incubating them in soil containing corn leaf residues for 5 days before they were used in the experiment.

A commercially available atrazine-metolachlor liquid formulation, Primextra® (label content: 153 g L^{-1} atrazine, 364 g L^{-1} metolachlor, and 10 g L^{-1} unidentified other active triazines; Ciba-Geigy Co, Greensboro, NC), was used. Herbicide solutions were prepared with reverse-osmosis-purified water at a recommended field rate (1H), three times the recommended field rate (3H), and six times the recommended field rate (6H) applications (Table 1). Water without herbicides (0H) was used as a control.

Table 1. Overview of herbicide treatments and equivalent field rates.

Herbicide Treatment	Atrazine [mg kg^{-1} dry soil]	Metolachlor [mg kg^{-1} dry soil]
0H	0	0
1H	1.7	5.1
3H	5.1[a]	15.2
6H	10.1[a]	30.4
In top 5 cm of field soils after herbicide application	1.6 to 6.4[b]	1.6 to 6.4[b]

[a] the concentration of the herbicide solution is greater than the solubility of atrazine in water (30 mg L^{-1} at 25 °C). Atrazine in herbicide application solution was thus in suspension.
[b] assuming a minimum and a maximum recommended herbicide application rate of 1 and 4 kg ha^{-1} respectively and a soil bulk density of 1.25 kg m^{-3}. Calculation for field soils assumes that herbicides are uniformly mixed in the surface soil layer (0-5 cm).

The experimental containers, one hundred-twenty 0.5 L glass jars, were prepared by adding 200 g moist (13 % w/w), sieved (< 3 mm) soil to each jar. Food source treatments consisted of 2 g soybean leaves (S), 2 g corn leaves (C), and no residues (N) additions. Herbicide solutions (20 mL) were uniformly applied onto the soil surface or crop residues by pipette. Approximately half of the herbicide solution was dripped onto the first layer of crop residues (1 g) and allowed to be sorbed into the leaves before the second layer of crop residues (1 g) and the remaining solution was added.

Clitellate adult earthworms (4 to 5 g) were washed, blotted dry with a paper towel, weighed, and placed onto the soil surface one day after herbicide application. Each jar received five earthworms. Earthworms and soil were incubated in environmental chambers at 12°C and re-weighed at either 21 or 46 days. Differences between initial and final *L. terrestris* weights were used to assess earthworm growth rates (initial weight = 100 %). Data were subjected to three-way analysis of variance with 2 time periods (0 to 21 and 0 to 46 days) X 3 crop residues (Soybean, Corn and No residues) X 4 herbicide rates (0H, 1H, 3H and 6H) using SAS 6.10 for Windows Version 4.0.

RESULTS AND DISCUSSION

No significant differences in initial earthworm weights were noted amongst different crop residues treatments X herbicide dose treatments X time treatment.

Mean changes in earthworm weight measured at 21 and 46 days were thus a result of either herbicide dose applications or crop residue treatments, or both.

The mean weight of earthworms in soils with corn residues increased (relative to initial weights) for all herbicide dose and time treatments, except the 46 day incubation at the six times recommended herbicide rate (Figure 1). *L. terrestris* in soils with either soybean or no residues decreased in weight between 21 and 46 days, independent of the presence or absence of herbicides. Visual observations indicated that earthworms ingested corn at a much slower rate than soybean residues, but generally both crop residues were consumed by the earthworms or incorporated into the soil within 21 days.

Three-way analysis of variance revealed that earthworm weight changes were significantly affected by time, crop residues and herbicide dose rates (Table 2). The interactions, time X crop residues, and, time X crop residues X herbicide dose rates, were also statistically significant, indicating that the treatments depended on the status of others.

Growth rates were significantly greater in soils with corn residues compared to those in soils with either soybean or no residues, after both 21 and 46 days incubation. Earthworms in soils with no residues for 46 days showed significantly greater weight losses than those in soils with soybean residues for 46 days, indicating that earthworms were affected by food deprivation for a prolonged period. Earthworm weight changes were reported to be dependent on type of food in a number of other studies (Martin 1986, Shipitalo et al. 1988). *L. terrestris* biomass and numbers were significantly greater in fields with corn-soybean-cereal rotations compared with continuous soybean management (Tomlin et al. 1995).

