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Effects on litter-dwelling earthworms and microbial decomposition of soil-applied imidacloprid for control of wood-boring insects

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Abstract

BACKGROUND: Imidacloprid is an effective, systemic insecticide for the control of wood-boring insect pests in trees. Systemic applications to trees are often made by soil injections or drenches, and the resulting imidacloprid concentrations in soil or litter may pose a risk of harm to natural decomposer organisms. The authors tested effects of imidacloprid on survival and weight gain or loss of the earthworms *Eisenia fetida* (Savigny) and *Dendrobaena octaedra* (Savigny), on leaf consumption rates and cocoon production by *D. octaedra* and on microbial decomposition activity in laboratory microcosms containing natural forest litter.

RESULTS: *Dendrobaena octaedra* was the most sensitive of the two earthworm species, with an LC_{50} of 5.7 mg kg^{-1}, an LC_{10} of about 2 mg kg^{-1} and significant weight losses among survivors at 3 mg kg^{-1}. Weight losses resulted from a physiological effect rather than from feeding inhibition. There were no effects on cocoon production among survivors at 3 mg kg^{-1}. The LC_{50} for *E. fetida* was 25 mg kg^{-1}, with significant weight losses at 14 mg kg^{-1}. There were no significant effects on microbial decomposition of leaf material at the maximum test concentration of 1400 mg kg^{-1}.

CONCLUSION: The results indicate that, when imidacloprid is applied as a systemic insecticide to the soil around trees, it is likely to cause adverse effects on litter-dwelling earthworms if concentrations in the litter reach or exceed about 3 mg kg^{-1}.
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Keywords: imidacloprid; toxicity; forest litter; earthworms; microbial activity

1 INTRODUCTION

Trees in eastern North America are increasingly threatened by invasive, exotic insect pests including wood-boring insects such as emerald ash borer (*Agrilus planipennis* Fairmare), Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky), and brown spruce longhorn beetle (*Tetropium fuscum* Fabricius). These invasive wood-boring insects have potential to cause substantial economic and ecological impacts similar in magnitude to those of previous invasive pests on American chestnut (*Castanea dentate* Marsh.) and white elm (*Ulmus americana* L.).[1–3] Wood-boring insects are particularly difficult to control by conventional foliar insecticides or trunk sprays because the most damaging life stages of these insects are the phloem-feeding larvae burrowing under the bark.[2]

The systemic insecticide imidacloprid [1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylidene-amine], a chloro-neonicotinyl insecticide that blocks the nicotinergic neuronal pathway in insects,[4] has been shown to be effective against emerald ash borer and Asian longhorned beetle.[3,5] It is effective on larvae in the phloem and cambium, and on adults feeding on twigs.[3] Systemic applications to individual trees may not be feasible for all forest pest management situations, but they may be well suited for control of exotic species within a restricted area before the species becomes widely distributed. They may also be well suited for protection of high-value trees in urban and recreational settings, in riparian areas of urban watersheds or in other environmentally sensitive areas where broad-scale pesticide applications or tree removal approaches are not acceptable.

Applications of imidacloprid to trees are often made by soil injection or soil drench, and the resulting imidacloprid concentrations in forest litter

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and upper soils could pose a risk of harm to natural decomposer organisms. Microbial communities[6–8] and earthworm populations[9–11] are known to be critical for forest litter decomposition processes which contribute to organic matter breakdown, nutrient cycling and the development of nutrient-rich organic soils. Effects of imidacloprid on soil microorganisms and earthworms have previously been reported,[12–20] but most previous studies have been conducted in an agricultural or horticultural context with emphasis on endogeic (soil-dwelling) and anecic (deep-burrowing) earthworms and microbial communities in mineral soils. The present authors tested the effects of imidacloprid on decomposer organisms in a forest pest management context by focusing on epigeic (litter-dwelling) earthworms and microbial communities exposed to imidacloprid in natural forest litter.

