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Highlighted article

Non-target effects on aquatic decomposer organisms of imidacloprid as a systemic insecticide to control emerald ash borer in riparian trees

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Abstract

Imidacloprid is effective against emerald ash borer when applied as a systemic insecticide. Following stem or soil injections to trees in riparian areas, imidacloprid residues could be indirectly introduced to aquatic systems via leaf fall or leaching. Either route of exposure may affect non-target, aquatic decomposer organisms. Leaves from ash trees treated with imidacloprid at two field rates and an intentionally-high concentration were added to aquatic microcosms. Leaves from trees treated at the two field rates contained imidacloprid concentrations of 0.8–1.3 ppm, and did not significantly affect leaf-shredding insect survival, microbial respiration or microbial decomposition rates. Insect feeding rates were significantly inhibited at foliar concentrations of 1.3 ppm but not at 0.8 ppm. Leaves from intentionally high-dose trees contained concentrations of about 80 ppm, and resulted in 89–91% mortality of leaf-shredding insects, but no adverse effects on microbial respiration and decomposition rates. Imidacloprid applied directly to aquatic microcosms to simulate leaching from soils was at least 10 times more toxic to aquatic insects than the foliar concentrations, with high mortality at 0.13 ppm and significant feeding inhibition at 0.012 ppm.

Keywords: Imidacloprid; Aquatic invertebrates; Microbial; Decomposition; Microcosms; Emerald ash borer control

1. Introduction

The emerald ash borer (EAB) (*Agrilus planipennis)* is an invasive, exotic insect pest that has recently been responsible for widespread mortality of ash trees (*Fraxinus* spp.) in northeastern North American. The EAB has the potential to cause economic and ecological impacts on a scale similar to those of previous invasive pests on American chestnut (*Castanea dentata*) and white elm (*Ulmus americana*) (Liebhold et al., 1995; Cappaert et al., 2005; Poland and McCullough, 2006). Wood-boring insect pests like the EAB provide unique challenges to forest pest managers because the most damaging life stages of the insects are the phloem-feeding larvae which are difficult to target by conventional foliar applications of insecticides. In addition, conventional insect control methods are often not considered appropriate or publicly acceptable in many urban or recreational environments.

In response to the need for effective and safe pest control strategies to address these types of pest problems, there have been concerted efforts in developing and testing pesticides with reduced risk to human or environmental health (Thompson and Kreutzweiser, 2007). One alternative strategy to reduce environmental exposure and provide effective control of problematic insect pests such as wood-boring insects is the application of selective, systemic insecticides to trees through stem- or soil-injections (e.g., Helson et al., 2001, Wanner et al., 2002). Systemic insecticides are not applicable to all forest pest management situations but they may be well suited for exotic species within a restricted area before the species becomes widely distributed. They may also be well suited to smaller-scale situations in which an infestation occurs or threatens an environmentally sensitive area where broad-scale pesticide applications or tree-removal approaches are not

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acceptable. This would include, for example, riparian forests of municipal watersheds or agricultural irrigation streams, shoreline areas of "cottage country", public parks and other high-profile recreational areas, high-value stands, and conservation areas.

Imidacloprid (1-(6-chloro-3-pyridinylmethyl)-*N*-nitroimidaolidin-2-ylideneamine) is a systemic, chloro-neonicotinyl insecticide, that specifically blocks the nicotinergic neuronal pathway. This pathway is more abundant in insects than in warm-blooded animals accounting for its selective toxicity. The properties, efficacy, toxicology, and environmental profile of imidacloprid have been previously reviewed (Elbert et al., 1991; Felsot, 2001; Sheets, 2001). While imidacloprid is most widely used as an agricultural or horticultural insecticide, it has recently been demonstrated to be highly effective as a systemic insecticide or prophylactic treatment against EAB in ash trees of northern Michigan, USA (McCullough et al., 2003) and southern Ontario, Canada (Helson and Thompson, unpublished data). Stem injections of imidacloprid have also been used successfully to affect control over wood-boring Asian longhorned beetle (*Anoplophora glabripennis)* infestations near Chicago, IL, USA (Poland et al., 2006), and recent studies have indicated that it is effective against the brown spruce longhorned beetle (*Tetropium fuscum*) in eastern Canada as well (Thompson et al., unpublished data). In exploiting its systemic properties, imidacloprid may be applied by direct stem injection into tree trunks, or by soil drench or soil injections around the drip-lines of tree canopies.

When applied as stem injections to riparian trees, foliar residues of imidacloprid can enter water bodies when trees lose their leaves in autumn. When applied to soils, imidacloprid concentrations can leach to nearby water bodies. Field studies on the leaching potential of imidacloprid provide variable results with some studies reporting little or no movement below 30 cm depth (Rouchaud et al., 1996; Kalpana et al., 2002) while others indicate significant mobility in soil column (Gupta et al., 2002; Vollner and Klotz, 1997) and field (Felsot et al., 1998) studies. Imidacloprid is most susceptible to leaching in soils with low organic content, coarse texture, high rock or gravel component, and in those which are saturated or near-saturated or with significant macropore content (Felsot et al., 1998). The Canadian Pest Management Regulatory Agency considers imidacloprid to have high potential for surface water contamination, leaching to groundwater and persistence in soils (PMRA, 2001).

Imidacloprid concentrations in leaves from treated trees or from soil applications leaching to receiving waters may pose a risk of harm to non-target, aquatic decomposer organisms. The objective of our study was to determine if leaves that fall from riparian ash trees treated with imidacloprid to control EAB pose a risk of harm to natural decomposer organisms and processes in nearby waterbodies. We then compared that to the risk of harm posed by direct exposure to imidacloprid concentrations in water that might arise from ground applications and leaching to nearby aquatic systems.

2. Materials and methods

2.1. Experimental treatments and design

The fate and effects of imidacloprid were determined in aquatic microcosms in two separate experiments. In the first experiment, leaves from imidacloprid-treated ash trees were added to replicate microcosms to mimic an autumn leaf-fall scenario in which leaves from treated riparian trees would fall into nearby waterbodies. The second experiment was conducted immediately after and included untreated leaves as well as the direct application of imidacloprid to the microcosms to mimic a leaching scenario from soil applications. Here, and throughout, the term "leaves" refers to leaflets taken from the compound ash leaves. Details on handling of leaves and microcosm applications are given in Section 2.3.