LS mean comparison for time × crop residues × herbicide dose rates showed that sub-lethal effects of herbicides on earthworms were more affected by the kind of crop residue present than by herbicide dose rates (Table 3). *L. terrestris* growth rate, in soils with corn at 3× herbicide rates, was significantly greater at 21 days compared with earthworms in soils with no herbicides and either soybean or no residues. Earthworms in soils with corn residues and 6× herbicide rates showed similar growth rates at 21 days as those in soils with no herbicides and either soybean or no residues. In field studies, earthworm biomass and numbers were also less affected by herbicide application rates relative to crop rotation management (Tomlin et al. 1995).

Earthworms incubated for 21 days in soils with corn residues demonstrated a statistically similar weight increase for all herbicide dose treatments. Earthworm weight gain between 0 to 21 days decreased with increasing soil herbicide concentration for soybean and no residues treatments, but differences amongst herbicide treatments were not significant, except for soybean-fed earthworms in soils with 6× herbicide rates. In this latter treatment, more than 50 % of the

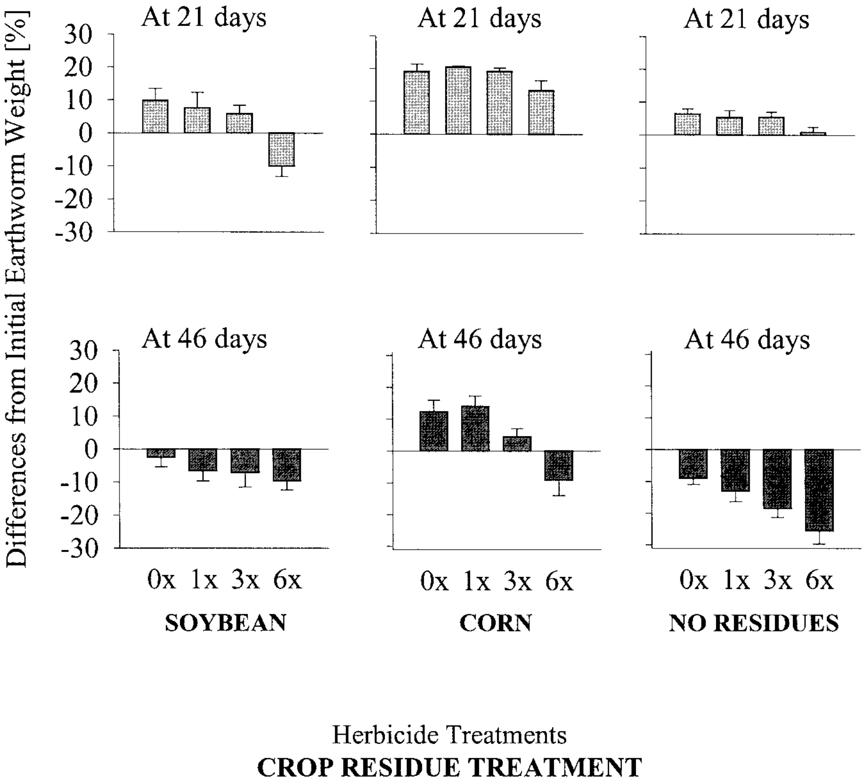

Figure 1. Changes in earthworm weight in soils with various crop residues and herbicide field rate applications. Earthworms were incubated in soil for either 21 or 46 days.

soybean residues were left on the soil surface, suggesting that earthworms were affected by herbicides and therefore less active, or avoided contaminated food.

Earthworm weight losses up to 46 days increased with increasing herbicide field rates for soils with soybean or with no residues. Herbicide applications, in the absence of food, significantly decreased earthworm weights, relative to soil without herbicide treatments. Earthworms in soils with corn residues showed significantly greater weight losses at 6× herbicide rates, relative to that with lower herbicide application rates. Earthworms were probably more affected by atrazine than by metolachlor since previous studies have shown that the LC_{50} (14 days) for earthworms is 78 and 140 mg Kg^{-1} soil, for atrazine and metolachlor respectively (Tomlin 1994).