2 METHODS

2.1 Microcosms

Microcosm design, handling and measurement endpoints were taken from previous microcosm experiments to assess forest pesticide effects on earthworms.[21] Microcosms were constructed of acrylic tubing, 7 cm diameter and 10 cm high, fitted with a plastic bottom containing two screened drainage holes, and covered on top with plastic, 1 mm mesh screen held by elastic bands. Each microcosm contained 60 g of field-collected litter from a sugar maple (*Acer saccharum* Marsh.) forest, with the litter held at or corrected to ambient moisture by addition of deionized water just prior to being placed in the microcosm. Litter consisted of partially decomposed organic material (about 60% organic determined by ash-free dry mass) collected from under the recent leaf litter and above the mineral soil. The material was frozen for several weeks to kill litter invertebrates, and thawed for 1 week to re-establish microbial communities before being added to the microcosms. The litter was frozen in sealed plastic containers, and, when thawed, was kept at constant weight (ambient moisture) by adding deionized water when necessary. The litter was thoroughly mixed before being distributed among terrestrial microcosms. Microcosms were placed on a shelf in an experimental room with controlled temperature ($20 \pm 3\,^{\circ}C$), light (daylight simulation fluorescent bulbs, 12:12 h light:dark, photon flux approximately $30\,\mu mol\,m^{-2}\,s^{-1}$) and humidity (50–70% RH). During the microcosm experiments, ambient moisture in the litter was maintained by addition of deionized water about every 2 days to bring microcosms to original weights.

2.2 Experimental design

Separate experiments were conducted to test the hypothesis that imidacloprid applied to forest litter would have adverse effects on epigeic earthworms *Eisenia fetida* (Savigny) and *Dendrobaena octaedra* (Savigny) and on microbial decomposition of leaf material. Effects on microbial communities were tested as part of the *E. fetida* experiment. For each experiment there were four replicate microcosms for each treatment group. Comparisons were made among imidacloprid-treated microcosms and controls. Imidacloprid was added as a $750\,g\,kg^{-1}$ WP (Merit Solupak®; Bayer Crop Sciences, Calgary, Alberta, Canada) in tap water. Stock solutions were prepared and appropriate volumes of test material were added to the 60 g of litter in designated microcosms to achieve nominal test concentrations. The test material was added to the surface of the litter with a pipette and then thoroughly mixed into the litter using a stainless steel spatula. Nominal test concentrations for the *E. fetida* experiment were 0, 1.4, 14, 25 and $45\,mg\,kg^{-1}$, for the *D. octaedra* experiment they were 0, 3, 7, 14, 28 and $56\,mg\,kg^{-1}$ and for the microbial decomposition experiment (as part of the *E. fetida* experiment) they were 0, 1.4, 14, 140 and $1400\,mg\,kg^{-1}$. Based on results from the initial *E. fetida* experiment, the test concentrations for *D. octaedra* were adjusted (range narrowed), resulting in different test concentrations for the two experiments. The observation periods for the experiments were 35 days following addition of earthworms and application of imidacloprid.

2.3 Response measurements

Eisenia fetida were obtained from a commercial supplier (Cathy's Crawly Composters, Bradford, Ontario, Canada), and *Dendrobaena octaedra* were field-collected from the litter of a northern-hardwood forest in the Turkey Lakes Watershed Research Area approximately 60 km north of Sault Ste Marie, Ontario, Canada. The worms were held in the experimental laboratory for at least 4 weeks prior to the experiments. They were placed in containers with litter and leaf material from the worm collection site and held at similar moisture levels by periodic visual inspection and addition of deionized water when necessary. Two clitellate (light-coloured band present, indicating sexual maturity) worms were randomly allocated to each microcosm on the day that concomitant test materials were added to the microcosms. Just prior to placement in the microcosms, each pair of worms was lightly rinsed with water to remove litter particles, then weighed to determine initial pair weights. It was not possible to track individual weights because individuals could not be differentiated.

At weekly intervals after dosing, the contents of each microcosm were emptied onto a porcelain tray and searched for earthworms. Worms not moving and not responding to gentle prodding were considered dead and were removed and discarded. Missing worms were assumed dead and decomposed and were added to the mortality count. At the same time that the mortality checks were made, the pair of worms from each microcosm was removed from the contents, placed in a petri dish and gently rinsed with deionized water to remove litter particles. The litter was returned to

each microcosm, and the pair of earthworms was transferred to a second petri dish, weighed and then returned to the microcosms. Weight measurements were discontinued when one or both of the worms in a microcosm were dead.

