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Insecticides (dimethoate and lambda-cyhalothrin) for soybean aphid control – are they toxic to earthworms? Evidence from laboratory and field bioassays

I CEU IN PEST MANAGEMENT

SOYBEAN aphid is a native of Asia that was accidently released in Michigan, USA around 1995. This introduced pest has spread rapidly through soybean production areas of the USA and first appeared in Quebec and Ontario, Canada in 2001. Aphids suck sap from leaves, stems and pods, which diverts photosynthates needed for plant growth and seed production, and transmit viruses such as the soybean mosaic and bean yellow mosaic viruses during feeding (Wang et al., 2006). Due to its high reproduction capacity, notably a birth rate of 3-8 aphids per day and generation times of 7-10 days, aphid populations can double in 2-3 days when conditions are favorable (Ragsdale et al., 2004).

Biological control by predators such as lady beetles, green and brown lacewings, syrphid flies and damsel bugs can partially control aphid populations. However, aphid outbreaks across large areas in the USA Midwest and Canada in 2003, 2005, 2007 and 2009 have necessitated the use of insecticides to minimize soybean yield losses, at a cost of millions of dollars to agricultural producers (Steffey, 2009; Gray, 2010). Agricultural entomologists recommend taking action when populations exceed 250 aphids per plant over 80% of the field, based on an economic threshold that gives the producer a lead time of 7 days to apply insecticides and thus prevent yield losses (Ragsdale et al., 2007). Field strip trials (> 50 sites) in Ontario, Canada showed that timely application of insecticidal sprays containing dimethoate and lambda-cyhalothrin during the pod formation stages controlled the pest and increased soybean grain yields by 8-10% on average (Baute et al., 2006). Dimethoate is an organophosphate insecticide that kills insects and mites systemically and on contact by interfering with cholinesterase and consequently nervous system function. Lambda-cyhalothrin is a pyrethroid that kills insects and mites on contact by acting on ion channels in nerve cells, disrupting cellular functions in peripheral and central nervous systems. These broad spectrum insecticides are moderately persistent in agricultural soils, with $t_{1/2}$= 4 to 83 d for dimethoate (Patil et al., 1987; Kolbe et al., 1991; Martikainen, 1996) and $t_{1/2}$= 28 to 84 d for lambda cyhalothrin (Hornsby et al., 1995). The impact of these insecticides on non-target invertebrates in soybean agroecosystems is not known.

The objective of this study was to evaluate the toxicity to earthworms of dimethoate and lambda-cyhalothrin. Bioassays were conducted in the laboratory and in field plots planted to soybean where the insecticides were applied at the rates recommended to control soybean aphid.

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MATERIALS AND METHODS

LABORATORY BIOASSAY MATERIALS

All glassware was washed with phosphate-free detergent then soaked in 15% HCl and rinsed thoroughly with deionized water. Earthworms (*E. fetida*) were purchased from Carolina Biological Supply Company (Burlington, North Carolina, USA), kept in laboratory cultures (covered 37 L plastic containers with peat moss bedding at 50% humidity and 20°C) and fed with a high protein substrate, Magic Worm Food™ (Magic Products, Amherst Junction, WI, USA) for two months prior to starting the laboratory bioassay. Artificial soil was composed of 700 g kg^{-1} silica sand, 200 g kg^{-1} colloidal kaolinite clay and 100 g kg^{-1} Canadian peat moss (<2mm size). About 10 g kg^{-1} of calcium carbonate was added to adjust the pH to 6.0 ± 0.5. Commercial insecticides used in the laboratory bioassay were Cygon 480 containing 23.4% dimethoate (Cheminova Canada, Kilworth, Ontario, Canada) and Matador™ 120 EC containing 13.1% lambda-cyhalothrin (Syngenta Crop Protection Canada, Guelph, Ontario, Canada).

LABORATORY EXPERIMENTAL DESIGN

The experiment was a completely randomized block design where artificial soil was treated with dimethoate (0.006, 0.058, 0.585, 5.85, 58.5 and 117 mg active ingredient, a.i. kg^{-1} soil), lambda-cyhalothrin (0.003, 0.033, 0.131, 0.327, 3.27 and 32.7 mg a.i. kg^{-1} soil), with distilled water (water control) or 5 mg carbaryl kg^{-1} soil (positive control; van Gestel, 1992). Each treatment was replicated four times. In total, 56 jars were prepared.