Leaves for the first experiment were collected in late September, 2005 just before leaf-fall from young, potted green ash (*Fraxinus pennsylvanica*) trees (approximately 5 cm diameter) that had been systemically-treated with imidacloprid, or from non-treated, potted ash trees that served as controls. The ash trees were potted in 50-cm diameter degradable pots, planted in the ground in early May 2005, and treated with imidacloprid in late June 2005. Applications were made to the treated trees by soil application or stem-injection to provide concentrations in leaves that were similar to or above those from operational applications to green ash trees for the control of EAB (Thompson and Helson, unpublished data). The stem injections were made with an experimental formulation EcoPrid (Great Lakes Forestry Centre, emulsifiable concentration containing 50 mg/mL imidacloprid) and the soil applications were made with the formulated product, Merit Solupak® (Bayer Crop Sciences, wettable powder containing 750 mg/g imidacloprid). Stem injections were made by drilling a 6-mm hole in the trunk and injecting EcoPrid with a micropipette in the hole at a treatment rate of 0.06 g imidacloprid/cm diameter at breast height (dbh). The 0.06 g/cm dbh rate has been demonstrated in field trials to be effective for canopy protection from EAB defoliation (94–100% foliage protection in treated ash trees; Helson and Thompson, unpublished data). Soil applications were applied with a Kioritz® hand soil injector (Kioritz Corporation, Tokyo) that was used to pierce 20 1.6-cm diameter holes in the soil evenly distributed around the pot to a depth of approximately 10 cm, and to inject 20 mL Solupak per hole for a total dose rate of 0.56 g imidacloprid/cm dbh. The rate of 0.56 g/cm dbh is the maximum label rate for Merit Solupak. An intentionally-high soil application was made at 10 × that rate.

These applications to ash trees resulted in three treatment groups; a low-end field rate (stem injection, referred to throughout as low-field and representing concentrations near the low end of the range found in leaves at senescence from ash trees treated for EAB control), a high-end field rate (soil injection, referred to throughout as high-field and representing the high end of the range of field concentrations), and an intentionally over-dosed rate (soil injection at 10 × the label rate, referred to throughout as over-dose) to which responses at realistic concentrations could be compared. Responses to these three treatments were made in comparison to controls (containing leaves from non-treated ash trees).

Leaves for the second experiment (imidacloprid added directly to the microcosms) were taken from the same control trees used for the first experiment. Leaves in the second experiment were added to maintain similarity in microcosm composition between the first and second experiments, and to provide a substrate for aquatic invertebrate and microbial decomposers and an organic compartment for potential sorption of imidacloprid. The test material in the second experiment was the imidacloprid added directly. Nominal test concentrations of imidacloprid were 0.0012, 0.012, 0.12, 1.2, and 12.0 mg/L, prepared from the EcoPrid formulation. Volumes of EcoPrid were added to the surface of each designated microcosm to attain nominal test concentrations (calculated from the concentration of imidacloprid in EcoPrid and the

known volume of water in the microcosms), while the water was being gently stirred with a glass rod.

In both experiments, two sets of microcosms were concurrently operated with one set (differing only in inclusion of aquatic insects) being used for biological response measurements (effects microcosms) and the other set deployed for quantifying imidacloprid concentrations in water and leaf material (fate microcosms). This approach was taken to avoid destructive sampling influences for residue analyses on the behaviour and survival of test animals. For the first experiment (leaves from treated trees added to microcosms), there were 5 replicates for the effects microcosms and 3 replicates for the fate microcosms for each treatment. For the second experiment (imidacloprid added directly to microcosms), there were 4 replicates for the effects microcosms and 2 replicates for the fate microcosms. The nominal test concentrations in the second experiment were referred to as Conc1–Conc5 in effects microcosms and Conc1R–Conc5R in fate microcosms. The experimental periods for both microcosm experiments (from the addition of the test material to the end of the observation period) were 14 days.

2.2. Microcosm design and deployment

Aquatic microcosms were 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 centre of the lid was left open to allow some air exchange, while the other was near the back edge of the lid and was fitted with a rubber stopper through which an air hose with air stone was placed to supply continuous air to each microcosm and provide gentle water circulation.

The microcosms were placed in a single row on a shelf in an experimental room that was temperature- ($20 \pm 3\,^{\circ}C$) and light-controlled (daylight simulation fluorescent bulbs, 12/12 h light/dark, photon flux approximately 30 $\mu mol/m^2/s$). Each microcosm contained 6 L of natural stream water (collected from a forest stream at a single time and stored at 2 °C for less than 7 days), 300 mL of natural stream detritus (organic material collected from a forest stream, sieved to 1–5 mm particle sizes, frozen for several weeks to kill sediment organisms, then thawed for 5 days before being added to the microcosms), and 10 twigs from speckled alder (*Alnus incana*) trees (approximately 10 mm diameter and 15 cm long) to provide natural cover and sites of attachment for the test insects.

Stonefly (*Pteronarcys dorsata*, Order: Plecoptera, Family: Pternonarcyidae), and crane fly (*Tipula* sp., Order: Diptera, Family: Tipulidae) larvae were selected as representative leaf-shredding insects. These were collected in September 2005 from a local stream near Sault Ste. Marie, Ontario, held in the laboratory at ambient conditions for 72–96 h, then distributed among microcosms 48 h before the microcosms were treated. Nine specimens of each of the 2 taxa were added to each effects microcosm. 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. Both effects and fate microcosms were assembled and operated for 1 wk prior to the addition of test materials.

2.3. Microcosm dosing procedures

For the first experiment, leaves from imidacloprid-treated and control ash trees were added as test materials to the microcosms. Leaves used for each treatment were collected at senescence just prior to leaf fall (late September) from 3 replicate trees treated at the same rate, thoroughly mixed to account for potential variability in imidacloprid concentrations in leaves among replicate trees, and impartially allocated among replicates of the treatment group. Possible differences in imidacloprid concentrations in leaves among replicate trees had no bearing on the test concentrations because actual concentrations in leaves added to microcosms were measured (see Section 2.4). The leaves were air-dried at room temperature for about 2 h to stabilize ambient moisture levels in the leaves, then placed in sealed plastic bags and stored in the dark at $2 \pm 2\,^{\circ}C$ for 12 days until being added to the microcosms. The control leaves used in the second experiment were collected at the same time and stored at the same conditions for 38 days until being added to the microcosms of the second experiment. For the second experiment, leaves were added to the microcosms, then imidacloprid was added to the water as described above. Just before the leaves were added to microcosms of both experiments, they were batch-weighed to obtain initial fresh weights of leaf material.

