Are Leaves that Fall from Imidacloprid-Treated Maple Trees to Control Asian Longhorned Beetles Toxic to Non-target Decomposer Organisms?

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The systemic insecticide imidacloprid may be applied to deciduous trees for control of the Asian longhorned beetle, an invasive wood-boring insect. Senescent leaves falling from systemically treated trees contain imidacloprid concentrations that could pose a risk to natural decomposer organisms. We examined the effects of foliar imidacloprid concentrations on decomposer organisms by adding leaves from imidacloprid-treated sugar maple trees to aquatic and terrestrial microcosms under controlled laboratory conditions. Imidacloprid in maple leaves at realistic field concentrations (3–11 mg kg^{-1}) did not affect survival of aquatic leaf-shredding insects or litter-dwelling earthworms. However, adverse sublethal effects at these concentrations were detected. Feeding rates by aquatic insects and earthworms were reduced, leaf decomposition (mass loss) was decreased, measurable weight losses occurred among earthworms, and aquatic and terrestrial microbial decomposition activity was significantly inhibited. Results of this study suggest that sugar maple trees systemically treated with imidacloprid to control Asian longhorned beetles may yield senescent leaves with residue levels sufficient to reduce natural decomposition processes in aquatic and terrestrial environments through adverse effects on non-target decomposer organisms.

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The Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky) is a significant invasive forest insect pest in deciduous trees of eastern North America. Early instar larvae of the beetle destroy cambial tissue of trees by feeding under the bark, and later instar larvae cause further damage by tunneling and feeding in sapwood and hardwood (Cavey et al., 1998). Owing to its mobility in transported wood and wood products and its propensity to infest and kill healthy trees of several hardwood species, the Asian longhorned beetle has the potential to cause significant economic and ecological damage in urban, recreational, and forested landscapes of eastern North America (Nowak et al., 2001; Haack, 2006).

Wood-boring insects like the Asian longhorned beetle are difficult to control by topical and foliar insecticide applications because the most damaging life stages are the phloem-feeding larvae burrowing under the bark. However, the systemic insecticide, imidacloprid (1-(6-chloro-3-pyridinylmethyl)-N-nitroimidaolidin-2-ylideneamine), a chloro-neonicotinyl insecticide that blocks the nicotinergic neuronal pathway in insects (Elbert et al., 1991), is registered for controlling several wood and cambial boring insects and has been shown to be effective against the Asian longhorned beetle (Poland et al., 2006).

Imidacloprid can be applied as a systemic insecticide to trees by direct stem injections or by soil injections and drenches. Stem-injected systemic insecticides in general will pose less risk of environmental exposure and effects than broad-scale foliar or soil-application insecticides because the stem injections restrict the insecticide application to the tree being protected. Systemic applications by stem injection to individual trees are not feasible for all forest pest management situations, but they may be well suited for control of exotic invasive 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 or where infestations threaten environmentally sensitive areas where broad-scale pesticide applications or tree-removal approaches are not acceptable.

Foliar concentrations of systemic insecticides can enter the natural environment during autumn leaf fall. Senescent leaves

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Abbreviations: dbh, diameter at breast height; HPLC, high-performance liquid chromatography;

that fall from imidacloprid-treated trees on to the forest floor or into nearby water bodies expose leaf-decomposing organisms to imidacloprid concentrations. Given that microbial- and invertebrate-mediated breakdown of leaf litter is a critical ecosystem process in terrestrial (Dilly and Munch, 1996; Förster et al., 1996; Adl, 2003) and aquatic (Petersen and Cummins, 1974; Richardson, 1992; Suberkropp, 1998; Abelho, 2001) environments, disruptions to these processes by the toxic effects of imidacloprid could have impacts on organic matter processing and nutrient cycling in these environments.

We determined the effects of foliar imidacloprid concentrations on decomposer organisms by adding leaves taken from imidacloprid-treated sugar maple (*Acer saccharum* Marsh.) trees that were stem-injected with imidacloprid to aquatic and terrestrial microcosms under controlled laboratory conditions. We measured effects on aquatic leaf-shredding insect survival and feeding rates; on litter-dwelling earthworm survival, feeding rates, growth rates and cocoon production; and on aquatic and terrestrial microbial decomposition activity.