EARTHWORM REPRODUCTION TEST

The earthworm reproduction test was based on the OECD (2004) standard method, with some modifications that are explained below. We selected earthworms weighing between 350 and 520 mg that were sexually mature (fully developed clitellum) and visually appeared to be in good health. After 28 d, soil was removed from the jar, sexually mature earthworms were collected, counted, surface rinsed, patted dry and weighed. We determined survival and growth, based on the instantaneous growth rate (IGR, g final mass g^{-1} initial mass day^{-1}) calculated as follows: IGR = ln (Wf-Wi)/Dt, where Wi and Wf are initial and final earthworm mass (g), respectively, and Dt is the growth interval measured in days (Brafield and Llewellyn, 1982). The soil was replaced and jars were returned to the laboratory cupboard for an additional 28 d. Then, jars were immersed in a water bath and heated gradually from 20 to 60°C to retrieve juvenile earthworms, which were counted, then soil was wet sieved to collect hatched and unhatched cocoons. Cocoon hatchability (%) was the percentage of hatched cocoons amongst the cocoons collected. The number of juveniles per hatched cocoons was also calculated.

SOYBEAN FIELD SITES

The field experiment was conducted on two commercial farms in Quebec, Canada during the 2007 and 2008 growing seasons. Farm A, located in La Présentation (45° 36' 5"N 73° 4' 37"W) was on a loamy soil of the St-Urbain series (Humic Gleysol) containing 19 g organic matter kg^{-1} and having pH 5.8 (1:2 soil:water slurry) in the topsoil (0-15 cm depth). Farm B was in Boucherville (45° 36' 57"N 73° 26' 29"W) on a clay loam soil of the Providence series (Humic Gleysol) and the topsoil (0-15 cm) contained 38 g organic matter kg^{-1} and pH 5.8. The distance between farms was about 28 km. Both farms followed a corn-soybean-wheat rotation and used conservation tillage (soil was not cultivated following corn harvest, soybeans were directly seeded using no-till equipment).

FIELD EXPERIMENTAL DESIGN

Experimental plots were arranged in a randomized complete block design with the following five treatments: lambda-cyhalothrin (Matador™ 120 EC) applied at the R3 stage, lambda-cyhalothrin applied at the R5 growth stage, dimethoate (Cygon 480) applied at the R3 growth stage, dimethoate applied at the R5 growth stage and a control that did not receive insecticide. There were five blocks (replicates) for a total of 25 experimental plots.

INSECTICIDE APPLICATION

Aphid populations were assessed prior to insecticide applications by counting aphids on 20 plants per plot. In 2007, insecticide treatments were applied with a MAT-OSU bicycle, equipped with a 3 m boom and six flat fan spray nozzles (TeeJet DG 8002, TeeJet Technologies, Wheaton, Illinois, USA) at 50 cm spacing along the boom. It was calibrated to spray 200 L ha^{-1} at 240 kPa, and the boom was positioned 50 cm above the crop canopy. In 2008, the nozzles were changed to six flat fan nozzles (HARDI S4110-20, Hardi Inc., Davenport, Iowa, USA); the spray volume was 200 L ha^{-1} and the pressure was 200 kPa. Insecticide application rates were the same at both growth stages and in both years: 83 mL ha^{-1} of Matador™ 120 EC (contains lambda cyhalothrin, 13.1% a.i., density = 0.91 g mL^{-1}) and 1000 mL ha^{-1} of Cygon 480 (contains dimethoate, 23.4% a.i., density = 1.1 g mL^{-1}), following recommendations from OMAFRA (2009). Applications at the R3 stage were made on 19 July 2007 and 17 July 2008, while the R5 stage was sprayed on 15 August 2007 and 5 August 2008.

EARTHWORM POPULATION ASSESSMENT

Earthworm populations were examined in the spring of 2007 to evaluate indigenous species. The effect of insecticides controlling soybean aphid on naturally-occuring earthworm populations in all plots was evaluated 6 to 17 d following insecticide applications at the R3 stage (25 July 2007, 29 July 2008) and the R5 stage (23 August 2007, 22 August 2008). Earthworms were collected from treated plots as follows: soil blocks (38 cm × 38 cm) were removed (15 cm depth) from each plot and handsorted to collect surface-dwelling earthworms. A dilute formaldehyde solution (7 mL of 37% formaldehyde mixed with 1 L of water) was poured into the bottom of each hole to collect earthworms living deeper than 15 cm such as *L. terrestris*. All earthworms were preserved in 5% formaldehyde solution upon collection. Preserved earthworms were separated into age classes on the basis of clitellum development, and further categorized as fragments (incomplete earthworm fragments), juveniles, pre-clitellate adults (clitellum present but not fully developed) and clitellate adults (fully developed clitellum). Sexually mature specimens were identified to species level (Reynolds, 1977). Preserved earthworms were then oven-dried (60 °C for 48 h) and ashed at 500 °C for 4 h to determine ash-free dry weight (AFDW).

