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Highlighted Article

Imidacloprid in leaves from systemically treated trees may inhibit litter breakdown by non-target invertebrates

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ABSTRACT

Imidacloprid is a systemic insecticide that is used in trees to control several invasive, wood-boring insect pests in North America. Applications to deciduous trees result in foliar concentrations of imidacloprid that could pose a risk of harm to non-target decomposer invertebrates when autumn-shed leaves fall to forest floors or adjacent water bodies. Selection experiments were conducted in aquatic and terrestrial microcosms to test the hypothesis that non-target, leaf-shredding invertebrates can detect and avoid leaves from imidacloprid-treated trees thereby circumventing effects on leaf litter decomposition. There was no significant preferential feeding on non-contaminated leaves in selection microcosms indicating that the invertebrates could not detect and avoid imidacloprid-containing leaves. Mass loss and area consumed of both imidacloprid-containing and natural leaves in selection microcosms were significantly less than in control microcosms, indicating a sub-lethal feeding inhibition effect from consumption of leaf material at realistic field concentrations of 18–30 μg/g fresh weight. Our results indicate that imidacloprid at realistic concentrations in leaves can inhibit leaf litter breakdown through adverse sub-lethal effects on decomposer invertebrates.

1. Introduction

Non-native, invasive forest insect pests such as the emerald ash borer (*Agrilus planipennis*) and Asian longhorned beetle (*Anoplophora glabripennis*) threaten the health and survival of deciduous trees in large regions of eastern North America (Nowak et al., 2001; Peterson et al., 2003; Poland and McCullough, 2006; McCullough and Siegert, 2007). Many of the susceptible trees (e.g., ash (*Fraxinus* spp.), maple (*Acer* spp.), willow (*Salix* spp.)) are important species that influence or regulate forest and riparian ecosystem dynamics and nutrient cycling through leaf litter inputs to forest floors and water bodies (Ellison et al., 2005). Among the options to control or reduce tree mortality from invasive, wood-boring insect pests is the application of the systemic insecticide, imidacloprid (1-(6-chloro-3-pyridinyl-methyl)-*N*-nitroimidaolidin-2-ylideneamine) (Poland et al., 2006). Imidacloprid applications to deciduous trees result in foliar concentrations of the insecticide that could pose a risk of harm to non-target decomposer organisms when autumn-shed leaves fall to forest floors or adjacent water bodies. The risk of harm will be related to the concentrations in the leaves, which in turn will be related to the time since treatment and foliar dissipation rates. Given that invertebrate-mediated breakdown of leaf litter is a critical ecosystem process (Graça, 2001; Hattenschwiler et al., 2005), imidacloprid effects on invertebrate decomposers could have ecologically significant implications for leaf litter breakdown and nutrient cycling in detritus-based food webs of forest floors and nearby aquatic ecosystems.

We previously showed that imidacloprid concentrations in senescent green ash (*Fraxinus pennsylvanica*) leaves (approximately 1 μg/g fresh weight) from trees treated to control emerald ash borer posed little threat of direct mortality to leaf-shredding aquatic insects and did not inhibit aquatic microbial decomposition of ash leaves in aquatic microcosms (Kreutzweiser et al., 2007). However, we did detect significantly lower feeding rates (mass loss of leaf material) by leaf-shredding insects. In further aquatic and terrestrial microcosm tests, we showed that imidacloprid concentrations in senescent sugar maple (*Acer saccharum*) leaves of approximately 3–11 μg/g fresh weight did not affect survival of leaf-shredding aquatic insects over a 14-day exposure period or litter-dwelling earthworms over 35 days, but did cause significant feeding inhibition among the aquatic insects and earthworms, and induced measurable weight losses among earthworms (Kreutzweiser et al., 2008).