Materials and Methods

Test Materials

Leaves collected at senescence from imidacloprid-treated sugar maple trees and from non-treated sugar maple trees (to serve as controls) were added as test materials to aquatic and terrestrial microcosms. The leaves were collected from the trees just before leaf fall in October 2006 as representative of leaves entering water bodies or dropping to the forest floor in autumn. One set of leaves was obtained from three sugar maple trees (approximately 15 cm diameter at breast height [dbh]) treated in June 2006 at an operational rate to control Asian longhorned beetles by stem-injections of imidacloprid at 25 g m^{-1} dbh. The leaves were pooled from the three trees and mixed thoroughly before being added to the microcosms. This treatment group was taken from operationally treated trees in open growing conditions (maximizing translocation uptake) and was therefore considered to represent the upper end of expected field concentrations and is referred to as "high-field" throughout.

A second set of leaves was collected from two sugar maple trees (approximately 6 cm dbh) that were stem-injected with imidacloprid at the same operational rate, but in the fall (about 2 wk before the leaves were collected) to minimize the uptake and to provide concentrations in leaves at the lower end of expected field concentrations. The leaves were pooled from the two trees and mixed thoroughly. This treatment group is referred to as "low-field" throughout. Leaves were also collected from two intentionally overdosed sugar maple trees (approximately 6 cm dbh) to provide leaves with high concentrations to which responses at realistic concentrations could be compared. This treatment is referred to as "overdose" throughout. Leaves were also collected from three sugar maple trees (approximately 15 cm dbh) that were located adjacent to the low-dose and overdose trees and were not treated with imidacloprid to serve as leaf material in control microcosms.

The leaves for all treatment groups were collected within a few days of each other (early October 2006), sealed in Ziploc plastic bags, and held in the dark in cold storage (2 ± 2°C) until initiation of the microcosm experiments. The maximum storage time before use in the microcosms was 41 d. We previously determined that imidacloprid is stable in leaf litter for up to 6 mo at these storage conditions (unpublished data).

Imidacloprid Concentrations

Imidacloprid concentrations in leaves of each treatment group were measured in composite batches of 4 to 18 leaves drawn from the treatment groups about 1 wk before and again at several hours before being added to the microcosms and in some cases from further batches drawn from the treatment leaves in storage (to verify initial foliar concentrations). The average of these measurements was used as the test concentration for each treatment group.

Imidacloprid concentrations were quantified by high-performance liquid chromatography (HPLC) with photo-diode array detection. High-performance liquid chromatography calibration standards were prepared from imidacloprid technical (99.5%) (Lot #30714; Crescent Chemical, Islandia, NY). All samples were analyzed using an Agilent 1100 HPLC (Agilent Technologies Canada Inc., Mississauga, Ontario, Canada) equipped with photo-diode array detector and autosampler. Imidacloprid was extracted from leaf material by accelerated solvent extraction techniques involving methylene chloride at high temperature and pressure, and extracts were cleaned up on Florisil columns before HPLC analysis. Concurrent quality control analyses using blank leaf samples fortified with known amounts of imidacloprid were run to determine recovery efficiencies and precision of the methods. The average recovery efficiency (and coefficient of variation) for foliar (n = 16) quality control samples was 88.6 (9.27%). All residue data were corrected for analytical recovery losses and reported on a fresh weight basis. The analytical limit of quantification for imidacloprid in foliage was calculated as 0.033 mg kg^{-1} fresh weight.

Aquatic Microcosms

Aquatic microcosms consisted of glass aquariums (13 cm wide, 30 cm long, and 21 cm high) fitted with a Plexiglas lid in which two 2.5-cm diameter holes were drilled. One hole in the lid was left open to allow some air exchange, and the other was fitted with a rubber stopper through which an air hose with an air stone was placed to supply continuous air to each microcosm and to provide gentle water circulation. The rate of aeration was not measured but was set and monitored to ensure continuous and similar air flow to each microcosm. The microcosms were placed in a single row on a shelf in an experimental room with controlled temperature, humidity, and light (daylight simulation fluorescent bulbs, 12/12 h light/dark cycle). Each microcosm contained 6 L of stream water (collected from a forest stream at a single time and stored at 2°C for 5 d), 300 mL of stream detritus (organic material collected from a forest stream, sieved to 1- to 5-mm particle sizes, frozen for several weeks to kill sediment organisms, then thawed for 5 d before being added to the microcosms), and 10 twigs from speckled alder (*Alnus incana* ssp. *rugosa* (Du Roi) Clausen) trees (approximately 10 mm diameter and 15 cm long) to provide natural cover and sites of attachment for the test insects. There were five replicate microcosms assigned randomly to each treatment group.