In the *D. octaedra* experiment, two further response measurements were made. Reproduction was determined by removing and counting all cocoons produced in the microcosms at each mortality-check interval. Cocoon searches were discontinued when one or both of the worms in a microcosm were dead. The cocoons were placed in glass jars lined with paper towel, kept moist by addition of deionized water and held in the experimental lab for 6 weeks beyond the end of the experimental period. The number of juveniles hatched from the cocoons each week was recorded, and they were summed at the end of the 6 week period to measure the percentage of cocoons hatched (assuming one juvenile per cocoon).

The decomposition of leaf material by *D. octaedra* feeding and microbial activity was measured by mass loss of leaf material buried in the litter of the microcosms. This was determined as the difference between the estimated initial dry weights of sugar maple leaves and the final dry weights of remaining leaf material at the end of the 35 day experimental period. The leaves added to the microcosms were not initially dried and weighed for direct measurement of initial dry weights because the microcosm experiments were to simulate leaf fall under as natural conditions as possible (fresh leaves added to microcosms), and to avoid the potential that predrying the leaves could affect the palatability to the earthworms. Four leaf pieces (two maple leaves cut in half) from leaves collected at senescence just before leaf fall were placed in each microcosm at about 1 cm below the surface of the litter. Fresh batch weights were measured for the leaf pieces, and initial dry weights were estimated from a regression of fresh leaf weights on dry leaf weights. A subset of 50 leaves from the same batch was used for the regression. These were individually weighed, dried at 60 °C for 48 h and reweighed to determine dry weights. A linear regression of fresh weight on dry weight was computed to estimate the initial dry weights from fresh weights of leaves added to the microcosms (regression $r^2 = 0.86$). Leaf material remaining at the end of the experimental period was removed from each microcosm, dried at 60 °C for 48 h and reweighed to determine final dry weights.

Microbial decomposition of leaf material was measured separately by mass loss of leaf disks in 1 mm mesh bags. Five leaf disks were cut from the leaves with a cork borer, batch-weighed to determine initial fresh weight (initial dry weight was estimated from the same regression as the leaf pieces), placed in the fine-mesh bags to exclude the earthworms and buried at about mid-depth in the litter of each microcosm. At the end of the experimental period, leaf disks were removed, washed gently to remove litter particles, dried at 60 °C for 48 h and then reweighed to determine final dry weights. Mass loss by microbial decomposition was determined as the difference between estimated initial dry weights and measured final dry weights of the leaf disk batches.

2.4 Imidacloprid concentrations

Imidacloprid concentrations were verified in stock solutions and measured in litter samples by high-performance liquid chromatography with photodiode array detection (HPLC-DAD). Litter samples were obtained from a subset of the microcosms to 'spot-check' initial test concentrations, to provide a measure of how evenly the imidacloprid was mixed within the litter and to determine its disappearance rate. Litter samples were taken immediately after the imidacloprid was added and the litter was thoroughly mixed, and again at the end of the experimental period. Samples were obtained by withdrawing four aliquots (approximately 1 g each) with stainless steel forceps from mid-depth in the litter and pooling these to form one sample per microcosm. These were sealed in a plastic bag and frozen for subsequent analyses.

HPLC calibration standards were prepared from imidacloprid technical, 99.5% (lot 30714; Crescent Chemical, Islandia, New York). All samples were analyzed using an Agilent 1100 HPLC equipped with a photodiode array detector and autosampler. Imidacloprid in litter was extracted by accelerated solvent extraction with methylene chloride at high temperature and pressure, and cleaned up via column chromatography on Florisil columns before HPLC analysis. Concurrent quality control analyses using blank litter samples fortified with known amounts of imidacloprid were run to determine recovery efficiencies and precision of the methods. Average recovery (and coefficient of variation) for quality control litter samples ($n = 8$) was 85.3 (±10.2)%. All residue data were corrected for analytical recovery losses and reported on a fresh weight basis to reflect ambient moisture conditions in the microcosms.