A subset of 50 control leaves was taken from the cold storage on the day the leaves were added to the microcosms for both experiments, to determine the initial dry weights of leaves used in the microcosms. These leaves from the control subsets were individually weighed, leached in running water for 24 h, dried at 60 °C for at least 48 h, then re-weighed to determine dry weights and to account for leaching losses. A linear regression of fresh weight on dry weight was computed for each batch to estimate the initial dry weights from fresh weights of leaves added to the microcosms (regression $r^2 = 0.96$ and 0.93 for the two experiments). The leaves added to the microcosms were not initially dried and weighed to directly measure 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 imidacloprid concentrations in the leaves could be compromised and (or) the palatability to test organisms could be affected if the leaves were oven dried at 60 °C.

All microcosms of both experiments received 12 ash leaves from the designated treatment groups. The leaves were grouped in 3 batches of 4, weighted with a small plastic clip, and placed on the detritus and alder twigs in the microcosms. Each effects microcosm also received two batches of 10 leaf disks, cut with a 23-mm diameter cork borer from leaves of the same treatment groups, held in 0.5-mm mesh bags, weighted with plastic paper clips, and placed on the bottom of the microcosms to exclude the aquatic insects and to measure microbial respiration and decomposition activity on leaf material. Before the leaves were distributed among the replicate microcosms of the first experiment, a subsample of 12 leaves was withdrawn from each treatment group and frozen for subsequent analysis of initial imidacloprid concentrations in leaves added to aquatic microcosms.

2.4. Imidacloprid residue sampling and analysis

Water samples for imidacloprid analyses were collected by drawing 25 mL of water (5 aliquots of 5 mL each) from the centre, mid-depth position of each designated fate or effects microcosm with a 10-mL glass pipette, transferred to a 50-mL poly centrifuge tube and frozen for subsequent analyses. Leaf samples were obtained by drawing 3 leaves from each fate microcosm (one leaflet from each group of 4 in the microcosm) with stainless steel forceps, and were blotted dry on a paper towel, sealed in a plastic bag, and frozen for subsequent analyses.

Imidacloprid concentrations were quantified in water and leaf material by High Performance Liquid Chromatography with photo-diode array detection (HPLC-DAD). HPLC calibration standards were prepared from imidacloprid technical, 99.5% (Crescent Chemical, Islandia, NY, USA), Lot #30714. All samples were analyzed using an Agilent 1100 HPLC equipped with photo-diode array detector and autosampler. Imidacloprid in leaf material was extracted using accelerated solvent extraction techniques involving methylene chloride at high temperature and pressure, and further cleaned on Florisil columns. Imidacloprid in water samples was extracted using solid phase C18 sep paks.

Concurrent quality control analyses, using blank 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 water samples ($n = 20$) was 92.7 (5.7)% and for ash leaves ($n = 20$) was 91.2 (8.4)%. All residue data reported were corrected for analytical recovery losses using a correction factor of 100/average recovery efficiency. Estimated limits of detection (LOD) and limits of quantification (LOQ) for imidacloprid residues in water and ash foliage were determined based on analysis of detector response variation for blank samples ($n = 4$) in each matrix. For water, LOD and LOQ estimates

were 0.014 and 0.016 μg/mL and for foliage 0.028 and 0.045 μg/g fresh weight, respectively.

2.5. Water quality analysis

An Onset Optic Stowaway® thermologger was placed in a control microcosm near the centre of the row, and programmed to record water temperatures every hour. All other water quality conditions, including manual temperature checks, were made on days -1, 2, 5, 8, and 12. Dissolved oxygen (DO) concentrations were measured with a WTW Oxi197 dissolved oxygen meter and self-stirring probe. The pH was measured with an Orion Model 230A hand-held pH meter, and conductivity was measured with a TDSTestr 40 conductivity meter.

2.6. Response measurements

Aquatic insect mortality was determined on the last day of the experimental period. The bottom substrates were removed, searched for all insects, and the numbers of dead and living individuals were recorded. Because these were closed systems, missing individuals were presumed dead and decomposed. Mortality was defined as no movement and no response to prodding, while insects were classified as moribund when they exhibited very sluggish movement and no response to prodding.

Decomposition of leaf material from combined insect feeding and microbial activity was determined as the difference between the estimated, initial batch dry weight of the12 leaves added to the microcosms, and the dry weight of leaf material remaining at the end of the 14-day experimental period. The remaining leaf material was collected from each microcosm, dried at 60 °C for 48 h, and weighed.

Microbial decomposition of leaf material was determined by mass loss of leaf material in fine mesh bags. One batch of leaf disks was removed on day 7, and the other removed on day 14. The leaf disks were removed from the mesh bags, and added to aquatic respiration chambers (see below). At the end of the respiration measurements, the leaf disks were 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 estimated, initial dry batch weights of the leaf disks (using estimates from the same regressions as for whole leaves) and remaining batch weights after drying at 60 °C for 48 h.

Microbial respiration on leaf disks was determined by oxygen uptake in sealed, darkened respiration chambers. Leaf disks removed from the effects microcosms for determining microbial decomposition were immediately placed in water at ambient temperature and transferred within 30 min to an aquatic respiration unit. Thirty-two circular Plexiglas chambers (6.5 cm diameter, 7 cm high) were mounted in two Plexiglas tanks (16 chambers in each tank) which were held in the same experimental room as the microcosms to maintain ambient water temperatures. Each circular chamber contained a magnetic stir bar in a screened cell at the bottom of the chamber to provide continuous, gentle water circulation during incubation periods. The chambers were filled with water (stored from the source water for the microcosms that had been brought to ambient temperature) and bubbled with compressed air for 30 min to saturate the water with dissolved oxygen. The stir bars were de-activated and initial DO concentrations in each chamber were measured with the DO meter and self-stirring probe. The 10 leaf disks from each mesh bag were added to each corresponding chamber, and the chambers were sealed with Plexiglas lids on rubber O-rings, darkened with a black cover, the stir bars re-activated, and the disks incubated for 4 h. At the end of the incubation period, the stir bars were de-activated and DO measurements were taken through a portal in the lid of each chamber. Microbial respiration was expressed as DO uptake (mg/L/h).