RESULTS AND DISCUSSION

LETHAL AND SUBLETHAL EFFECTS OF INSECTICIDES ON EARTHWORMS IN LABORATORY BIOASSAYS

After 28 d exposure, dimethoate was lethal to adult *E. fetida* but lambda cyhalothrin was not (data not shown). The acute lethal concentration (LC_{50}) for dimethoate in this study was 81.6 mg a.i. kg^{-1} soil, slightly lower than the LC50 of 97 to 304 mg a.i. kg^{-1} soil reported for *E. fetida* under standard test conditions (Larink and Kula, 1994; Frampton et al., 2006). The field earthworm *Aporrectodea tuberculata* was more sensitive to dimethoate, with LC_{50} ranging from 38 to 100 mg a.i. kg^{-1} after 14 d exposure in artificial, clayey and humus soils (Martikainen, 1996) whereas the field earthworms *A. trapzeoides, A. caliginosa, A. longa* and *A.rosea* were unaffected by 10-21 d exposure to a single rate of 72.5 mg dimethoate kg^{-1} soil in pots filled with loamy soil (Dalby et al., 1995). Lambda cyhalothrin exposure gave an LC_{50} of 100 mg a.i. kg^{-1} soil for *E. fetida* under standard test conditions (Frampton et al., 2006; Garcia et al., 2011), so the highest nominal concentration of lambda cyhalothrin in this study (32.7 mg a.i. kg^{-1} soil) was probably not high enough to be lethal to earthworms.

The lethal and sublethal effects of insecticides on earthworms were calculated from nominal exposure concentrations rather than the actual dimethoate and lambda cyhalothrin concentrations. Quantifying dimethoate and lambda-cyhalothrin concentrations, as well as other contaminants in commercial insecticides, by high performance liquid chromatography or gas chromatography after 28 d (adult earthworms) and 56 d (juvenile earthworms) would provide more accurate dose-responses, but was not possible in this study due to financial constraints.

INSECTICIDE EFFECTS IN FIELD BIOASSAYS: APHID POPULATIONS, SOYBEAN YIELDS AND EARTHWORMS

Soybean aphid was a problem for producers participating in this study. Soybeans were infested with aphids in 2007, with populations of 216 to 447 aphids per plant at the R3 stage, which declined to about 100 aphids per plant at the R5 stage on Farm A and increased to 652 aphids per plant at the R5 stage on Farm B (data not shown). The economic threshold suggests insecticide application when there are 250 or more aphids per soybean plant (Ragsdale et al., 2007; Baute et al., 2006). Without insecticide application (unsprayed control plots), soybean yield was reduced by up to 8% on Farm A and up to 22% on Farm B, compared to the highest yield in the treated plots (data not shown). This is comparable to soybean yield gains, on average more than 8%, achieved in field strip trials in Ontario following application of the same commercial insecticides as used in this study (Baute et al., 2006).

On Farm A, the best yield was achieved when soybean was sprayed at the R3 stage with dimethoate, which gave significantly ($p < 0.05$) greater yield than the untreated soybeans or soybean sprayed at the R5 stage with dimethoate (data not shown). On Farm B, soybean yield was significantly ($p < 0.05$) greater when plots were sprayed at the R3 stage with dimethoate or lambda-cyhalothrin than untreated (data not shown). Yields improved significantly ($p < 0.05$) when plots were treated with lambda cyhalothrin at the R3 stage than the R5 stage (data not shown). The naturally-occurring earthworm population on Farm A was dominated by *Aporrectodea turgida* (64% of adults), with *Lumbricus terrestris* (22%), *Allolobophora chlorotica* (12%) and *Aporrectodea rose*a (2%) also present. On Farm B, adult earthworms were *A. turgid*a (42%), *A. chlorotica* (31%), *Eiseniella tetraedra* (26%) and *A. rosea* (1%). These species represent the three major ecological groups of earthworms: endogeic, anecic and epigeic (Bouché, 1977). *The Aporrectodea spp*. and *A. chlorotica* are endogeic earthworms that inhabit the top 15-20 cm of soil, feeding primarily on buried plant litter and creating extensive horizontal burrows. Reproduction occurs within the soil. Endogeic earthworms likely have limited exposure to foliar-applied insecticides, although they could come in contact with dimethoate if this systemic insecticide was translocated through plant roots. The anecic earthworm *L. terrestris* and the epigeic *E. tetraedra* feed and reproduce at the soil surface, thus could be impacted by insecticide residue on the soil surface or on senescent soybean foliage/stems. The major assumption was that earthworms were sessile and there was no immigration-emigration in plots following insecticide application, which is probably a good assumption since earthworm migrations in Quebec seem to occur mainly in spring when the ground is wet and the average temperature is between 5 and 10°C (personal observation). The naturally-occuring earthworm populations were not impacted negatively by exposure to dimethoate or lambda cyhalthron on either farm during 2007 and 2008 (Table 1). There were more earthworms in the dimethoate-treated plots at the R3 stage on Farm A in 2007, but no difference in earthworm populations between the lambda cyhalothrin plots and the control plots (Table 1).