2.5 Data analyses

Differences among treatments for response measurements taken at a single time (number of cocoons, cocoon survival, leaf decomposition) were tested by one-way ANOVA. When significant differences among groups were detected, each treatment was compared with the controls by Dunnett's test. Response measurements taken over time (earthworm weights) were analyzed by repeated-measures ANOVA (RM-ANOVA) with the treatment-by-time interaction as the primary effect of interest. A significant interaction indicated that trends over time were not parallel among treatments, and differences were examined further by planned comparisons (Holm–Sidak tests) between the control and other treatments at each sampling time. For all statistical tests, significance was accepted at $P < 0.05$. Testing for differences among all treatments at each sampling time was avoided to reduce the effects of multiple comparisons on the

overall error rate. Percent data were arcsine/square-root transformed prior to analysis. Datasets were tested for normality and homogeneity of variances prior to analysis. None of the tests for normality and homogeneity of variances was significant, and transformations were not required. Median and 10-percentile lethal concentrations to earthworms were estimated from the concentration–response data by probit analysis. The ANOVAs were conducted with SigmaStat 3.5, and the probit analysis was run on Minitab14.

3 RESULTS

3.1 Imidacloprid concentrations in litter

Initial concentrations of imidacloprid measured in litter were significantly correlated (Pearson product moment correlation coefficient $n = 28$, $R = 0.98$, $P < 0.001$) with nominal test concentrations (Table 1). There was no evidence that the imidacloprid dissipated in the litter over the 35 day experimental period. Differences observed between initial and final concentrations may be artifacts of incomplete mixing of the test solution throughout the litter, but are likely to simulate non-uniform concentrations of imidacloprid in natural forest floor litter after a soil injection or drench as observed in field experiments discussed in Section 4 below. Because the maximum measured concentrations for each treatment level were close to nominal, especially at the lowest effective concentrations (see below), nominal concentrations will be used as test concentrations throughout the remainder of the paper.

3.2 Earthworm mortality

Test concentrations of 25 and 45 mg kg^{-1} caused 50 and 100% mortality of *E. fetida* respectively (Table 2). The median lethal concentration of imidacloprid for *E. fetida* was 25 mg kg^{-1}. The LC_{10} could not be calculated owing to limited intermediate mortality among treatments, but was between 15 and 25 mg kg^{-1}. The data further indicated that no mortality among *E. fetida* would be expected from concentrations in litter of up to 14 mg kg^{-1}. *Dendrobaena octaedra* was more sensitive to imidacloprid, with complete mortality at 14 mg kg^{-1} and measurable mortality at 3 mg kg^{-1}. A probit analysis of the concentration–response data from *D. octaedra* estimated an LC_{10} of 1.9 ± 1.4 mg kg^{-1} and an LC_{50} of 5.7 ± 0.98 mg kg^{-1}.

3.3 Earthworm sublethal responses

There were significant weight losses among *E. fetida* at test concentrations of 14 and 25 mg kg^{-1} (Fig. 1A). The RM-ANOVA detected a significant treatment-by-time interaction ($P < 0.001$), and the Holm–Sidak test indicated that the average pair weights of *E. fetida* were significantly lower at the two higher test concentrations than in controls by day 14. The trend in pair weights at the 1.4 mg kg^{-1} treatment was similar to controls, although there was a significant difference on day 21 (Holm–Sidak $P < 0.05$). Average weights of surviving *D. octaedra* pairs were reduced at all test concentrations, but were increased or maintained in controls (Fig. 1B). There was significant treatment-by-time interaction ($P = 0.007$), and the Holm–Sidak test indicated significantly lower pair weights by day 21 at the 3 mg kg^{-1} treatment, and by day 7 at 7 and 14 mg

Table 2. Total percentage mortality of earthworms across treatments

Test species	Test concentration (mg kg^{-1})	Mortality (%)
Eisenia fetida	0	0
	1.4	0
	14	0
	25	50
	45	100
Dendrobaena octaedra	0	0
	3	25
	7	62
	14	100
	28	100
	56	100