The potential ecological significance of this in natural systems would largely depend on the mechanism by which the feeding inhibition occurred and on the availability of alternate food

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sources. If the feeding inhibition was a repellent effect and if there were alternate sources of leaf material available, detritivorous invertebrates are likely to select and preferentially feed on non-contaminated leaves. On the other hand, if the feeding inhibition resulted from sub-lethal toxic effects after consumption of imidacloprid-containing leaf material, the ensuing lethargy or altered behaviour of these invertebrates could reduce their decomposition activity, predator avoidance, reproduction, or growth to maturity and ultimately survival in natural systems. To assess this potential impact, we offered natural and imidacloprid-containing leaves to leaf-shredding aquatic insects and litter-dwelling earthworms in microcosms, and measured their survival and feeding rates on the two types of leaves. Our hypothesis was that the imidacloprid would be detected and avoided by the invertebrates, and that they would preferentially feed on natural leaves in selection microcosms (microcosms that contained both imidacloprid-contaminated and control leaves) at the same rate as feeding on natural leaves in control microcosms (microcosms that contained control leaves only). Previous antifeedant effects testing of imidacloprid with the tobacco whitefly (*Bemisia tabaci*) demonstrated a clear preference by *B. tabaci* for non-treated leaf material (Nauen et al., 1998). An ability by non-target leaf-shredding invertebrates to detect and avoid imidacloprid-containing leaves would reduce the potential for significant adverse effects on leaf litter breakdown in natural systems.

2. Materials and methods

2.1. Microcosm design and deployment

Aquatic and terrestrial microcosms contained field-collected natural substrates (stream water, detritus, and wood pieces in aquatic microcosms, forest floor litter in terrestrial microcosms), and their description, deployment, and operation have been described previously (Kreutzweiser et al., 2007, 2008). Briefly, aquatic microcosms consisted of glass aquariums, 13 cm wide, 30 cm long, and 21 cm high, fitted with a Plexiglas lid. Each microcosm contained 6 L of stream water (collected from a forest stream at a single time), 300 mL of stream detritus (organic material collected from a forest stream, sieved to 1–5 mm particle sizes, frozen for 14 weeks to kill sediment organisms, then thawed for 5 days before being added to the microcosms), and 10 twigs from speckled alder (*Alnus incana* ssp. *rugosa*) trees (approximately 10 mm diameter and 15 cm long) to provide natural cover and sites of attachment for the test invertebrates. Water was not renewed over the 14-day experimental period, and temperatures ranged 18–20 °C, pH was 6.5–7.4, specific conductance was 94–130 μS/cm, and dissolved oxygen was held near saturation (8.1–9.3 mg/L) by aeration. Terrestrial microcosms were constructed of acrylic tubing, 7 cm diameter and 10 cm high, fitted with a plastic bottom containing two screened drainage holes, and covered on top with 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 addition of de-ionized water just prior to 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 12 weeks to kill litter invertebrates, and then thawed and held in open containers for 1 week before being added to the microcosms. Litter temperatures were not monitored, but air temperatures were 19.1–20.2 °C and relative humidity was 69–74% in the experimental laboratory that contained the microcosms. For both aquatic and terrestrial microcosm tests, daylight simulation fluorescent bulbs provided a 12 h light/12 h dark regime.

Representative decomposer invertebrates in aquatic microcosms were field-collected stonefly nymphs, *Pteronarcys dorsata*, and crane fly larvae, *Tipula* sp., and in terrestrial microcosms were field-collected litter-dwelling earthworms, *Dendrobaena octaedra*. In aquatic microcosms, the 2 taxa were tested separately and 9 individuals of each taxon were added to each replicate microcosm. The earthworms were tested in pairs with 2 clitellate (light-coloured band present indicating sexual maturity) worms impartially allocated to each replicate microcosm.