2.7. Statistical analyses of response measurements

Differences among treatment groups were analyzed by 1-way ANOVA. When significant differences overall were detected ($P<0.05$), Dunnett's pairwise comparisons were made between controls and all other treatment groups, with significance at $P<0.05$. Percent response data were arcsine square-root transformed to improve normality. Other response data that failed tests of homogeneity of variances or normality ($P<0.05$) were log-transformed prior to the ANOVAs. All statistical analyses were conducted using SigmaStat 3.5 (Copyright© 2006 Systat Software Inc.).

3. Results

3.1. Water quality

Over the coarse of the first experiment, water temperatures ranged from 18.7 to 19.3 °C, with average daily temperatures of 18.9 to 19.1 °C. DO concentrations remained at or near saturation, ranging from 8.85 to 9.42 mg/L. Conductivity gradually increased over the 14-day period ranging from 53 to 80 μS/cm, and pH ranged from 6.2 to 6.8. During the second experiment, water temperatures ranged from 18.9 to 20.4 °C, with average daily temperatures of 19.1 to 20.0 °C. DO ranged from 8.52 to 10.56 mg/L, pH was 6.1–7.1, while conductivity again gradually increased over time ranging from about 50 to 138 μS/cm. None of the water quality measurements indicated treatment-related trends.

3.2. Experiment 1—imidacloprid in ash leaves added to microcosms

Average initial concentrations of imidacloprid in leaves that were added to aquatic microcosms in the first experiment, expressed as ppm on a fresh weight (f.w.) basis, were 0.85 for the low-field treatment, 1.28 for high-field, and 81.3 for the intentional over-dose (Table 1). The low- and high-field concentrations were similar to those found in green ash (trees after field trials to control EAB, where foliar concentrations at senescence were typically about 1 ppm or less; Thompson and Helson, unpublished data). Imidacloprid was rapidly lost from leaves added to aquatic microcosms. By day 2, imidacloprid concentrations in leaves were approximately 40–65% less than initial concentrations (Table 1). Concentrations in leaf material continued to decline over the 14-day period, and were reduced by 95% in low-field microcosms, 94% in high-field microcosms and 99% in over-dose microcosms. Imidacloprid losses from leaves into water were only detectable at the over-dose treatment, where small concentrations of imidacloprid were found in water by day 2 and persisted at trace levels over the 14-day period (Table 2).

There was no significant mortality of either aquatic insect taxon in microcosms containing leaves from trees treated at realistic (low-field and high-field) doses of imidacloprid (Table 3). In contrast, the over-dose treatment caused 89% mortality of *Pteronarcys*, and 91% mortality (dead and moribund individuals combined) among *Tipula*. While most tipulids were not completely dead by the end of the 14-day observation period (about 13% dead), almost all were observed to be moribund (no

Table 1
Average (±1SE) concentrations (μg/g or ppm f.w.) of imidacloprid in leaves added to fate microcosms ($n = 3$)

Treatment	Day 0	Day 2	Day 5	Day 9	Day 14
Low-field	0.850 (n.a.)	0.363 (0.018)	0.056 (0.014)	0.000 (0)	0.043 (0.002)
High-field	1.280 (n.a.)	0.489 (0.052)	0.106 (0.022)	0.028 (0.029)	0.072 (0.037)
Over-dose	81.3 (n.a.)	49.3 (14.2)	4.44 (1.65)	1.98 (0.91)	0.666 (0.142)

Samples for day 0 are from a composite subsample of 12 leaves for each treatment from treated batches immediately before addition to microcosms, therefore there is no average or standard deviation (indicted by "n.a."). Values at or below analytical limit of quantification (LOQ) = 0.043 μg/g f.w. for imidacloprid in foliage should be considered as trace detections only.

Table 2
Average (±1SE) concentrations (μg/mL or ppm) of imidacloprid in water from fate microcosms ($n = 3$) and effects microcosms ($n = 5$) to which leaves from systemically-treated ash trees were added

Treatment	Day 2	Day 5	Day 9	Day 14
Low-field fate	n.d.	n.d.	n.d.	n.d.
High-field fate	n.d.	n.d.	n.d.	n.d.
Over-dose fate	0.009 (0.001)	0.012 (0.003)	0.009 (0.002)	0.008 (0.002)
Low-field effects	n.d.	n.a.	n.a.	n.d.
High-field effects	n.d.	n.a.	n.a.	n.d.
Over-dose effects	0.022 (0.002)	n.a.	n.a.	0.030 (0.002)

Samples not taken are indicated by "n.a." Samples with non-detectable concentrations are indicated by "n.d." Values at or below analytical LOQ = 0.016 μg/mL. for imidacloprid in water should be considered as trace detections only.

Table 3
Mean (±SE) percent insect mortality (including moribund) in effects microcosms ($n = 5$) 14 days after leaves from systemically-treated ash trees were added

Treatment	*Pteronarcys dorsata*			*Tipula* sp.		
	% Dead	% Moribund	Total mortality	% Dead	% Moribund	Total mortality
Controls	4.4 (2.7)	0	4.4 (2.7)	6.7 (6.7)	0	6.7 (6.7)
Low-field	6.7 (2.7)	0	6.7 (2.7)	2.2 (2.2)	0	2.2 (2.2)
High-field	8.9 (4.2)	0	8.9 (4.2)	6.7 (4.4)	0	6.7 (4.4)
Over-dose	88.9 (4.9)	0	88.9 (4.9)*	13.3 (6.5)	77.8 (7.9)	91.1 (8.9)*

* indicate significant difference from control (Dunnett's $P < 0.05$).

response to prodding, little or sluggish movement, often positioned on top of the detritus or floating in the water) and were therefore included in the total mortality count. In the natural environment, insects rendered moribund by the treatment would be unable to avoid predation, be swept away by the current, or be otherwise dysfunctional and would almost certainly be incapable of surviving. Time to mortality was not systematically recorded throughout the observation period because it was not possible to determine total mortality without disturbing the microcosms, but it was observed that mortality among some insects at the over-dose treatment occurred within 24 h after the leaves were added.