At the 6× herbicide rate, no significant difference in earthworm weight loss was observed at 46 days between corn and soybean-fed *L. terrestris*. This suggests that, at much larger than recommended field rate applications and prolonged times, the

Table 2. Degrees of Freedom (DF), Sum of Squares (SS), F ratio and P levels of the three-way analysis of variance on the effect of time (after 21 and 46 days), crop residues (Soybean, Corn, No residues), and herbicide dose (0×, 1×, 3× and 6× recommended field rates) on earthworm growth rates.

Source	DF	Type III SS[1]	F ratio and P levels
MODEL	**23**	**17542.27**	
Time	1	6080.63	141.64***[2]
Crop residues (Food type)	2	6463.27	83.94 ***
Herbicide doses	3	2699.47	19.74 ***
Time × Crop residues	2	769.8	9.01***
Time × Herbicide doses	3	139.54	1.09*ns*[2]
Crop residues × Herbicide doses	6	117.52	0.46*ns*
Time × Crop residues × Herbicide doses	6	763.12	2.98*[2]
ERROR	**94**	**4015.55**	

[1] Type III SS is reported instead of type I SS as response data was unbalanced: 2 out of 5 earthworms died in jars with corn residues and 6 × recommended field rates for 46 days.
[2] *ns* = not significant, * = $P< 0.05$, *** = $P < 0.001$.

adverse effects of herbicides on earthworms are independent of the kind of crop residues present. Earthworms incubated 46 days in soils with soybean residues exhibited similar weight losses to those for 46 days in soils with no residues, at both 0× and 1× herbicide rates. At the greater herbicide rates (3× and 6×), earthworms in soils without residues at 46 days showed significantly greater weight losses than any other crop residue treatment, suggesting that adverse effects of herbicides on earthworms can be enhanced by a continuous lack of food supply.

This study demonstrated that earthworms are not affected adversely by atrazine-metolachlor applications unless the maximum recommended field rate is exceeded by six fold. Differences between initial and final earthworm weights were more influenced by the presence and nature of crop residues than by herbicide application rates. Since the sublethal effects of herbicides on earthworm populations were strongly related to the type of food present, we conclude that a greater attention to food quality is needed when earthworms are used as test species for ecotoxicology assessment.

Table 3. LS means on earthworm weight changes (initial earthworm weight = 100 %) for each for each time × crop residues × herbicide dose combination.

Time	Crop residue	Herbicide dose	Earthworm weight as % of initial earthworm weight	
0 to 21 days	Corn	1×	120.42	a [1]
0 to 21 days	Corn	3×	119.09	a
0 to 21 days	Corn	0×	119.04	a
0 to 46 days	Corn	1×	113.93	ab
0 to 21 days	Corn	6×	113.25	abc
0 to 46 days	Corn	0×	112.35	abcd
0 to 21 days	Soybean	0×	109.75	bcd
0 to 21 days	Soybean	1×	107.49	bcde
0 to 21 days	No residues	0×	106.34	bcde
0 to 21 days	Soybean	3×	105.77	bcde
0 to 21 days	No residues	3×	105.42	cdef
0 to 21 days	No residues	1×	105.33	cdef
0 to 46 days	Corn	3×	104.45	def
0 to 21 days	No residues	6×	100.89	efg
0 to 46 days	Soybean	0×	97.45	fgh
0 to 46 days	Soybean	1×	93.34	ghi
0 to 46 days	Soybean	3×	92.91	ghi
0 to 46 days	No residues	0×	90.95	hi
0 to 46 days	Corn	6×	90.94	hi
0 to 46 days	Soybean	6×	90.39	hi
0 to 21 days	Soybean	6×	89.95	hi
0 to 46 days	No residues	1×	86.98	ij
0 to 46 days	No residues	3×	81.44	j
0 to 46 days	No residues	6×	74.42	j

[1] Means followed by same letters are not significantly different at $P < 0.05$ (Student Newman Keul's).

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