Table 1. Imidacloprid concentrations (expressed on fresh weight basis) in litter of microcosms in which test concentrations were checked

Test organism	Nominal concentration (mg kg^{-1})	Number of microcosms[a]	Initial concentration[a] (mg kg^{-1}) (±SEM)	Final concentration[a] (mg kg^{-1}) (±SEM)
Eisenia fetida	1.4	2	1.8 (±1.4)	1.0 (±0.01)
	14	2	7.3 (±1.1)	13.7 (±1.1)
	25	–	–	–
	45	–	–	–
Dendrobaena octaedra	3	4	3.4 (±0.8)	–
	7	4	7.2 (±0.5)	–
	14	4	15.4 (±0.5)	–
	28	4	33.0 (±4.1)	–
	56	4	64.7 (±3.6)	–
Microbial decomposition	1.4	2	1.8 (±1.4)	1.0 (±0.01)
	14	2	7.3 (±1.1)	13.7 (±1.1)
	140	2	111 (±8.2)	189 (±26.7)
	1400	2	1057 (±223)	1314 (±97.8)

[a] A dash (–) denotes that no samples were taken.

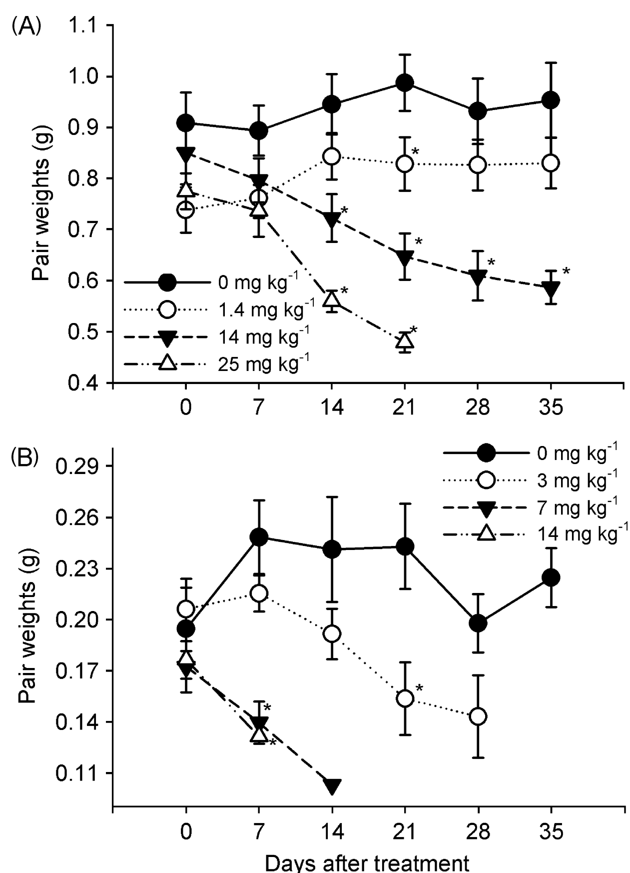

Figure 1. Mean pair weights (±SEM) of (A) *Eisenia fetida* and (B) *Dendrobaena octaedra* in microcosms treated with imidacloprid and in controls over the 35 day experimental period. Asterisks indicate significant differences from controls (Holm–Sidak $P < 0.05$) following significant time-by-treatment interaction in RM-ANOVA ($P < 0.05$).

kg^{-1}. Weight losses of *D. octaedra* at the two higher test concentrations could not be assessed beyond day 7 because of high mortality among replicates.

Neither cocoon production by surviving *D. octaedra* pairs nor cocoon survival appeared to be affected at the 3 mg kg^{-1} treatment (Table 3). There were no significant differences in the number of cocoons produced (ANOVA $P = 0.962$) or in cocoon survival (ANOVA $P = 0.659$) between that treatment group and controls. Only one pair of *D. octaedra* survived the 7 mg kg^{-1} treatment. Both the number of cocoons produced and their survival were less at 7 mg kg^{-1} than in the other treatment groups, but these differences could not be assessed for statistical significance because there was only one replicate with a surviving *D. octaedra* pair in the 7 mg kg^{-1} treatment.