Total mass loss of leaf material (from insect feeding and microbial decomposition) in the low-field treatment was not affected by the imidacloprid concentrations, but the decomposition of leaves from the high-field and over-dose treatments was significantly lower than in controls (Dunnett's $P < 0.05$) (Fig. 1). Leaves from the control, low-field and high-field treatments all showed evidence of direct feeding by the aquatic invertebrates (shredded leaf material), but decomposition in the over-dose treatments appeared to be by microbial activity only, with no visual evidence of insect feeding. The significant mortality of insects from exposure to the over-dose leaves occurred despite no observable feeding on the leaf material, indicating that feeding inhibition and (or) mortality were rapid at this treatment level.

There was no indication that imidacloprid in leaf material at any test concentration inhibited microbial decomposition rates. Mass loss by microbial decomposition at all test concentrations did not differ significantly from controls except on day 7 when microbial decomposition in the high-field treatment was significantly higher than in controls (Dunnett's $P < 0.05$) (Fig. 2A).

A pattern similar to microbial decomposition was observed in microbial respiration on leaf material. There was no indication that imidacloprid residues in leaves from

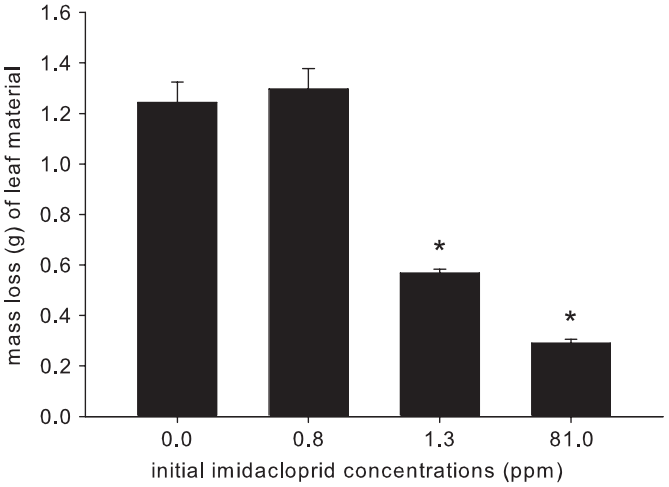

Fig. 1. Mean (±1SE) mass loss of leaf material from insect feeding activity and microbial decomposition in effects microcosms ($n = 5$) of the first experiment to which leaves from treated and control trees were added. * indicate significant differences from controls (Dunnett's test, $P < 0.05$).

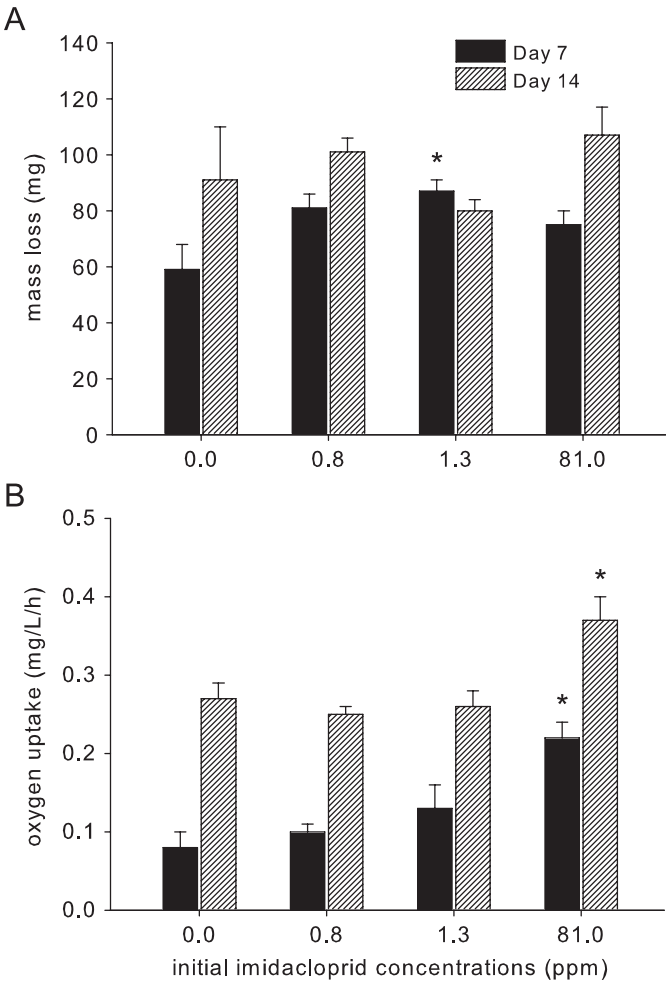

Fig. 2. Mean (±1SE) mass loss of leaf material from microbial decomposition (A) and oxygen uptake from microbial respiration (B) in effects microcosms ($n = 5$) of the first experiment to which leaf disks from leaves of treated and control trees were added. * indicate significant differences from controls (Dunnett's test, $P < 0.05$).

treated trees inhibited aquatic microbial respiration activity (Fig. 2B). Respiration rates on leaf disks from treated trees were never significantly lower than on control leaf disks, and were significantly higher at the over-dose treatment (Dunnett's $P < 0.05$).

3.3. Experiment 2—imidacloprid added directly to water

Average initial imidacloprid concentrations in water within 1 h after adding EcoPrid to the microcosms were 0.001, 0.012, 0.135, 1.55, and 15.4 μg/mL or ppm (Table 4). These are slightly higher than the nominal concentrations, and will be referred to as test concentrations in the remainder of this paper. Imidacloprid concentrations in water declined over time across all treatments in fate microcosms, generally with the rate of decline diminishing over time. By day 14, concentrations in Conc1R were undetectable, while concentrations in Conc2R were reduced by 42%, in Conc3R by 61%, in Conc4R by 52% and in Conc5R by 47%. Initial (day 0) concentrations were not measured in effects microcosms, but the trend of decline over time appears to be similar to the decline in fate microcosms (Table 4). At least some of the decline in aqueous imidacloprid concentrations was due to adsorption to leaf material in the microcosms. Imidacloprid concentrations in leaf material were measurable by day 2 at all test concentrations, and tended to increase or stabilize over the 14-day experimental period (Table 5).

