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Toxicity of the Systemic Insecticide, Imidacloprid, to Forest Stream Insects and Microbial Communities

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Abstract Imidacloprid was added to laboratory aquatic microcosms at concentrations of 12, 24, 48 and 96 μg/L to determine effects on leaf-shredding aquatic insect survival and feeding rates, and on aquatic microbial decomposition of leaf material. Survival of the stonefly, *Pteronarcys dorsata*, was significantly reduced at 48 and 96 μg/L. There was no significant mortality of the cranefly, *Tipula* sp., but most surviving tipulids were very sluggish and non-responsive to prodding at 48 and 96 μg/L. Leaf decomposition by these leaf-shredding insects was significantly reduced at all test concentrations. There were no significant adverse effects on microbial decomposition of leaf material.

Keywords Imidacloprid · Toxicity · Aquatic insects · Microbial decomposition

Imidacloprid (1-(6-chloro-3-pyridinylmethyl)-N-nitroimidaolidin-2-ylideneamine) is a systemic, chloro-neonicotinyl insecticide that blocks nicotinergic neuronal pathways that are abundant in insects (Sheets 2001). While it is commonly used to control various agricultural and horticultural insect pests (Elbert et al. 1991), it has recently been demonstrated to be effective against wood-boring insect pests in trees (Poland et al. 2006). Systemic treatments with imidacloprid may be particularly appropriate for protecting trees in urban or environmentally sensitive areas where broad-scale pesticide applications or tree removal are not acceptable. This could 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.

Systemic treatment of trees can be made by stem or soil injections, or by soil drench. Following any of these treatments, imidacloprid residues could be transferred into aquatic environments indirectly through leaf fall. However, in conjunction with soil applications, imidacloprid may move into aquatic systems more directly through off-site leaching (Felsot et al. 1998; Wilkins 2000), particularly in soils with low organic or high clay content (Cox et al. 1998; Smelt et al. 2003).

We assessed the risk of effects on aquatic insects by exposing two representative insect species to aqueous imidacloprid concentrations in laboratory microcosms, and measured insect survival, feeding rates, and microbial decomposition activity on leaf material. We used leaf-shredding insects as test species because they are common in forest or tree-lined streams where applications of imidacloprid could be made around riparian trees to control insect pests, and because insect-mediated leaf decomposition is a critical ecosystem process in forest water bodies (Abelho 2001). The microcosms were designed to closely simulate natural water bodies by use of field-collected stream water, detritus, wood pieces and test organisms.

Materials and Methods

Aquatic microcosms consisted of glass aquariums, 13 cm wide, 30 cm long, and 21 cm high, fitted with a Plexiglas

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lid in which two 2.5-cm diameter holes were drilled. One hole in the lid was left open to allow some air exchange, while 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 provide gentle water circulation. The microcosms were placed in a single row on a shelf in an experimental room that was temperature-(20 ± 3°C) and light-controlled (daylight simulation fluorescent bulbs, 12/12 h light/dark). Each microcosm contained 6 L of stream water (collected from a forest stream at a single time and stored at 2°C for 5 days), 300 mL of 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* Say) nymphs and cranefly (*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 72–96 h, then impartially allocated among microcosms 48 h before the microcosms were treated. Nine specimens of each of the two taxa were added to each microcosm and the microcosms were assembled and operated for 1 week prior to the addition of imidacloprid. At the end of the 14-day post-treatment observation period, the bottom substrates were removed, 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 leaves from 15-cm diameter sugar maple (*Acer saccharum* Marsh.) trees were weighted with plastic paper clips and placed in the microcosms to provide a food source for the leaf-shredding insects. The leaves were collected at senescence just before leaf fall, air-dried for 2 h to stabilize fresh weights, then batch-weighed to provide initial fresh weights. Initial dry weights were estimated from a regression of dry weights on fresh weights (60°C for 48 h, linear regression, $p < 0.001$, $r^2 = 0.85$) 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, then 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 to simulate natural leaf-fall (fresh leaves added to microcosms), and to avoid the potential that drying the leaves could affect the palatability to test organisms. At the end of the 14-day 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. 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 exclude the aquatic insects and 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 estimated, initial dry batch weights of the leaf disks (using estimates from the same regression as for whole leaves) and remaining batch weights after drying at 60°C for 48 h.