Stonefly (*Pteronarcys dorsata* Say) nymphs and crane fly (*Tipula* sp. L.) larvae were selected as representative leaf-shredding insects. These were collected from a local stream, held in the laboratory at ambient conditions for 72 to 96 h, and then impartially allocated among microcosms 48 h before the microcosms were treated. The microcosms were assembled and operated for 5 d before the addition of insects, and nine specimens of each of the two taxa were added to each microcosm 2 d before the addition of maple leaves. Body sizes varied among specimens, but similar body sizes (indicating similar larval instars) were evenly distributed among all microcosms such that each microcosm received equal numbers of individuals in similar body size groups. At the end of the 14-d experimental period (from when the maple leaves were added to the end of the observation period), the bottom substrates were removed and searched for all insects, and the numbers of dead and living individuals were recorded. Mortality was defined as no movement and no response to prodding.

Nine sugar maple leaves from each treatment group were weighted with plastic paper clips (three groups of three) and placed in the corresponding microcosms. The leaves were taken from cold storage, air-dried for 2 h to stabilize fresh weights, and batch-weighed to provide initial fresh weights. Initial dry weights were estimated from a regression of dry weights on fresh weights (linear regression, $p < 0.001$; $r^2 = 0.91$) of 50 maple leaves from the same trees. These were individually weighed, leached in running water for 24 h, dried at 60°C for 48 h, and re-weighed to determine initial dry weights and to account for leaching losses. The leaves added to the microcosms were not initially dried and weighed to directly measure initial dry weights because the microcosm experiments were intended to simulate natural leaf-fall (fresh leaves added to microcosms) and to avoid the possibility that drying the leaves could affect the stability of the imidacloprid and the palatability to test organisms. At the end of the experimental period, remaining leaf material was removed, dried at 60°C for 48 h, and weighed. Decomposition (mass loss) of leaf material from combined insect feeding and microbial activity was determined as the difference between the estimated, initial batch dry weight of the nine leaves added to the microcosms and the dry weight of leaf material remaining at the end of the experiment.

Microbial decomposition was determined by mass loss of leaf material in fine mesh bags to exclude the leaf-shredding insects. Twenty leaf disks, cut with a 23-mm diameter cork borer from the same group of maple leaves, were batch-weighed to determine fresh weights, put in 1-mm mesh bags, weighted with plastic paper clips, and placed on the bottom of the microcosms to measure microbial decomposition activity on leaf material. The leaf disks were retrieved on Day 14, removed from the mesh bags, gently washed to remove the biofilm while being careful not to damage the leaf material, dried at 60°C for 48 h, and weighed. Mass loss was determined as the difference between the estimated, initial dry batch weights of the leaf disks (using estimates from the same regression as for whole leaves) and the remaining batch weights after drying at 60°C for 48 h.

Terrestrial Microcosms

The terrestrial microcosm design and the handling and measurement endpoints were adapted from previous microcosm experiments to assess forest pesticide effects on earthworms (Addison and Holmes, 1995). 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 with a metal lid containing four 3-mm diameter holes for air circulation. Each microcosm contained 60 g of field-collected litter from a sugar maple forest, with the litter held at or corrected to ambient moisture by the addition of deionized water just before being placed in the microcosms. The 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 then thawed and held in open containers for 1 wk before being added to the microcosms. When the litter was thawed, it was kept at constant weight (ambient moisture) by adding deionized water when necessary. The litter was thoroughly mixed before being distributed among terrestrial microcosms. The microcosms were placed on a shelf in the same experimental room and under the same conditions as the aquatic microcosms. During the terrestrial microcosm experiments, ambient moisture in the litter was maintained by addition of deionized water about every 2 d to bring microcosms to original weights. There were five replicate terrestrial microcosms randomly assigned to each treatment group.