There was no significant mortality of either taxon when imidacloprid was added directly to aquatic microcosms at test concentrations of 0.001 or 0.012 ppm (Table 6). At 0.135 ppm, there was 94.4% mortality of *Pteronarcys* and 100% mortality (number dead and moribund combined) of *Tipula*. Nearly complete mortality of both taxa occurred almost immediately (observed within a few hours after treatment) at test concentrations of 1.55 and 15.4 ppm.

While there was no significant mortality of aquatic insects at the test concentration of 0.012 ppm, there were significant sub-lethal effects on insects at that concentration. Mass loss of leaf material in the microcosms was significantly less at 0.012 ppm (and at all higher concentrations as well) than in the controls (Dunnett's $P < 0.05$) (Fig. 3). This indicates that feeding activity was significantly reduced among aquatic insects at 0.012 ppm and was corroborated by observations that the leaves in microcosms at this treatment level had much less evidence of "shredding" in comparison to leaves in microcosms at 0.001 ppm and controls. Mass loss was reduced further at the higher test concentrations, with little or no observable evidence of insect feeding on leaf material.

There were no significant differences in mass loss of leaf disks between controls and the first 4 treatment concentrations indicating that imidacloprid in water at up to about 1.55 ppm did not significantly inhibit microbial

decomposition (Dunnett's $P>0.05$) (Fig. 4A). Microbial decomposition activity was significantly increased at the 15.4 ppm test concentration (Dunnett's $P<0.05$). Similarly, there were no indications of adverse effects of aqueous imidacloprid concentrations on microbial respiration. There were no significant differences from controls in oxygen uptake at any test concentration (ANOVA $P=0.077$ for day 7 and $P=0.629$ for day 14) (Fig. 4B).

Table 4
Average imidacloprid concentrations (μg/mL or ppm ± 1SD) in water of fate ("R", $n=2$) and effects ($n=4$) microcosms after direct application of imidacloprid (EcoPrid)

Treatment	Day 0	Day 2	Day 7	Day 14
Conc1R	0.001(0.000)	0.002(0.000)	0.000(0)	0.000(0)
Conc2R	0.012(0.000)	0.008(0.001)	0.004(0.001)	0.007(0.005)
Conc3R	0.135(0.006)	0.085(0.000)	0.070(0.001)	0.053(0.000)
Conc4R	1.55(0.036)	1.04(0.083)	0.816(0.117)	0.750(0.130)
Conc5R	15.4(0.918)	11.0(0.737)	8.91(0.152)	8.18(0.790)
Conc1	n.a.	0.001(0.002)	n.a.	0.001(0.001)
Conc2	n.a.	0.007(0.001)	n.a.	0.004(0.001)
Conc3	n.a.	0.081(0.003)	n.a.	0.051(0.003)
Conc4	n.a.	0.899(0.073)	n.a.	0.682(0.045)
Conc5	n.a.	8.87 (1.01)	n.a.	6.01(2.92)

Samples not taken are indicated by "n.a." Values at or below analytical LOQ = 0.016 μg/mL for imidacloprid in water should be considered as trace detections only.

4. Discussion

4.1. Water quality

Water quality parameters (DO, pH, conductivity) for both experiments were maintained at conditions similar to those of regional forest water bodies (Kreutzweiser et al., unpublished data), and would not have caused the observed insect mortality or sublethal effects. There were no concentration-dependent patterns among water quality parameters measured, indicating no treatment effects on these water quality parameters. Conductivity tended to increase over time in all microcosms and may have been an indirect result of increased planktonic (water-borne), benthic (in the detritus) or epilithic (in the biofilm on leaves and microcosm walls) microbial growth over time. We observed, but did not quantify, increasing biofilms on the glass walls and increasing water turbidity in all microcosms over the 14-day period. Increased microbial activity could increase ion exudation through microbial nutrient regeneration with resultant increases in conductivity.

4.2. Fate of imidacloprid concentrations

Imidacloprid in leaves from treated ash trees was rapidly lost when added to aquatic microcosms. Imidacloprid concentrations in leaves were reduced by about 50% after 2

Table 5
Average concentrations (μg/g or ppm) of imidacloprid (±1SD) in leaf material of fate microcosms ($n=2$, except Conc3R Day 2 for which there was only one sample) to which direct applications of EcoPrid were made

Test concentration (mg/L)	Day 2	Day 5	Day 9	Day 14
0.001	0.076 (0.040)	0.002 (0.003)	0.000 (0)	0.229 (0.222)
0.012	0.078 (0.023)	0.025 (0.002)	0.044 (0.018)	0.077 (0.006)
0.135	0.213 (n.a.)	0.463 (0.094)	0.445 (0.008)	0.349 (0.015)
1.55	3.29 (0.569)	4.85 (0.353)	4.47 (0.193)	3.88 (0.867)
15.4	21.5 (4.88)	43.7 (2.60)	40.6 (1.55)	37.4 (4.17)

Values at or below analytical LOQ = 0.043 μg/g f.w. for imidacloprid in foliage should be considered as trace detections only.

Table 6
Mean (± SE) percent insect mortality (including moribund) in effects microcosms ($n=4$)14 days after direct applications of imidacloprid (EcoPrid) to microcosms

Test concentration (mg/L)	*Pteronarcys dorsata*			*Tipula* sp.		
	% Dead	% Moribund	Total mortality	% Dead	% Moribund	Total mortality
0	4.4 (2.7)	0	4.4 (2.7)	0	0	0
0.001	8.3 (2.8)	0	8.3 (2.8)	2.8 (2.8)	0	2.8 (2.8)
0.012	7.4 (3.7)	0	7.4 (3.7)	0	0	0
0.135	94.4 (3.2)	0	94.4 (3.2)*	33.3 (7.9)	66.7 (7.8)	100 (0)*
1.55	100	0	100*	94.4 (5.6)	0	94.4 (5.6)*
15.4	100	0	100*	100	0	100 (0)*

* indicate significant difference from control (Dunnett's $P<0.05$).