2.2. Experimental treatments

Leaves were collected at senescence just before leaf-fall (24 September 2007) from 5-cm diameter sugar maple trees, some of which had been treated several weeks before (27 August) by stem injections of imidacloprid (injections described in Kreutzweiser et al., 2007). Injections were made at a low-dose of 0.125 g/cm dbh (diameter at breast height) to obtain foliar concentrations near the upper end of expected concentrations from operational applications, and at a high-dose of 0.25 g/cm dbh to obtain a maximum-challenge concentration. The stem injections were made with an experimental imidacloprid formulation, an emulsifiable concentration containing 50 mg/mL imidacloprid (Great Lakes Forestry Centre, Sault Ste. Marie, Ontario). Leaves were collected approximately 1 week before the microcosm experiments, and were held in the dark at 2 °C. Fourteen subsamples of leaves from low-dose and 15 from high-dose treatments were analyzed for imidacloprid concentrations by high-performance liquid chromatography with photo-diode array detection (HPLC-DAD) (Kreutzweiser et al., 2008).

There were 5 replicates of each of 3 treatments: controls, low-dose selection, and high-dose selection microcosms. Controls contained only natural leaves (leaves referred to as C). Low-dose selection microcosms contained both natural leaves (LC) and leaves from the low-dose-treated maple trees (LT). High-dose selection microcosms contained natural leaves (HC) and leaves from high-dose-treated trees (HT). This facilitated comparison of leaf decomposition among natural leaves in low-dose and high-dose selection microcosms (LC and HC leaves) to decomposition of imidacloprid-containing leaves in those microcosms (LT and HT leaves), and to decomposition of natural leaves in control microcosms (C leaves).

Aquatic selection microcosms contained either *P. dorsata* or *Tipula*, and 6 whole maple leaves; 3 natural (control) leaves and 3 imidacoprid-containing leaves. The 2 leaf types were paired (3 pairs of 2 leaves, each pair consisting of a control and a treated leaf), held together and weighed with plastic paper clips, and placed on the bottom substrates in sequential positions (one pair near the back, one in middle, and one near the front of each microcosm). The leaves were paired to ensure equal access to both types of leaves by the invertebrates regardless of their position in the microcosms. Aquatic control microcosms contained 6 control leaves in the same configuration. Terrestrial selection microcosms contained 2 *D. octaedra* individuals and 2 whole maple leaves; one control leaf and one imidacloprid-containing leaf. The leaves were placed below the surface of the litter at the same depth but on separate sides. In all selection microcosms, leaves were stem-clipped to differentiate and track control and imidacloprid-containing leaves. Terrestrial control microcosms contained 2 whole, control leaves in the same configuration.

2.3. Response measurements

At the end of the 14-day exposure period in aquatic microcosms, the bottom substrates were removed, searched for all insects, and the numbers of normal, sluggish, moribund, and dead individuals were recorded. Control microcosms were searched first and thereafter invertebrate behaviour was classified in comparison to controls. Accordingly, normal was defined as alive and similar in behaviour to controls, sluggish was alive but slow-moving, moribund was alive but little movement or response to prodding, and dead was defined as no movement or response to prodding.

Leaf decomposition by aquatic insects was measured as mass loss and surface area consumed. For mass loss determination, leaves from each replicate among the leaf groups (C, LC, LT, HC, HT) were 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, $p<0.001$, $r^2=0.98$) 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 and the stability of the imidacloprid. At the end of the 14-day exposure period, remaining leaf material was removed, gently washed free of debris and biofilms, 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 leaves added to the microcosms, and the dry weight of leaf material remaining at the end of the experiment. Mass loss measurements were adjusted for insect abundance at the end of the exposure period by dividing mass loss by the number of living insects in each microcosm (mass loss expressed as mg/insect).

Leaf surface area consumed was also measured to provide a second metric of leaf decomposition and because it is a more direct measure of invertebrate feeding by excluding microbial decomposition activity. At the end of the exposure period, each leaf was removed and individually photographed at a consistent scale before being dried and batch-weighed for mass loss determination. The initial surface area of each leaf was outlined on the digital image by tracing and joining non-contiguous edges or by outlining the initial edges based on residual veins and comparisons to images of same-size maple leaves. Then missing pieces or sections of leaves were digitally outlined, their surface areas computed and summed to provide a measure of total surface area lost (cm^2). The image analyses were done with NIS-Elements Imaging Software (Laboratory Imaging, Nikon Instruments Inc., Melville, New York). Surface area lost was adjusted for insect abundance in each

microcosm by dividing the total area lost by number of living insects at the end of the exposure period (area lost expressed as cm^2/insect).