Litter-dwelling earthworms, *Dendrobaena octaedra* (Sav.), were field-collected from the litter of a sugar maple forest approximately 60 km north of Sault Ste. Marie, Ontario, Canada. The worms were held in the experimental laboratory for at least 4 wk before 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-colored band present indicating sexual maturity) worms were impartially allocated to each microcosm on the day that the maple leaves were added to the relevant microcosms. Just before placement in the microcosms, each pair of worms was lightly rinsed with water to remove litter particles and weighed to determine initial pair-weights. It was not possible to track individual weights because individuals could not be differentiated.

At weekly intervals after the maple leaves were added, the contents of each microcosm were emptied into a porcelain tray and searched for earthworms. Worms not moving and not responding to gentle prodding were considered dead and were removed and discarded. Worms missing 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 returned to the microcosms. Weight measurements were discontinued when one or both of the worms in a microcosm were dead. Reproduction was determined by counting and removing 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 the addition of deionized water, and held in the experimental lab for 6 wk beyond the end of the experimental period. The number of juveniles hatched from the cocoons was recorded weekly and summed at the end to measure the percent of cocoons hatched (assuming one juvenile per cocoon).

The decomposition of leaf material by earthworm feeding and microbial activity was measured as the 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-d experimental period. Four leaf pieces (two maple leaves cut in half) from each treatment group were placed in each microcosm at about 1 cm below the surface of the litter to prevent the leaf material from drying out. 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 following the procedures outlined previously for aquatic microcosms, except that the individually weighed leaves were not pre-leached in running water. The regression for this batch of leaves was significant and highly predictive ($p < 0.001$; $r^2 = 0.97$).

Microbial decomposition of leaf material was measured separately by mass loss of leaf disks in 1-mm mesh bags to exclude the earthworms. 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, and buried at about mid-depth in the litter of each microcosm. At the end of the experimental period, the leaf disks were removed, washed gently to remove litter particles, dried at 60°C for 48 h, and re-weighed 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.

For the terrestrial microcosm experiments, the overdose treatment group was tested separately from the low-field and high-field treatment groups (about 6 wk prior) because the overdose group was tested as part of a pilot experiment with high-concentration leaves. Accordingly, results from the overdose treatments were compared with controls for that experiment (referred to below as Control 1) and from the low-field and high-field groups were compared with controls for the second experiment (Control 2).

Experimental Conditions

Over the course of the experimental periods, microcosms were held at air temperatures of 19.1 to 20.5°C, light levels of about 30 μmol m^{-1} s^{-1} from daylight simulation fluorescent bulbs, and relative humidity of 35 to 68%. In aquatic microcosms, water temperatures ranged from 18.8 to 19.9°C, dissolved oxygen was held at or near saturation ranging from 7.7 to 9.0 mg L^{-1}, pH was 6.2 to 6.6, and conductivity ranged from 51 to 87 μS cm^{-1}.

Data Analyses

All response measurements taken from microcosms containing imidacloprid-contaminated leaves from each treatment group (three treatments, five replicates per treatment) were compared with those from five replicate control microcosms. Differences among treatments for response measurements taken at a single time were tested by one-way ANOVA. When significant differences among groups were detected ($p < 0.05$), each treatment was compared with the controls by a Dunnett's test with significance at $p < 0.05$. Response measurements taken over time were analyzed by repeated-measures ANOVA with the among-treatments and the treatment × time interaction as the tests of interest. A significant interaction ($p < 0.05$) 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. We avoided testing for differences among all treatments at each sampling time to reduce the effects of multiple comparisons on the overall error rate. Percent data were arcsine/square-root transformed before analysis. Data sets were tested for normality and homogeneity of variances before analysis, and when significant ($p < 0.01$), the data were log transformed. All statistical analyses were conducted with SigmaStat 3.5 (Systat, 2006).

Results

Imidacloprid Concentrations in Leaves

Imidacloprid concentrations in batches of leaves taken from the low-field treatment group ($n = 7$ batches) ranged from 1.4 to 5.4 mg kg^{-1} with an average (±SE) of 3.2 (0.6) mg kg^{-1} fresh weight. Concentrations in batches analyzed from the high-field group ($n = 4$) ranged from 6.4 to 18.5 mg kg^{-1} with an average of 11.0 (2.7) mg kg^{-1}. Concentrations from the overdose treatment used in aquatic microcosms were analyzed in two batches and were 46.0 and 31.2 mg kg^{-1} with an average of 38.6 (7.4) mg kg^{-1}. The overdose treatment used in terrestrial microcosms was analyzed in four batches and concentrations ranged from 86.6 to 188.0 mg kg^{-1}, with an average of 132.0 (18.2) mg kg^{-1}. These average concentrations from the batches of leaves from each treatment group were used as test concentrations throughout the remainder of this paper.