3.4 Leaf decomposition

Leaf decomposition by *D. octaedra* feeding and microbial activity combined was significantly different among treatments (ANOVA $P < 0.001$) (Table 4). Mass loss of leaf material was significantly reduced at 7 mg kg^{-1} and above (Dunnett's $P < 0.05$), but was not significantly different from controls (Dunnett's $P > 0.05$) at the 3 mg kg^{-1} treatment. There were no significant differences among treatments in leaf decomposition by microbial activity alone (ANOVA $P = 0.137$), even at test concentrations of up to 1400 mg kg^{-1} (Table 5).

Table 3. Cocoons produced by earthworm pairs (*Dendrobaena octaedra*) and percentage cocoon survival by the end of the 35 day experimental period. The number of cocoons in the 7 mg kg^{-1} treatment is the total count in one microcosm. Data from the other replicates were not analyzed, as one or both of the worms were dead. There was no significant difference in the number of cocoons (ANOVA $P = 0.962$) or in the cocoon survival (ANOVA $P = 0.659$) between groups

Test concentration (mg kg^{-1})	Number of cocoons (±SEM)	Cocoon survival (%) (±SEM)
0	20.2 (±4.3)	58.9 (±8.5)
3	20.0 (±2.5)	63.9 (±6.0)
7	17	26.7

Table 4. Mass loss of leaf material by *Dendrobaena octaedra* feeding and microbial decomposition combined in microcosms

Test concentration (mg kg^{-1})	Mass loss (g) (±SEM)[a]
0	0.32 (±0.02)
3	0.26 (±0.02)
7	0.16 (±0.03)*
14	0.16 (±0.01)*
28	0.10 (±0.02)*
56	0.16 (±0.02)*

[a] Asterisk indicates treatments significantly different from control (Dunnett's $P < 0.05$).

Table 5. Mass loss of leaf material by microbial decomposition in microcosms. There were no significant differences among treatments (ANOVA $P = 0.137$)

Test concentration (mg kg^{-1})	Mass loss (mg) (±SEM)
0	52.9 (±3.1)
1.4	42.7 (±3.0)
14	59.1 (±3.3)
140	45.4 (±4.4)
1400	47.2 (±7.6)

4 DISCUSSION AND CONCLUSIONS

When imidacloprid is applied as a systemic insecticide to trees by soil drench or injection, it is likely to cause adverse effects on litter-dwelling earthworms if concentrations in the litter reach or exceed about 3 mg kg^{-1}. Species with sensitivities to imidacloprid similar to that of *Dendrobaena octaedra* would be expected to exhibit significant weight losses and some mortality at concentrations near 3 mg kg^{-1}. Weight losses among *D. octaedra* at this test concentration were apparently the result of a sublethal physiological effect rather than a result of feeding inhibition, because surviving *D. octaedra* in the 3 mg kg^{-1} group consumed leaf material at about the same rate as controls.

Imidacloprid concentrations of 3 mg kg^{-1} or greater in litter or upper soil layers are well within expected

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concentrations following ground applications around trees for control of wood-boring insect pests. Results from field trials with soil-injected imidacloprid (Merit Solupak) around ash trees for control of emerald ash borer indicated that soil concentrations at the points of injection reached maximums of about 200 mg kg^{-1}, with average concentrations within a 50 cm radius of 20–25 mg kg^{-1} at 0–15 cm deep, and average concentrations of about 12 mg kg^{-1} at 15–30 cm deep (Thompson DG, unpublished). The present study indicates that earthworms in or foraging through an area of about 50 cm radius and 30 cm deep around an injection point are likely to be exposed to toxic concentrations. However, the extent to which this risk could be mitigated by litter-dwelling earthworms detecting and avoiding the imidacloprid by moving out of injection areas is not known. Capowiez and Bérard[14] tested the avoidance behavior of two burrowing earthworm species and found no avoidance of imidacloprid, but they only tested at concentrations up to 1 mg kg^{-1}. Regardless, the overall risk of harmful effects on litter-dwelling earthworm populations in an area in which trees are systemically treated would be reduced by the limited spatial distribution of the imidacloprid. Under a typical soil injection scenario, several injection points (often 16–20 for a mature tree) would be evenly spaced within the drip lines of each tree canopy. Results of field trials indicated that imidacloprid concentrations beyond the 50 cm radii around the injection points averaged less than 0.02 mg kg^{-1} (Thompson DG, unpublished).