At weekly intervals through the 35-day exposure period in terrestrial microcosms, the contents were removed and searched for living earthworms, then replaced. Mortality was defined as no movement or response to prodding. Missing earthworms were presumed dead and decomposed. Leaf decomposition in terrestrial microcosms was measured as mass loss and surface area consumed as outlined above for aquatic microcosms, except the estimated initial dry weights of leaves were based on regression leaves that were not pre-leached (linear regression, $p<0.01$, $r^2=0.97$). Leaf mass loss and area consumed were not adjusted for worm abundance because there was no mortality in microcosms until between days 29 and 35. Mass loss was expressed as mg/pair and surface area lost as cm^2/pair.

2.4. Statistical analysis

Average imidacloprid concentrations in leaves of low-dose and high-dose selection microcosms were compared between *P. dorsata* and *Tipula* microcosms by a *t*-test. Percent mortality data were arcsine/square-root transformed to improve normality, and tested for differences among treatments (controls, low-dose, high-dose) by ANOVA. When this was significant ($p<0.05$), comparisons were made between the two treatment levels and controls by a Dunnett's test.

Differences among leaf groups (C, LC, LT, HC, HT) in leaf mass loss and area consumed were used to infer detection and avoidance of imidacloprid-treated leaves. A significant difference in leaf consumption between LC and LT or HC and HT leaves in selection microcosms indicated detection of imidacloprid and preferential feeding on control (LC or HC) leaves. A significant difference between natural leaves in control microcosms (C) and control leaves in selection microcosms (LC or HC) indicated that imidacloprid could not be detected and avoided in selection microcosms and that exposure to imidacloprid in selection microcosms affected overall leaf consumption rates. Comparisons were tested by ANOVA, and when significant, Tukey's multiple comparison tests were applied to determine which leaf groups were significantly different from the others. The Tukey's procedure adjusts the overall error rate for the multiple comparisons. Prior to analysis, the leaf mass loss and area consumed data were tested for normality and homogeneity of variances, and when the test was significant ($p<0.05$), the data were square-root transformed to satisfy these distribution assumptions.

3. Results

In aquatic microcosms, the mean imidacloprid concentration ($\pm$SE) in LT leaves of subsamples from the low-dose microcosms was 18.0 ± 3.6 μg/g (all concentrations expressed on a fresh weight basis) and in HT leaves from high-dose microcosms was 122.9 ± 20.1 μg/g. There was no significant difference in average imidacloprid concentration between *P. dorsata* and *Tipula* microcosms among low-dose (*t*-test $p=0.49$) or high-dose (*t*-test $p=0.45$) treatments. In terrestrial microcosms, the mean imidacloprid concentration in LT leaves was 30.8 ± 9.9 μg/g and in HT leaves was 135.4 ± 42.7 μg/g. These average concentrations of imidacloprid in leaves from the low-dose treatments (18 and 31 μg/g) were similar to imidacloprid concentrations found in leaf samples from systemically treated maple trees in two separate field trials ($n=4$, mean = 17.8 μg/g, and $n=3$, mean = 31.1 μg/g) (D. Thompson, unpublished data). Analytical quality control for the microcosm subsamples ($n=6$) showed a mean recovery of 77.2% with a coefficient of variation of 4.1%, and analytical results were adjusted accordingly by a factor of 1.3.