Effects on Aquatic Decomposers

No significant mortality was detected among leaf-shredding insects exposed to maple leaves containing imidacloprid at realistic concentrations (3.2 and 11.0 mg kg^{-1}; Dunnett's test, $p > 0.05$), although mortality of the stonefly *Pteronarcys* tended to be slightly higher in microcosms with imidacloprid-contaminated leaves than in controls (Table 1). There was up to about 30% mortality among *Tipula* at the realistic concentrations, but there was similar mortality in controls, indicating some inherent stress among the field-collected tipulids (although no unusual behavior or symptoms were observed) and precluding evidence of a treatment effect. At the overdose concentration (38.6 mg kg^{-1}), there was over 80% mortality among specimens of both test species (Dunnett's test, $p < 0.05$).

Although no significant mortality of leaf-shredding insects was detected at realistic concentrations, leaf decomposition was significantly reduced. Total mass loss of leaf material from insect feeding

Table 1. Mean (±SE) % insect mortality over a 14 d experimental period in aquatic microcosms. Test concentrations are average imidacloprid concentrations in sugar maple leaves added to aquatic microcosms.

Treatment	Test concentration	*Pteronarcys dorsata*	*Tipula* sp.
	mg kg^{-1}		
Control	0	4.4 (2.7)	33.3 (6.1)
Low-field	3.2	11.1 (3.5)	22.2 (4.5)
High-field	11.0	8.8 (4.1)	31.1 (10.8)
	38.6	83.3 (11.8)*	83.3 (5.6)*

* Significant difference from control (Dunnett's $p < 0.05$) after a test for significant differences overall (ANOVA, $p < 0.05$).

and microbial decomposition combined was significantly less than in controls when imidacloprid concentrations were 11 mg kg^{-1} and higher in maple leaves (Dunnett's test, $p < 0.05$) (Fig. 1A). Mass loss of leaf material at the overdose treatment seemed to be mainly by microbial decomposition because there was no visual evidence of leaf consumption by the insects (no holes or shredded edges of leaves). Leaf decomposition at the low-field concentration also tended to be lower than in controls, but a significant difference could not be detected (Dunnett's test, $p > 0.05$).

Inhibition of aquatic microbial decomposition activity by imidacloprid was responsible for at least some of the significant reductions in leaf mass loss at the high-field concentration and the overdose. Mass loss of leaf material from microbial activity in fine-mesh bags was significantly lower at the high-field and overdose concentrations than in controls (Dunnett's test, $p < 0.05$) and tended to be lower than in controls at the low-field concentration (Fig. 1B).

Effects on Terrestrial Decomposers

There was no mortality among litter-dwelling earthworms exposed to imidacloprid-contaminated leaves in terrestrial microcosms, even at the overdose concentration of about 132 mg kg^{-1}, and there was no mortality in control microcosms. However, the exposure to imidacloprid-contaminated leaves had adverse sublethal effects on earthworms. Significant weight losses occurred among earthworms exposed to leaves at the overdose concentration, whereas earthworms in the controls gained weight over the same period (Fig. 2). Differences between the overdose group and control 1 were apparent by Day 14 and significant by Day 28 (Holm-Sidak test, $p < 0.05$). A similar but more subtle trend of weight loss was observed among earthworms exposed to leaves at realistic concentrations, although significant differences from controls could not be detected by repeated-measures ANOVA (treatment × time interaction, $p = 0.086$). Weight losses among earthworms at realistic concentrations did not result in pair weights that were significantly different from controls, but, in comparison to the weight gain in control earthworms of the same experiment (control 2), it seems that imidacloprid in leaves at realistic field concentrations may have adversely affected earthworm growth, especially by Day 35 (Fig. 2).