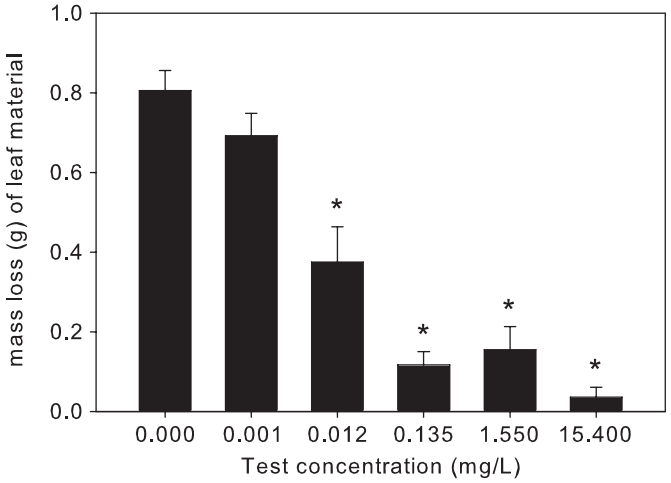

Fig. 3. Mean (±1SE) mass loss of leaf material from insect feeding activity and microbial decomposition in effects microcosms ($n = 4$) of the second experiment to which imidacloprid was added directly. * indicate significant differences from controls (Dunnett's test, $P<0.05$).

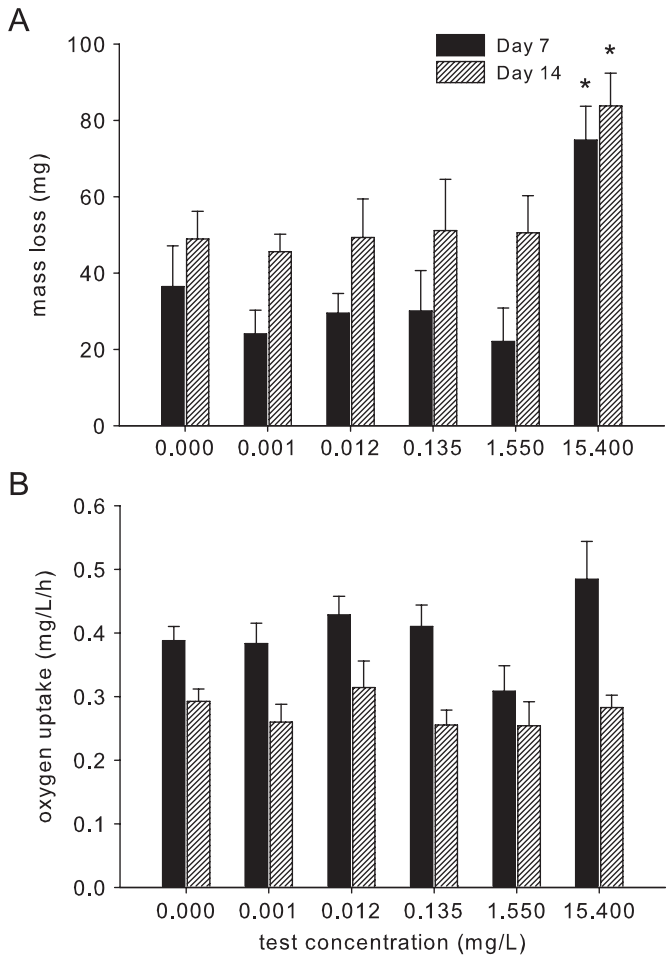

Fig. 4. Mean (±1SE) mass loss of leaf material from microbial decomposition (A) and oxygen uptake from microbial respiration on leaf disks (B) in effects microcosms ($n = 4$) of the second experiment to which imidacloprid was added directly. * indicate significant differences from controls (Dunnett's test, $P<0.05$).

days, and by over 90% after 14 days (Table 1). The rapid decline in foliar concentrations indicates that imidacloprid residues were being lost through leaching out into the surrounding water, and (or) rapid microbial/enzymatic degradation within the leaf tissue itself. Regardless of the dissipation mechanism, these results indicate that imidacloprid in ash leaves that fall into water bodies will not persist beyond a few days, and will rapidly diminish over time thereby reducing the risk of harm to decomposer organisms through consumption of contaminated leaf material.

Aqueous concentrations resulting from leaching of leaves that fall into water bodies from imidacloprid-treated trees are unlikely to reach effective concentrations in a natural environment because of low mass loading by the leaves and dilution by the water body. For example, 12 leaves in our 6-L microcosms, each at about 1.3 ppm (total approximately 15.6 ppm) lost over 90% of imidacloprid over a 14-day period but did not result in detectable aqueous concentrations (Table 2). In a natural standing water body that was large enough to be ecologically productive (e.g. ephemeral pond, assuming dimensions of 3 m × 3 m × 15 cm deep), it would require about 1000 g of leaves (approximately 2000 ash leaves) with 1.3 ppm imidacloprid leaching at a rate of 100% to raise the aqueous concentration to 0.001 ppm (near the limit of detection). Thus the risk of significant aqueous concentrations leaching from fallen ash leaves in standing water bodies is small. Since aqueous residues of imidacloprid are highly susceptible to photolysis (Moza et al., 1998), this risk would be further mitigated by photodegradation in natural water bodies exposed to sunlight and by dilution in flowing water bodies such as streams or ditches.

When imidacloprid was added directly to water in the microcosms, aqueous concentrations declined over time, generally with the rate of decline diminishing over time and with a dissipation of about 50–60% after 14 days (Table 4). This suggests that aqueous imidacloprid concentrations in natural water bodies that result through leaching from soil applications could persist at measurable, potentially effective, concentrations for several weeks. However, this would depend largely on environmental conditions in the receiving environment including those associated with photolysis, sorption and dilution as the primary loss processes. At least some of the imidacloprid loss from water in the microcosms was attributable to sorption on to leaf surfaces. When aqueous concentrations were at 0.012 ppm or above, imidacloprid concentrations in leaf material accumulated at concentrations up to 7 times higher than in the water (Table 5). Thompson et al. (1995) previously demonstrated in a field trial that waterborne pesticide residues can accumulate at up to 20 times higher in natural leaf material than in the water column of a forest stream. The sorption/desorption processes of imidacloprid in soils is known to be controlled largely by organic matter (Cox et al., 1998) and our study demonstrates that submerged organic matter such as leaves also has a

propensity to adsorb imidacloprid. This could potentially provide a secondary route of exposure to decomposer organisms if water bodies were contaminated by imidacloprid through leaching from soil applications at concentrations sufficient to accumulate effective concentrations on leaf material.