At the end of the 14-day exposure period, mortality of *P. dorsata* in selection microcosms containing low-dose (LT) and control (LC) leaves was not significantly different (Dunnett's $p>0.05$) from microcosms containing control leaves only (C), but *P. dorsata* mortality was significantly higher in selection microcosms containing high-dose (HT) and control (HC) leaves (Dunnett's $p<0.05$) (Table 1). *P. dorsata* that survived the high-dose treatment appeared sluggish in comparison to controls. Tipulids were more sensitive to imidacloprid in leaves with about 15% morality and 58% of survivors exhibiting sluggish behaviour in the low-dose selection microcosms, but a significant difference from controls could not be detected (Dunnett's $p>0.05$). Significant mortality of Tipulids occurred in high-dose selection microcosms (Dunnett's $p<0.05$), and the remaining individuals were all moribund (Table 1). Because all Tipulids in high-dose selection microcosms were either dead or moribund by the end of the experiment, the effects of imidacloprid on leaf decomposition by Tipulids were not measured in these high-dose microcosms.

Table 1
Mean ($\pm$SE, $n=5$) mortality and behaviour of leaf-shredding insects in aquatic microcosms at the end of the 14-day experimental period

Taxa	Treatment	% Normal	% Sluggish	% Moribund	% Dead
P. dorsata	Control	95.5 (4.5)	0	0	4.4 (4.4)
	Low	91.1 (4.2)	0	0	8.9 (4.2)
	High	0	82.2 (2.7)	0	17.8 (2.7)*
Tipula	Control	97.8 (2.2)	0	0	2.2 (2.2)
	Low	26.7 (19.4)	57.8 (14.6)	0	15.5 (6.7)
	High	0	0	46.7 (12.4)	53.3 (12.4)*

Treatments are control = leaves from non-treated trees, low = leaves from control and low-dose trees (average concentration 18 μg/g), high = leaves from control and high-dose trees (average concentration 123 μg/g). Asterisks indicate mortality significantly different from control (Dunnett's $p<0.05$). See Methods for description of behaviour categories.

The decomposition of leaf material in aquatic microcosms was adversely affected by imidacloprid. Mass loss of leaf material in all selection microcosms containing *P. dorsata* and both control and imidacloprid-treated leaves was significantly lower (ANOVA $p<0.001$, Tukey's $p<0.05$) than in microcosms containing *P. dorsata* and control leaves only (Fig. 1). Within the selection microcosms, there were no significant differences (Tukey's $p>0.05$) in mass loss between treated (LT and HT) and control (LC and HC) leaves. Mass loss of leaf material was also significantly reduced in *Tipula* selection microcosms with no preferential feeding on control leaves within the selection microcosms. Leaf area consumed by *P. dorsata* and *Tipula* followed the same patterns, but the effect size (magnitude of differences between control and treated leaves) was greater (Fig. 1). Among all selection microcosms, less than 40% of the available control leaf material was consumed (range 28–37%), and in control microcosms less than 55% (range 31–52%) was consumed indicating that food availability was not limiting.

In terrestrial microcosms, the survival of litter-dwelling earthworms may have been affected by the imidacloprid in leaves, but the response was not dose-dependent with 3 of 10 dead in low-dose, and 1 of 10 dead in high-dose microcosms and only by day 35 (Table 2). However, the decomposition of leaf material in terrestrial microcosms was clearly affected by imidacloprid. Mass loss of leaf material was significantly lower in selection microcosms than in controls (ANOVA $p<0.001$, Tukey's $p<0.05$) (Fig. 2). Among the selection microcosms, there was a tendency for greater mass loss of control leaf material than treated leaves, but this was significant (Tukey's $p<0.05$) only in high-dose microcosms. There was a similar pattern of reduced leaf area consumption by the earthworms in selection microcosms overall and a tendency for greater feeding on control leaves than on treated leaves in selection microcosms (Fig. 2).