Further evidence of adverse sublethal effects on earthworms was demonstrated by reductions in mass loss of leaf material at all test concentrations. The decomposition of leaf material by earthworm feeding and microbial activity (imidacloprid-contaminated leaf material buried in the litter) was significantly lower at realistic

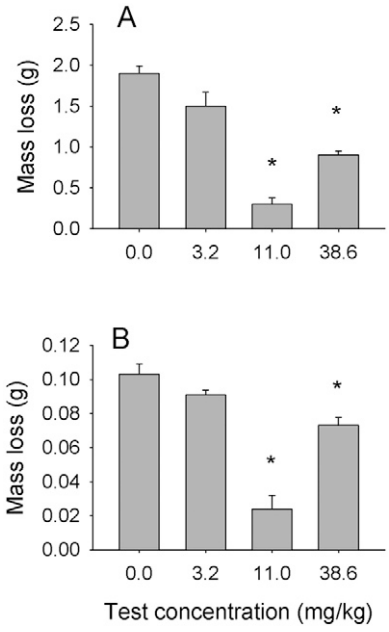

Fig. 1. Mean (±1 SE) mass loss of leaf material by insect feeding activity and microbial decomposition combined (A) and by microbial decomposition alone (B) in aquatic microcosms. Test concentrations are average imidacloprid concentrations in sugar maple leaves added to aquatic microcosms. *Significant difference from control (Dunnett's $p < 0.05$) after a test for significant differences overall (ANOVA, $p < 0.05$).

field concentrations and at the overdose concentration than in controls (Fig. 3A; Dunnett's test, $p < 0.05$). This seems to have been largely the result of feeding inhibition effects on earthworms because reductions in leaf decomposition by microbial activity alone in fine mesh bags were observed only at the high-field and overdose concentrations, and microbial decomposition was increased at the low-field concentration (Fig. 3B). We observed (but did not quantify) that leaves from control terrestrial microcosms were much more fragmented by earthworm feeding

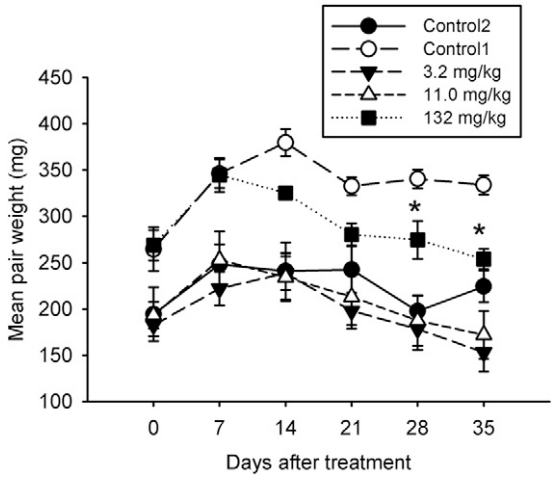

Fig. 2. Mean (±1 SE) pair weights of litter-dwelling earthworms in terrestrial microcosms. *Significant difference from control (Holm-Sidak $p < 0.05$) after a significant time × treatment interaction in repeated-measures ANOVA ($p < 0.05$). Comparisons were made between control 1 and the overdose and between control 2 and the low- and high-field test concentrations.

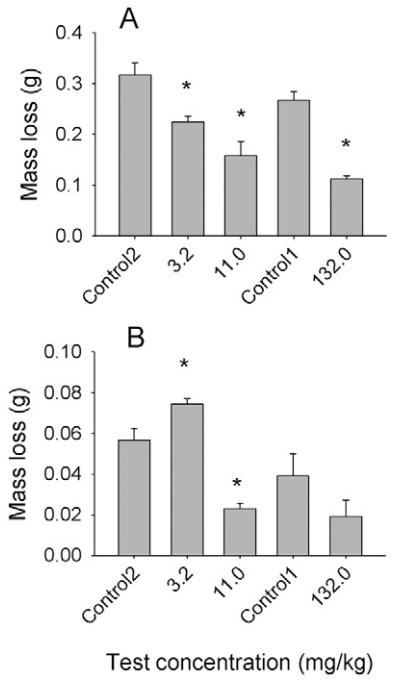

Fig. 3. Mean (±1 SE) mass loss of leaf material by earthworm feeding activity and microbial decomposition combined (A) and by microbial decomposition alone (B) in terrestrial microcosms. Test concentrations are average imidacloprid concentrations in sugar maple leaves added to terrestrial microcosms. *Significant difference from control (Dunnett's $p < 0.05$) following a test for significant differences overall (ANOVA, $p < 0.05$). Comparisons were made between control 1 and the overdose and between control 2 and the low- and high-field test concentrations.

activity than imidacloprid-contaminated leaves from any of the other treatment groups.

