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Environmental safety to decomposer invertebrates of azadirachtin (neem) as a systemic insecticide in trees to control emerald ash borer

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ABSTRACT

The non-target effects of an azadirachtin-based systemic insecticide used for control of wood-boring insect pests in trees were assessed on litter-dwelling earthworms, leaf-shredding aquatic insects, and microbial communities in terrestrial and aquatic microcosms. The insecticide was injected into the trunks of ash trees at a rate of 0.2 g azadirachtin cm^{-1} tree diameter in early summer. At the time of senescence, foliar concentrations in most (65%) leaves where at or below detection (<0.01 mg kg^{-1} total azadirachtin) and the average concentration among leaves overall at senescence was 0.19 mg kg^{-1}. Leaves from the azadirachtin-treated trees at senescence were added to microcosms and responses by test organisms were compared to those in microcosms containing leaves from non-treated ash trees (controls). No significant reductions were detected among earthworm survival, leaf consumption rates, growth rates, or cocoon production, aquatic insect survival and leaf consumption rates, and among terrestrial and aquatic microbial decomposition of leaf material in comparison to controls. In a further set of microcosm tests containing leaves from intentional high-dose trees, the only significant, adverse effect detected was a reduction in microbial decomposition of leaf material, and only at the highest test concentration ($\sim$6 mg kg^{-1}). Results indicated no significant adverse effects on litter-dwelling earthworms or leaf-shredding aquatic insects at concentrations up to at least 30 $\times$ the expected field concentrations at operational rates, and at 6 $\times$ expected field concentrations for adverse effects on microbial decomposition. We conclude that when azadirachtin is used as a systemic insecticide in trees for control of insect pests such as the invasive wood-boring beetle, emerald ash borer, resultant foliar concentrations in senescent leaf material are likely to pose little risk of harm to decomposer invertebrates.

1. Introduction

The emerald ash borer (*Agrilus planipennis*) is an exotic, invasive wood-boring insect pest in North America that is causing extensive ash tree (*Fraxinus* spp.) mortality across an area extending from southwestern Ohio, USA to southwestern Quebec, Canada, and is spreading rapidly (Cappaert et al., 2005). Owing to its rapid spread, the difficulties in early detection, and the scarcity of natural pathogens, predators and parasites, this invasive beetle poses a threat of nearly complete loss of ash trees from urban and rural landscapes with consequential ecological and economic impacts (Poland and McCullough 2006; Gandhi and Herms, 2010; Kovacs et al., 2010). For example, Kovacs et al. (2010) estimate the cost of dealing with dead ash trees over the next decade in USA urban settings alone will exceed 10 billion dollars, while the ecological costs could include the long-term disruption and cascade of changes in forest ecosystem structure and the loss or reduction in some fundamental ecosystem processes (Ellison et al., 2005).

Among the options being explored to slow the spread of the emerald ash borer and other wood-boring insect pests is the use of systemic insecticides to protect high-value trees and stands, and to reduce the source of adult (mobile) beetles from infested trees (Poland et al., 2006; McKenzie et al., 2010; Mercader et al., 2011). Systemic trunk injections of insecticides offer the environmental benefit of specific, targeted applications to reduce overall environmental exposure, and may be particularly well suited for environmentally sensitive areas such as riparian (shoreline) forests, wooded wetlands, urban parks and conservation areas. However, systemic injections in trees will result in foliar insecticide concentrations that could pose a risk of harm to non-target decomposer organisms when autumn-shed leaves fall to forest floors or adjacent water bodies. Given that invertebrate-mediated decomposition of leaf litter is a critical forest and aquatic ecosystem

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process (Graça, 2001; Hattenschwiler et al., 2005), adverse insecticide impacts on decomposer invertebrates could have ecologically significant implications for leaf litter breakdown and nutrient cycling in detritus-based food webs of forest floors and nearby aquatic ecosystems. The risk of harmful insecticide effects on decomposer organisms and processes will depend on the level of exposure (the insecticide concentrations in leaves at senescence and number of trees treated in a given area) and on the toxicity of the insecticide at those concentrations.

One of the systemic insecticides that is registered and recommended for emerald ash borer in the USA is the chloro-neonicotinyl insecticide, imidacloprid (Poland et al., 2006; Smitley et al., 2010). We previously showed that when imidacloprid was applied to trees for wood-borer control, it resulted in foliar concentrations at senescence that significantly reduced the decomposition of leaf litter in terrestrial and aquatic microcosms (Kreutzweiser et al., 2007, 2008). We further determined that the reduced litter decomposition resulted from sub-lethal, antifeedent effects on litter-dwelling earthworms and leaf-shredding aquatic insects, and from significant inhibition of terrestrial and aquatic microbial decomposition activity (Kreutzweiser et al., 2009). Those studies suggested that imidacloprid may not be the best option for a systemic insecticide in environmentally sensitive areas because of its potential adverse effects on decomposition processes.