4. Discussion

We reject our hypothesis that non-target leaf-shredding invertebrates can detect and avoid leaves from imidacloprid-treated trees. If the aquatic insects or earthworms were able to avoid imidacloprid-containing leaves, they would have been expected to preferentially feed on control leaves in selection microcosms at the same rate as those in control microcosms. The

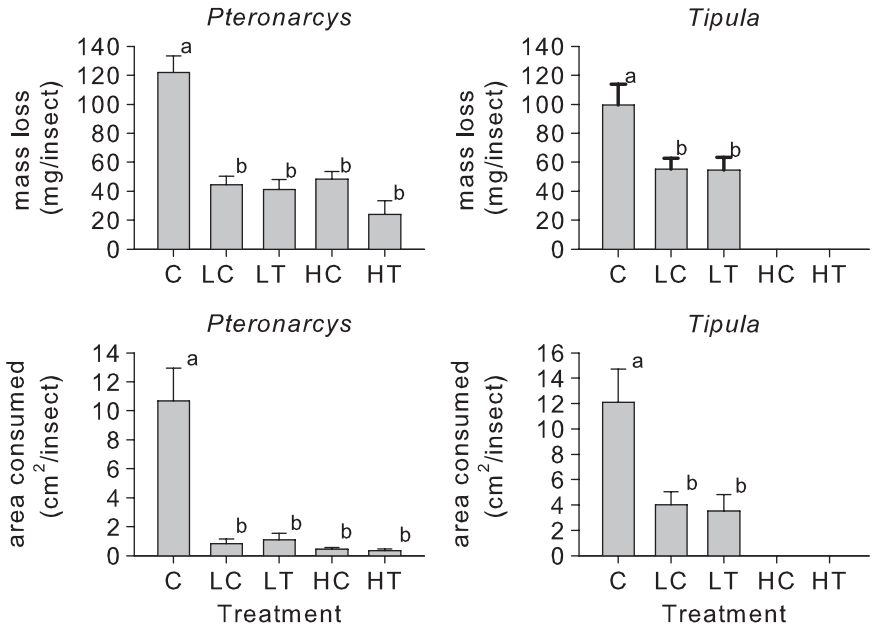

Fig. 1. Mean (±SE, $n = 5$) leaf decomposition (top panels: mass loss, bottom panels: area consumed) in aquatic microcosms containing control leaves only (C), and in selection microcosms containing control leaves (LC) and leaves from low-dose trees (LT), and containing control leaves (HC) and leaves from high-dose trees (HT). Bars with different letters are significantly different (Tukey's test $p < 0.05$).

Table 2
Mean (±SE, $n = 5$) mortality of earthworms in terrestrial microcosms at days 29 and 35 of the 35-day experimental period

Day	Treatment	% Mortality
29	Control	0
	Low	0
	High	0
35	Control	0
	Low	30 (12.2)
	High	10 (10.0)

Treatments are control = leaves from non-treated trees, low = leaves from control and low-dose trees (average concentration 31 μg/g), high = leaves from control and high-dose trees (average concentration 135 μg/g).

significantly reduced consumption of control leaves in selection microcosms indicates that the invertebrates consumed portions of imidacloprid-containing leaves and the toxic effects of that leaf material were sufficient to inhibit further feeding. We do not interpret the higher breakdown of control leaves than imidacloprid-containing leaves in earthworm selection microcosms as an indication of detection and avoidance of imidacloprid by earthworms because leaf consumption overall in those microcosms was reduced in comparison to the consumption of leaves in control microcosms. In those instances, earthworms may have fed for some time on control leaves before encountering and feeding on imidacloprid-containing leaves resulting in a tendency for higher consumption of control leaves in some selection microcosms. The fact that measurable amounts of imidacloprid-containing leaves in earthworm microcosms were consumed indicates that the earthworms could not detect and avoid the leaves, but rather consumed some imidacloprid-containing material. Leaf consumption by earthworms overall in selection microcosms was significantly less than in control microcosms, indicating sub-lethal feeding inhibition effects. Sub-lethal feeding inhibition effects of imidacloprid have previously been demonstrated in the pest insect tobacco whitefly (Nauen et al., 1998) and in other non-target aquatic invertebrates (Alexander et al., 2007).