Although exposure to imidacloprid-contaminated leaves at realistic field concentrations seemed to cause feeding inhibition and weight losses among earthworms, it did not adversely affect cocoon production or survival. There were no significant differences among treatments in the number of cocoons produced (ANOVA, $p = 0.1834$) or in the percentage of cocoons hatched (ANOVA, $p = 0.5726$) (Table 2).

Discussion

There was considerable variation in imidacloprid concentrations among batches of leaves taken from each treatment group. This concurs with measurements from ongoing field trials in which substantial variation occurs in senescent sugar maple leaves

Table 2. Mean (±SE) number of cocoons produced by earthworms in terrestrial microcosms by the end of a 35-d experimental period and percent cocoons hatched after a further 42 d. Test concentrations are average imidacloprid concentrations in sugar maple leaves added to terrestrial microcosms.

Treatment	Test concentration	No. of cocoons	Percent hatched
	mg kg^{-1}		
Control 1	0	20.2 (4.3)	58.9 (8.5)
Control 2	0	25.4 (3.2)	58.8 (6.2)
Low-field	3.2	31.4 (2.1)	62.2 (6.5)
High-field	11.0	23.8 (3.6)	71.6 (6.4)
Overdose	132	23.2 (2.1)	66.7 (4.4)

depending on several factors, including growing conditions and formulation applied. Data from our ongoing field studies indicate average concentrations of 8.1 mg kg^{-1} with an upper 95% confidence limit of 17.0 mg kg^{-1} in senescent foliage from sugar maples trees ($n = 17$) after systemic applications of imidacloprid at rates to control Asian longhorned beetles (D. Thompson, unpublished data). Variability in imidacloprid concentrations among leaves added to the microcosms undoubtedly increased the variability among response endpoints and may have reduced the ability to detect significant differences among treatments in some instances. Nevertheless, the average concentrations in our low-field and high-field treatment groups (3.2 and 11.0 mg kg^{-1}) seems to have captured a realistic range of concentrations expected among sugar maple leaves from trees treated with imidacloprid for control of the Asian longhorned beetle.

This range of foliar concentrations in our study did not cause direct mortality of leaf-shredding aquatic insects. However, sugar maple leaves with imidacloprid concentrations of 11 mg kg^{-1} and higher significantly reduced natural decomposition processes in aquatic microcosms through adverse effects on leaf-shredding insect feeding rates and on aquatic microbial decomposition activity. The ecological significance of this in natural systems would largely depend on the mode of action and the availability of alternate food sources. If the feeding inhibition is a repellent effect and if there are alternate sources of leaf material available, detritivorous insects are likely to search for and preferentially feed on noncontaminated leaf material. If, on the other hand, the feeding inhibition resulted from sublethal toxic effects after consumption of contaminated leaf material, the ensuing lethargy or altered behavior of these insects could reduce their predator avoidance, functional activity, and ultimately survival in natural systems. Given that invertebrate- and microbial-mediated breakdown of leaf litter is a critical ecological process in aquatic systems, reduced decomposition processes could have significant adverse implications for nutrient cycling in streams or ponds where riparian maple trees have been treated with imidacloprid.

Although leaf decomposition in aquatic microcosms was significantly reduced at 11.0 and 38.6 mg kg^{-1} test concentrations, the response was inversely related to the imidacloprid concentrations. It is not clear why the inhibition of leaf decomposition was greater at 11.0 mg kg^{-1} than at 38.6 mg kg^{-1}, but it may have been related to differences in leaf litter quality and palatability to decomposers. Leaves at the 38.6 mg kg^{-1} test concentration were taken from slightly larger, more mature maple trees, and these may have been more palatable to the decomposer organisms. The inverse relationship to imidacloprid concentration also suggests that a threshold for decomposition inhibition may have been reached at about 11 mg kg^{-1} such that increasing imidacloprid concentrations had no further adverse effects on feeding or microbial decomposition. Regardless, imidacloprid, at realistic concentrations in sugar maple leaves, can inhibit leaf litter decomposition in water bodies, especially when the concentrations in leaves are near 11 mg kg^{-1} or higher.