Imidacloprid in the commercial product Confidor™ 200SL (200 g/L imidacloprid, Bayer CropScience Inc.) was added directly to the surface of designated microcosms while the water was being gently stirred with a glass rod. Three replicates of each test concentration were treated. Nominal test concentrations were 12, 24, 48, and 96 μg/L. Responses among these microcosms were compared to those in three replicate control microcosms which were similar in every aspect except the addition of imidacloprid.

Water samples for imidacloprid analyses were collected by drawing 25 mL of water (five aliquots of 5 mL each) from the center, mid-depth position of each treated microcosm with a 10-mL glass pipette, transferred to a 50-mL poly centrifuge tube and frozen for subsequent analyses. Samples were taken within 1 h after application to verify initial test concentrations, and at day 14 to determine residual concentrations. Imidacloprid concentrations were quantified in water and leaf material using a High Performance Liquid Chromatograph (Agilent 1100) equipped with photo-diode array detection (HPLC-DAD). The instrument was calibrated prior to each analytical run using a series of five standards prepared from imidacloprid technical, 99.5% (Crescent Chemical, Islandia, NY, USA), Lot #30714. Imidacloprid in water was extracted using solid phase C18 sep paks. The analytical method was validated prior to use, and blank samples fortified with known amounts of imidacloprid were run concurrently with experimental samples to determine recovery efficiencies and precision of the method. Average recovery (and coefficient of variation) for quality control water samples ($n = 10$) was 93.3 (1.9)%. All concentration data reported were corrected for analytical recovery losses.

Results and Discussion

Average initial imidacloprid concentrations in microcosms were within 96%–108% of nominal concentrations with coefficients of variation of <10% (Table 1). By the end of the 14-day experimental period, concentrations in water were reduced by 53%–55%. This concurs with a previous experiment in which concentrations in water of microcosms treated with an experimental formulation of imidacloprid were reduced by 40%–60% at day 14 (Kreutzweiser et al. 2007). In the previous experiment, it was demonstrated that at least some of the decline in aqueous imidacloprid concentrations was due to absorption to leaf material in the microcosms.

The stonefly, *Pteronarcys dorsata*, exhibited concentration-dependent increases in mortality among treatments with no significant mortality (Dunnett's test $p > 0.05$) at 12 and 24 µg/L, marginally significant mortality (Dunnett's $p < 0.10$) of about 40% at 48 µg/L and significant mortality (Dunnett's $p < 0.05$) of about 70% at 96 µg/L (Table 2). A probit analysis of the response data estimated an LC_{10} and LC_{50} for *Pteronarcys* of about 21 and 70 µg/L, respectively. The cranefly, *Tipula* sp., was less sensitive to imidacloprid with mortality about 3× higher than in controls only at the highest concentration (Table 2), and with no significant mortality at any concentration (ANOVA $p = 0.142$). Mortality responses among *Tipula* were not as concentration-dependent, and the probit analysis estimated an LC_{10} and LC_{50} of about 16 and 139 µg/L, but with relatively large standard errors. However, we observed (but did not quantify) during the mortality counts that while most tipulids in the 48 and 96 µg/L treatments were not dead, many were very sluggish and gave little response to prodding. In the natural environment, insects rendered sluggish by the imidacloprid at these concentrations would be unable to avoid predation, be swept away by the current, or be otherwise dysfunctional and would almost certainly be incapable of surviving. If the tipulid mortality counts had included those that were sluggish, the mortality and estimated lethal concentrations would have been more similar between *Pternonarcys* and *Tipula*.

Table 1 Mean imidacloprid concentrations (µg/L, ±SE) in aquatic microcosms

Nominal concentrations	Initial concentrations	Final concentrations
12	13 (0.27)	6 (0.41)
24	24 (0.67)	11 (0.47)
48	47 (2.59)	22 (1.78)
96	93 (2.48)	42 (1.54)

Table 2 Mean % mortality (n = 3, ±SE, nine specimens per microcosm) and estimated LC_{10} and LC_{50} (±SE) for aquatic insects exposed to imidacloprid in microcosms