The present results demonstrated that the litter-dwelling earthworms *Eisenia fetida* and *Dendrobaena octaedra* exposed to imidacloprid in natural forest litter exhibited adverse effects at concentrations similar to or slightly higher than effective concentrations reported in previous studies. Most previous imidacloprid toxicity studies have focused on soil-dwelling or deep-burrowing earthworms in agricultural or horticultural contexts. Mostert *et al.*[18,19] reported an LC_{50} for *Pheretima* spp. tested in laboratory soils of 3 mg kg^{-1} after a 7 day exposure. Capowiez *et al.*[16] reported LC_{50} values of 2–4 mg kg^{-1} for soil-dwelling (*Allolobophora icterica* Savigny) and deep-burrowing (*Aporrectodea nocturna* Evans) earthworms exposed in field-collected agricultural soils for 14 days. They also reported significant weight losses of these test species at concentrations as low as 0.5 mg kg^{-1}. In a further study, these species also exhibited reduced burrowing efficiency in soils at imidacloprid concentrations of 0.5 mg kg^{-1}.[15] Previous toxicity tests with the litter-dwelling *E. fetida* in 14 day soil exposures indicated LC_{50} values of about 2 mg kg^{-1}.[13,20] In the present study, *D. octaedra* was the most sensitive of the two litter-dwelling species, with an LC_{50} of 5.7 mg kg^{-1}, an LC_{10} of about 2 mg kg^{-1} and significant weight losses among survivors at 3 mg kg^{-1}.

Leaf litter decomposition by microbial activity is unlikely to be affected by soil applications of imidacloprid for control of wood-boring insects. There were no indications of adverse effects on microbial decomposition of leaf material at test concentrations up to 1400 mg kg^{-1}. While the authors could not find previously published data on the toxicity of imidacloprid to microbial communities in forest litter, the present results concur with studies that reported little or no adverse effects on microbial activity in soils at realistic concentrations.[12,17]

Given that earthworm-mediated decomposition of leaf litter on forest floors is a critical ecosystem process for litter turnover, nutrient cycling and the development of nutrient-rich surface soil horizons,[9,22] lethal or sublethal effects of imidacloprid on litter-dwelling earthworms could have significant but localized impacts. The present results indicate that soil applications of imidacloprid to control wood-boring insect pests in trees could have significant adverse effects on earthworm growth and survival at realistic concentrations. This risk to litter-dwelling earthworms may be mitigated by using stem injections[23] rather than soil injections or drenches for applying systemic insecticides to trees. Studies to determine effects of imidacloprid-contaminated leaves from stem-injected trees on earthworm growth and survival would be useful to determine the extent to which this risk to earthworms can be reduced.

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REFERENCES

1 Cappaert D, McCullough DG, Poland TM and Siegert NW, Emerald ash borer in North America: a research and regulatory challenge. *Am Entomol* **51**:152–165 (2005).

2 Poland TM and McCullough DG, Emerald ash borer: invasion of the urban forest and the threat to North America's ash resource. *J For* **104**:118–124 (2006).

3 Poland TM, Haack RA, Petrice TR, Miller DH and Bauer LS, Laboratory evaluation of the toxicity of systemic insecticides for control of *Anoplophora glabripennis* and *Plectrodera scalator* (Coleoptera: Cerambycidae). *J Econ Entomol* **99**:85–93 (2006).

4 Elbert A, Becker B, Hartwig J and Erdelen C, Imidacloprid – a new systemic insecticide. *Pflanzenschutz-Nachrichten Bayer* **44**:113–136 (1991).