4.3. Effects on aquatic insects

The results indicate that imidacloprid concentrations in ash leaves that fall from trees treated at typical field rates will pose little risk of direct mortality to aquatic leaf-shredding insects. Significant insect mortality only occurred at the unrealistic over-dose treatment (Table 3). It is not clear if mortality in the over-dose microcosms resulted from the consumption of undetectable amounts of leaf material or from exposure to aqueous concentrations of about 0.02–0.03 ppm that leached out of imidacloprid-treated leaves. The second experiment demonstrated high mortality of aquatic insects when concentrations were about 0.13 ppm, but no significant mortality at 0.012 ppm (Table 6). Aqueous concentrations that leached out of imidacloprid-treated leaves at the over-dose treatment of the first experiment were 2–3 times higher than our no-observable-effect concentration in our second experiment, and may have at least contributed to the mortality of insects in that treatment. Regardless, the insect mortality in the over-dose microcosms occurred at a treatment level that was intentionally well beyond realistic concentrations in leaf material from systemic applications. Concentrations in water that leach from soil applications are likely to cause significant insect mortality when they exceed 0.012 ppm.

Leaves that fall from imidacloprid-treated trees into water bodies may pose a risk of significant sublethal feeding effects on obligate leaf-shredding insects when concentrations in leaf materials approach 1.3 ppm. 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 was a repellent effect and if there were alternate sources of leaf material available, detritivorous insects are likely to search for and preferentially feed on non-contaminated 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 behaviour of these insects could reduce their predator avoidance, functional activity, and ultimately survival in natural systems. Given that the invertebrate-mediated breakdown of leaf litter is a critical ecological function in aquatic ecosystems (Richardson, 1992; Webster et al., 1999; Abelho, 2001), reduced leaf-shredding insect survival or feeding activity on leaf material resulting from exposure to imidacloprid-contaminated leaves could have significant adverse implications for nutrient cycling in streams or ponds. This should be tested in further microcosm experiments with alternate sources of leaf material to determine if the feeding inhibition resulted from repellent or toxic effects on leaf-shredding insects.

4.4. Effects on aquatic microbial communities

Our results indicate that imidacloprid in leaves that fall from treated ash trees or in water from soil applications and leaching will not adversely affect aquatic microbial decomposition activity. There were no indications that imidacloprid at any test concentration inhibited microbial decomposition or respiration on leaf material (Fig. 2B). Microbially-mediated breakdown of leaf material is a critical part of litter decomposition processes in aquatic ecosystems (Suberkropp, 1998). In addition, the microbial colonization and conditioning of leaf litter in aquatic systems are vital precursors to leaf-shredding insect feeding activity (Petersen and Cummins, 1974; Cummins and Klug, 1979). Although we did not measure microbial community structure directly, the lack of adverse effects on decomposition or respiration activity indicated that the feeding inhibition by leaf-shredding insects was not the result of reduced palatability from inhibited microbial conditioning.

4.5. Comparison to previous studies

We were unable to find any previous studies that reported the effects of imidacloprid through consumption of foliage from imidacloprid-treated plants on aquatic decomposers. Likewise, there were no previous studies on effects of imidacloprid on aquatic microbial communities.

In standard toxicity tests, imidacloprid in water was toxic to aquatic crustaceans (48-h EC50 or LC50 to *Daphnia magna*) at concentrations of 10–85 mg/L (ppm) (Kidd and James, 1994; Song et al., 1997). Toxicity was much higher to aquatic insects in standard tests with an LC50 to mosquito larvae (*Aedes aegypti*) of 0.045 mg/L (Song et al., 1997) and to blackfly larvae (*Simulium vittatum*) of 0.007–0.009 mg/L (Overmyer et al., 2005). In a controlled field experiment, Sanchez-Bayo and Goka (2006), found that initial imidacloprid concentrations in water of about 0.240 mg/L in rice paddies caused significant adverse effects on aquatic invertebrate communities and significant declines in abundance of aquatic insects in particular. Our study showed significant adverse effects on aquatic insects (feeding inhibition) at concentrations of 0.012 mg/L (Fig. 3), and high mortality at 0.13 mg/L in water (Table 6). Taken together, these data indicate that standard toxicity tests with *Daphnia magna* to assess the risk of imidacloprid to aquatic invertebrates will greatly underestimate potential effects on aquatic invertebrates, and that significant adverse effects on aquatic insects would be expected where imidacloprid concentrations in water reach or exceed 0.005–0.010 mg/L (ppm). In this regard, stem-injections of imidacloprid to riparian trees will result in less environmental exposure and pose less risk of harm to aquatic detritivorous insects than soil injections. The

persistence and movement of imidacloprid from soil injection sites around ash trees and potential leaching to adjacent water bodies are being studied in EAB field trials (Thompson and Helson, unpublished data).

5. Conclusion

The results from these microcosm experiments indicate that imidacloprid concentrations in ash leaves that fall into water bodies from trees treated at typical field rates to control EAB will pose little risk of harm to aquatic leaf-shredding insects or to aquatic microbial communities. At the upper end of the range of typical field concentrations in leaves, there may be feeding-inhibition effects on leaf-shredding insects but the ecological implications of this will depend on the mode of action and the availability of alternate food sources, and have yet to be determined. Further microcosm experiments to explore this issue are recommended.

If imidacloprid concentrations leach from soil applications to water bodies, they are likely to be at least 10 times more toxic to aquatic leaf-shredding insects than foliar concentrations. Insects feeding on leaf material containing up to 1.3 ppm did not exhibit significant mortality, while insects exposed to 0.13 ppm in water were nearly all killed. Leaching from soil applications to water bodies could cause adverse effects on insect feeding rates if the resulting concentrations reach or exceed 0.01 ppm. Adverse effects on leaf-shredding insect feeding rates have potential to cause significant alterations to a critical ecosystem function, invertebrate-mediated breakdown of leaf litter. When imidacloprid is used as a systemic insecticide to control emerald ash borer in riparian ash trees, it will pose less risk of harm to non-target aquatic decomposers when applied as stem injections than when applied as soil injections.

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