It is possible that the feeding inhibition in aquatic selection microcosms resulted from exposure to aqueous imidacloprid concentrations leaching out of the leaf material, rather than from exposure through consumption of imidacloprid-containing leaves. However, we showed in previous experiments that even when 12 leaves containing ~80 μg/g imidacloprid were added to similar microcosms (same volume of water), maximum aqueous concentrations were only ~0.010–0.020 μg/mL (Kreutzweiser et al., 2007). In the present experiment at the low concentration where feeding inhibition was detected, only 3 leaves with ~18 μg/g were added to each selection microcosm so it was expected that resulting aqueous concentrations would have been at least 10 times lower (~1–2 μg/L). Stoughton et al. (2008) reported LC_{25} and EC_{50} (growth) concentrations of ~3 μg/L for other aquatic invertebrates, but we expect (based on our previous experiment in which leaching was measured) that aqueous concentrations leaching from treated leaves in selection microcosms would be lower than the values reported by Stoughton et al. (2008) and would not likely have been affecting feeding rates. Even if higher, effective aqueous concentrations did occur at or near the leaf surfaces, this effect could also occur in natural systems and the consequences in terms of effects on feeding rates are likely to be the same. The fact that feeding inhibition also occurred in terrestrial selection microcosms is further evidence that the feeding inhibition in all selection microcosms resulted from consumption of imidacloprid-containing leaf material.

The results from these microcosm tests may have implications for adverse effects on leaf litter breakdown in forest floors and adjacent water bodies where trees have been treated with imidacloprid. It appears that even when alternative food sources are available, leaf consumption by detrivorous invertebrates can be inhibited if they encounter imidacloprid-containing leaves. Since many of the landscapes where invasive insect pests are particularly problematic include riparian forest corridors of municipal and agricultural areas, these adverse effects may be especially important in streams and shoreline areas where leaf litter inputs have been shown to drive aquatic ecosystem

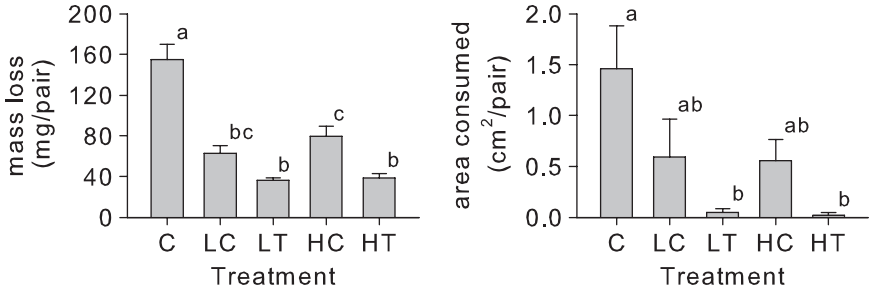

Fig. 2. Mean ($\pm$SE, $n = 5$) leaf decomposition (left panel: mass loss, right panel: area consumed) in terrestrial microcosms containing control leaves only (C), and in selection microcosms containing control leaves (LC) and leaves from low-dose trees (LT), and containing control leaves (HC) and leaves from high-dose trees (HT). Bars with different letters are significantly different (Tukey's test $p < 0.05$).

structure (Wallace et al., 1997). Adverse effects on leaf litter breakdown, in turn, have negative implications for organic matter processing, nutrient cycling, and the support of detritus-based food webs (Petersen and Cummins, 1974; Hattenschwiler et al., 2005).

5. Conclusions

The extent to which leaves from imidacloprid-treated trees will pose a risk of harm to non-target decomposer invertebrates and litter breakdown processes in natural systems will depend on the level of exposure. Our results indicate that imidacloprid at realistic concentrations in leaves that fall from systemically treated trees can inhibit litter breakdown processes through adverse sub-lethal effects on decomposer invertebrates. If the likelihood of decomposer invertebrates encountering imidacloprid-containing leaves is high, such as in riparian areas where a high percentage of trees have been treated with imidacloprid, then ecologically significant adverse effects on litter breakdown processes could occur. Because of this potential risk of harm to non-target decomposer organisms and processes, alternatives to imidacloprid should be explored for control of invasive forest insect pests. While 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 are likely to be much greater if large areas of trees are allowed to become infested and die, or to be cut down and destroyed as a pest eradication strategy.

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