5 McCullough DG, Smitley DR and Poland TM, *Evaluation of Insecticides to Control Emerald Ash Borer Adults and Larvae.* [Online]. Available: http://www.emeraldashborer.info/files/Summary2004InsecticideTrialsFinal.pdf [11 April 2007].

6 Berg B, Ekbohm G, Johansson MB, McClaugherty C, Rutigliano F, DeSanto AV, *et al.*, Maximum decomposition limits of forest litter types: a synthesis. *Can J Bot* **74**:659–672 (1996).

7 Dilly O and Munch JC, Microbial biomass content, basal respiration and enzyme activities during the course of decomposition of leaf litter in a black alder (*Alnus glutinosa* (L.) Gaertn.) forest. *Soil Biol Biochem* **28**:1073–1081 (1996).

8 Förster B, Eder M, Morgan E and Knacker T, A microcosm study of the effects of chemical stress, earthworms and

microorganisms and their interactions upon litter decomposition. *Euro J Soil Biol* **32**:25–33 (1996).

9 Knollengberg WG, Merritt RW and Lawson DL, Consumption of leaf litter by *Lumbricus terrestris* (Oligochaeta) on a Michigan woodland floodplain. *Amer Midl Nat* **113**:1–6 (1985).

10 Haimi J and Einbork M, Effects of endogeic earthworms on soil processes and plant growth in coniferous forest soil. *Biol Fertil Soils* **13**:6–10 (1992).

11 Addison JA, Safety testing of tebufenozide, a new molt-inducing insecticide, for effects on nontarget forest soil invertebrates. *Ecotoxicol Environ Saf* **33**:55–61 (1995).

12 Tu CM, Effect of five insecticides on microbial and enzymatic activities in sandy soil. *J Environ Sci Health* **B30**:289–306 (1995).

13 Luo Y, Zang Y, Zhong Y and Kong Z, Toxicological study of two novel pesticides on earthworm *Eisenia fetida*. *Chemosphere* **39**:2347–2356 (1999).

14 Capowiez Y and Bérard A, Assessment of the effects of imidacloprid on the behavior of two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*) using 2D terraria. *Ecotoxicol Environ Saf* **64**:198–206 (2006).

15 Capowiez Y, Rault M, Mazzia C and Belzunces L, Earthworm behavior as a bio-marker – a case study using imidacloprid. *Pedobiologia* **47**:542–547 (2002).

16 Capowiez Y, Rault M, Costagliola G and Mazzia C, Lethal and sublethal effects of imidacloprid on two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*). *Biol Fertil Soils* **41**:135–143 (2005).

17 Ingram CW, Coyne MS and Williams DW, Effects of commercial diazinon and imidacloprid on microbial urease activity in soil and sod. *J Environ Qual* **34**:1573–1580 (2005).

18 Mostert MA, Schoeman AS and van der Merwe M, The toxicity of five insecticides to earthworms of the *Pheretima* group, using an artificial soil test. *Pest Manag Sci* **56**:1093–1097 (2000).

19 Mostert MA, Schoeman AS and van der Merwe M, The relative toxicity of insecticides to earthworms of the *Pheretima* group (Oligochaeta). *Pest Manag Sci* **58**:446–450 (2002).

20 Zang Y, Zhong Y, Luo Y and Kong Z, Genotoxicity of two novel pesticides for the earthworm *Eisenia fetida*. *Environ Poll* **108**:271–278 (2000).

21 Addison JA and Holmes SB, Comparison of forest soil microcosm and acute toxicity studies for determining effects of fenitrothion on earthworms. *Ecotoxicol Environ Saf* **30**:127–133 (1995).

22 Boettcher SE and Kalisz PJ, Single-tree influence on earthworms in forest soils in eastern Kentucky. *Soil Sci Soc Amer J* **55**:862–865 (1991).

23 Wanner KW, Helson BV and Harris BJ, Laboratory evaluation of two novel strategies to control first-instar gypsy moth larvae with spinosad applied to tree trunks. *Pest Manag Sci* **58**:817–824 (2002).

Pest Manag Sci **64**:112–118 (2008)
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