As an alternative to imidacloprid, an azadirachtin-based systemic insecticide is being evaluated in Canada for control of emerald ash borer and other insects (Helson et al., 2001; McKenzie et al., 2010). Azadirachtin is a natural tetranortriterpenoid compound derived from seed kernels of the neem tree (*Azadirachta indica*) that has been shown to express insecticidal activity and be highly effective against a number of insect pests (Schmutterer, 1990; Ascher, 1993). Although the toxicological profile of azadirachtin is generally favorable (Boeke et al., 2004; Stark, 2007; Thompson and Kreutzweiser, 2007), its potential for non-target effects has not been assessed in the context of a systemic insecticide for forest insect pests. Given the rapid uptake and translocation of azadirachtin in trunk-injected ash trees and the presence of foliar concentrations at biologically active concentrations (McKenzie et al., 2010), we hypothesized that azadirachtin as a systemic insecticide could pose measurable risk of harm to non-target decomposer invertebrates at realistic foliar concentrations, similar to our findings for the imidacloprid assessment. Therefore, following similar field applications and microcosm experiments to those used previously for our assessment of imidacloprid, we determined the effects of leaves collected from azadirachtin-treated trees at senescence on litter-dwelling earthworms, leaf-shredding aquatic insects, and microbial community decomposition activity in terrestrial and aquatic microcosms.

2. Materials and Methods

2.1. Experimental treatments

Two separate microcosm experiments were conducted following the same protocols but using leaves from separate tree injections. The first experiment (Experiment 1) was conducted using leaves collected from ash trees treated at an operational rate at the recommended application time (early summer) for emerald ash borer control and thus represented foliar concentrations from a realistic operational setting. The second experiment (Experiment 2) was conducted using leaves collected from a separate set of ash trees treated at the operational rate but in early autumn (September) to reduce the azadirachtin dissipation time and thus intentionally result in higher foliar test concentrations.

2.2. 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 16-day experimental period, and water temperatures ranged 19–22 °C, pH was 7.3–7.6, specific conductance was 66–98 μS/cm, and dissolved oxygen was held near saturation (7.8–8.9 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 hardwood 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 19–23 °C and relative humidity was 45–76% 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., tested together with 9 individuals of each taxon in each microcosm. Decomposer invertebrates in the terrestrial microcosms were litter-dwelling earthworms, *Eisenia fetida*, obtained from a commercial supplier (Cathy's Crawly Composters, Bradford, Ontario). The earthworms were tested in pairs with two clitellate (light-colored band present indicating sexual maturity) worms impartially allocated to each microcosm.

2.2.1. Experiment 1: leaves from operational field trial

Leaves were collected at senescence just before leaf-fall (early October) from azadirachtin-treated ash trees and from non-treated ash trees (to serve as controls). Azadirachtin-treated trees were stem-injected with TreeAzin™ by an EcoJect System® (BioForest Technologies Inc., Sault Ste Marie, Ontario) at the recommended operational rate of 0.2 g azadirachtin cm^{-1} diameter at breast height in early summer (end of June). The trees were on average 20 cm diameter and 9 m high. The leaves were sealed in plastic bags and held on ice in the dark for transfer to the laboratory where there were placed in a dark, 2 °C storage chamber before addition to the microcosms. The leaves were held in cold storage for 30 days for the aquatic microcosms and 45 days for the terrestrial microcosms. A previous study demonstrated that azadirachtin in leaf tissue was stable for at least three months under dark cold storage conditions (Grimalt et al., in press).

For aquatic microcosms, batches of leaves were collected from among 6 treated trees. The batches consisted of 24 individual leaflets collected from a specific area of a tree (i.e., collected from a few compound leaves in close proximity and at the same location within a tree), each batch collected, labeled and stored separately. This was intended to provide a range of azadirachtin concentrations among leaf batches, assuming differences in uptake among trees and within a tree. For terrestrial microcosms, the leaves were collected at the same time using the same approach but in smaller batches of 14 leaves. Leaves from two nearby non-treated ash trees were collected in a similar fashion to be used as controls.

Leaf batches from non-treated trees were added to five aquatic microcosms and five terrestrial microcosms to serve as controls. Leaf batches from treated trees were added to 20 aquatic microcosms, and 16 terrestrial microcosms, hereafter referred to as treated microcosms. Each aquatic microcosm received 12 whole leaves, held in three groups of four leaves by a plastic clip, and placed on the bottom substrates. Each also contained a plastic cup, screened on both ends with 0.5-mm mesh to exclude the aquatic insects, and containing 10 leaf disks (23-mm diameter) cut from additional leaves in the same batch for each microcosm. Leaf material in the plastic cup was used to measure mass loss by microbial decomposition. Each terrestrial microcosm received eight half-leaves (the other halves were analyzed for azadirachtin concentration) that were placed just below the surface of the litter. In a manner similar to the setup for the aquatic microcosms, a 0.5-mm mesh pack containing five leaf disks cut from additional leaves in the same batch was placed in the litter below the leaf pieces to measure mass loss by microbial decomposition.

2.2.2. Experiment 2: leaves from intentional high-dose trees

For the second experiment, leaves were collected at senescence just before leaf-fall (early October) from Tree-Azin™-treated ash trees that were injected in September and from non-treated trees that served as controls. The trees were on average 5 cm in diameter and three trees were stem-injected at a rate of 0.2 g azadirachtin cm^{-1} diameter at three sequential times, one week apart (1 tree each week) using the same EcoJect System®. This was to provide three trees