Taxa	Initial concentrations (µg/L)	% Mortality	LC_{10}	LC_{50}
Pteronarcys dorsata	0	3.7 (3.7)	20.8 (6.9)	70.1 (6.3)
	12	3.7 (3.7)		
	24	7.4 (7.4)		
	48	40.7 (22.5)		
	96	70.4 (19.6)		
Tipula sp.	0	11.1 (6.4)	16.2 (16.5)	139.0 (36.0)
	12	7.4 (3.7)		
	24	7.4 (7.4)		
	48	18.5 (7.4)		
	96	33.3 (6.4)		

There were significant reductions (ANOVA $p = 0.041$) in mass loss of leaf material by combined insect feeding and microbial decomposition at all test concentrations (Fig. 1a). There were no visible signs of leaf-shredding by insects at 48 and 96 µg/L, with microbial decomposition accounting for mass loss of leaf material at those concentrations. Leaves in microcosms at the two lower concentrations did show signs of insect feeding, but at lower rates than those in controls. Thus, we observed feeding inhibition by leaf-shredding insects at concentrations as low as 12 µg/L. Given that insect-mediated leaf breakdown is a critical ecosystem process in temperate water bodies (Richardson 1992; Webster et al. 1999), reduced feeding by shredder insects exposed to imidacloprid in natural water bodies could have ecological implications with adverse effects on organic matter processing and nutrient cycling. There were no indications that microbial decomposition of leaf material was inhibited by imidacloprid at any test concentration (Fig. 1b).

We were unable to find previous studies on effects of imidacloprid on aquatic leaf-shredding insects (other than our own, Kreutzweiser et al. 2007) to which our results could be compared. In standard toxicity tests, imidacloprid was toxic to *Daphnia magna* (48-h EC_{50} or LC_{50}) at concentrations of 10–85 mg/L (Kidd and James 1994; Song et al. 1997). Toxicity was much higher to aquatic insects in standard tests with an LC_{50} to mosquito larvae (*Aedes aegypti*) of 45 µg/L (Song et al. 1997) and to blackfly larvae (*Simulium vittatum*) of 7–9 µg/L (Overmyer et al. 2005). In a controlled field experiment, Sanchez-Bayo and Goka (2006) found that initial imidacloprid concentrations in water of about 240 µg/L in rice paddies caused significant adverse effects on aquatic invertebrate communities and significant declines in abundance of aquatic insects in particular. The leaf-shredding insects in our microcosm

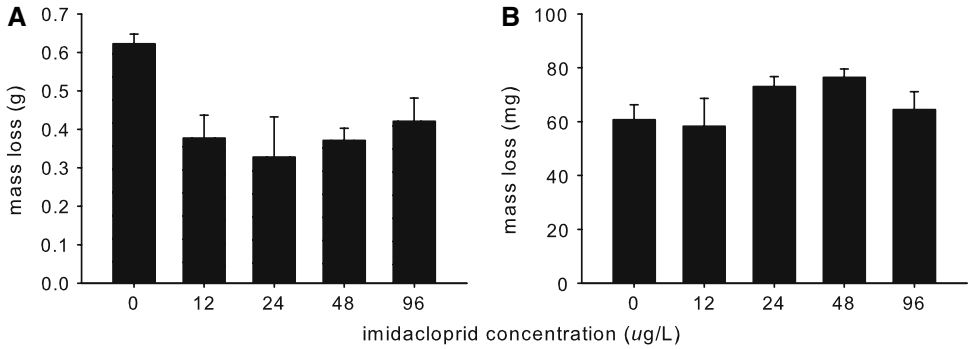

Fig. 1 Mean (±SE) mass loss of leaf material from insect consumption and microbial decomposition (**a**) and from microbial decomposition exclusively (**b**) in microcosms

experiments were among the more sensitive aquatic insects tested previously, and were much more sensitive than *Daphnia* in standard toxicity tests which are typically used to predict effects on non-target aquatic invertebrates.

Our results indicate that imidacloprid applications to soils around riparian trees that leach to adjacent water bodies could cause substantial mortality of aquatic insects if concentrations reach or exceed about 50 µg/L. Concentrations as low as 12 µg/L are likely to cause significant feeding inhibition in leaf-shredding insects which has the potential to interfere with a critical ecosystem process, i.e., leaf litter breakdown in natural water bodies. The risk of imidacloprid leaching from soil applications to water bodies when controlling forest insect pests in riparian trees can be reduced by the use of stem-injections rather than soil applications (e.g., Wanner et al. 2002).

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