CONCLUSIONS

Insecticide application rates to soybean in the field bioassay can be compared to nominal concentrations of dimethoate and lambda cyhalothrin in the laboratory bioassay, considering the specific gravity of each insecticide (1.1 g mL^{-1} for Cygon, 0.91 g mL^{-1} for Matador™), the concentration of the active ingredient in commercial formulation and the equivalent soil mass in one hectare (2.24 x 10^6 kg soil ha^{-1}, considering a bulk density of 1.12 Mg m^{-3} and earthworm activity to 20 cm depth). Thus, soybean fields received 0.12 mg dimethoate a.i. kg^{-1} soil and lambda cyhalothrin at 0.004 mg a.i. kg-1 soil. Field application rates of dimethoate were 51 times lower, and of lambda cyhalothrin were 1353 times lower, than the EC_{50} for adult *E. fetida* growth, the most sensitive toxicity endpoint in the laboratory bioassay. It is not surprising that insecticide application rates in the field had no effect on naturally-occuring earthworm populations. Field application rates may be low for insecticides in this study since the label for Matador™ recommends a maximum application rate for aphids of 233 mL ha-1, equivalent to 0.012 mg a.i. kg^{-1} soil of lambda cyhalothrin, with repetitions at 7 d intervals. Still, this would be 450 times lower than the EC_{50} for adult *E. fetida* growth. No comparable field bioassay was done to assess the impact of these insecticides on earthworms in temperate regions, but a study in the tropics by Föster et al. (2006) showed that lambda-cyhalothrin applied at rates of 0.018 to 0.18 mg a.i. kg^{-1} soil had no effect on naturally-occuring earthworm populations. We conclude that the insecticides registered in Canada for soybean aphid control should not affect earthworms when applied according to the label recommendations.

TABLE I.

Earthworm populations on two farms where soybean fields were sprayed with commercial formulations of lambda cyhalothrin (Matador™ 120 EC) and dimethoate (Cygon 480) insecticides to control the soybean aphid (*Aphis glycines L.*). Insecticides were applied two growth stages: beginning pod (R3 stage) and beginning seed (R5 stage). Earthworms were collected within about two weeks of insecticide application, enumerated and their biomass was determined on an ash-free dry weight (AFDW) basis.

FARM A								
Treatment	2007				2008			
	number m^{-2}		biomass (g AFDW m-2)		number m-2		biomass (g AFDW m-2)	
	R3 stage	R5 stage	R3 stage	R5 stage	R3 stage	R5 stage	R3 stage	R5 stage
Control	230 ± 62	283 ± 19	6.8 ± 2.2	7.6 ± 0.9	291 ± 32	361 ± 27	12.9 ± 2.4	15.2 ± 0.8
Dimethoate	297 ± 54	251 ± 47	11.3 ± 4.5	6.0 ± 1.4	288 ± 39	368 ± 40	10.2 ± 1.4	13.5 ± 1.8
Lambda cyhalothrin	225 ± 24	294 ± 60	7.8 ± 1.9	8.5 ± 2.5	214 ± 47	434 ± 103	8.4 ± 1.8	14.1 ± 1.3
Treatment effect	P=0.048*	NS	NS	NS	NS	NS	NS	NS

FARM B								
Treatment	2007				2008			
	number m^{-2}		biomass (g AFDW m-2)		number m-2		biomass (g AFDW m-2)	
	R3 stage	R5 stage	R3 stage	R5 stage	R3 stage	R5 stage	R3 stage	R5 stage
Control	121 ± 25	117 ± 23	2.5 ± 0.4	3.3 ± 0.8	122 ± 64	174 ± 58	1.4 ± 0.6	1.5 ± 0.6
Dimethoate	137 ± 29	187 ± 33	1.9 ± 0.3	3.4 ± 0.4	88 ± 25	130 ± 58	1.9 ± 0.8	1.6 ± 0.3
Lambda cyhalothrin	131 ± 21	119 ± 43	3.8 ± 0.6	2.3 ± 0.3	62 ± 16	105 ± 55	0.9 ± 0.3	1.1 ± 0.5
Treatment effect	NS	NS	NS	NS	NS	NS	NS	NS

Values are the mean ± one standard error. The treatment effect (one-way ANOVA) was significant (*$P < 0.05$) or non-significant (NS).