The results of our study indicate that leaves from imidacloprid-treated sugar maple trees that fall to the forest floor pose little risk of direct mortality or of adverse effects on cocoon production and

survival to litter-dwelling earthworms. However, imidacloprid in sugar maple leaves at realistic field concentrations did inhibit leaf litter decomposition through adverse effects on earthworm feeding rates and on terrestrial microbial decomposition activity when concentrations were at or above 3 mg kg^{-1}. These effects on leaf litter decomposition by earthworm feeding and microbial activity could have adverse implications for organic matter processing and nutrient cycling on the forest floor near imidacloprid-treated trees.

We could find no previous studies reporting the effects of imidacloprid on decomposer organisms through consumption of imidacloprid-contaminated leaves to which our results can be compared, other than our own. In our previous study, leaves from green ash (*Fraxinus pennsylvanica* Marsh.) trees treated at two field rates to control emerald ash borer (*Agrilus planipennis* Fairmare) contained imidacloprid concentrations of 0.8 to 1.3 mg kg^{-1} and did not significantly affect aquatic leaf-shredding insect survival or aquatic microbial decomposition rates. Aquatic insect feeding rates were significantly inhibited at foliar concentrations of 1.3 mg kg^{-1} but not at 0.8 mg kg^{-1} (Kreutzweiser et al., 2007).

Several studies have investigated the effects of imidacloprid on non-target organisms through direct exposure to concentrations in water, soil, and litter. Imidacloprid in water is highly toxic to some aquatic invertebrates, particularly insects, with lethal or behavioral effects at concentrations as low as 7 to 12 µg L^{-1} (ppb) (Overmyer et al., 2005; Kreutzweiser et al., 2007). In previous tests with earthworms exposed to imidacloprid in agricultural soils (Luo et al., 1999; Zang et al., 2000; Capowiez et al., 2002) and in forest litter (Kreutzweiser et al., 2008), lethal and sublethal effects were observed at concentrations as low as 0.5 to 3 mg kg^{-1}. Foliar concentrations of imidacloprid in maple leaves of our study were less toxic to non-target organisms than aqueous or soil concentrations, particularly to aquatic leaf-shredding insects. This implies that when imidacloprid is used as a systemic insecticide to control wood-boring insects in trees, it poses less risk of harm to non-target decomposers when applied as stem injections as compared with soil injections or soil drenches.

Under similar experimental conditions, we previously tested the effects of foliar concentrations of imidacloprid in ash leaves on aquatic and terrestrial microbial decomposition rates. When imidacloprid was in ash leaves, there were no adverse effects on microbial decomposition rates, even at unrealistically high test concentrations (up to 80 mg kg^{-1}) in aquatic (Kreutzweiser et al., 2007) and terrestrial (Kreutzweiser et al., unpublished data) microcosms. This contrasts with the results from the present study in which the microbial decomposition of imidacloprid-contaminated maple leaves was inhibited at realistic concentrations. The differences may have resulted from different microbial communities in the field-collected microcosm substrates, but this is unlikely given that the substrates were collected from the same locations (but at different times) and that the adverse effects were observed in aquatic and terrestrial microcosms. It is more likely that natural phytochemical compounds in sugar maple leaves interacted with imidacloprid to adversely affect microbial decomposition, whereas this effect was not observed in ash leaves.

Conclusions

Results from this study indicate that senescent leaves from imidacloprid-treated sugar maple trees at rates to control Asian longhorned beetles could reduce natural decomposition processes in aquatic and terrestrial environments through adverse sublethal effects on non-target decomposer organisms. The degree to which such effects might occur in the field depends on the frequency with which these organisms encounter contaminated leaves and the imidacloprid concentrations in those leaves. Nevertheless, the results indicate a risk of harm to natural decomposer organisms at realistic concentrations of imidacloprid, and this warrants investigations into alternatives to imidacloprid for control of wood-boring insect pests, such as Asian longhorned beetles. Although a potential risk of harm posed by imidacloprid to aquatic and terrestrial decomposer organisms has been demonstrated in this study, the results should be considered in the context that the risk of ecologically significant harm will be much greater if trees are allowed to become infested and die or are cut down and destroyed as a pest eradication strategy.

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