



To treat or not to treat: Diminishing effectiveness of emamectin benzoate tree injections in ash trees heavily infested by emerald ash borer

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ABSTRACT

Emerald ash borer (EAB), a non-native invasive tree-boring beetle, is the primary agent behind the widespread mortality of ash trees (*Fraxinus* spp.) in both natural forests and urban areas of North America. While a variety of insecticide options have been adopted for protection against EAB attacks, little has been reported on the success of insecticide treatments on EAB-infested trees. This is particularly important because EAB is difficult to detect in early stages of its infestation and protective treatment may be undertaken on trees already in decline. Here, we investigated the effectiveness over a four-year period of biennial emamectin benzoate injections in trees exhibiting different initial EAB infestation levels (estimated using visual ash canopy condition metrics) in an ash forest in central Ohio. Results indicate that emamectin benzoate treated trees exhibited less canopy decline relative to non-treated control trees over the course of the experiment. In fact, all untreated trees died over the course of the four-year experiment, while on average treated trees did not exhibit a significant decline. Furthermore, initially healthy and moderately EAB impacted trees treated with insecticide either maintained or improved their canopy condition, while initially heavily EAB impacted trees stabilized, declined slightly, or died. Our results suggest that by using trunk injections of emamectin benzoate, private and public landowners may effectively be able to preserve lightly or moderately EAB infested trees or delay the replacement of ash trees at varying levels of EAB infestation.

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Introduction

Disturbances caused by non-native and native pests and pathogens can cause extensive environmental and economic damage to trees in both urban and natural environments, with the costs of non-native species alone estimated at ~\$138 billion USD annually in the United States (Pimentel et al., 2005). In recent years, the inadvertent introduction of emerald ash borer (*Agrilus planipennis* Fairmaire; EAB) has shed light on the importance of tree protection from such disturbances. Relative to other phytophagous insects, phloem and wood-boring insects like the EAB cause the greatest degree of economic damage in North America (Aukema et al., 2011). In fact, the direct and indirect costs solely associated

with EAB may be upwards of \$1.7 billion USD per year (Aukema et al., 2011). Serving as both an economic and aesthetic commodity, the potential loss of ash trees in urban and natural environments represents an increasingly pressing concern for public and private landowners. Trees in urban and natural areas provide a variety of ecosystem services including the removal of carbon dioxide and other airborne pollutants, nutrient retention, water filtration, stormwater management, and microclimate regulation (Costanza et al., 1997; Bolund and Hunhammar, 1999; Flower and Gonzalez-Meler, 2015). Additionally, homeowners place emotional, aesthetic and other subjective values on trees and neighborhood tree cover has been linked to increased home values (Harris et al., 2013; Sander et al., 2010). As a consequence, EAB-induced ash decline has increased awareness and environmental stewardship in human populations surrounded by landscapes experiencing extensive ash mortality (Hunter, 2011). Maintaining the health of susceptible tree species vulnerable to species-specific pests and pathogens using insecticides can promote tree diversity in urban areas and may contribute to increased forest resilience (Peterson et al., 1998).

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Effective insecticides provide an alternative tool to homeowners and local communities to manage the health of their tree populations (McKenney and Pedlar, 2012).

Emerald ash borer is a phloem feeding beetle native to Asia, which was accidentally introduced into North America in the 1990s and has since spread to two Canadian provinces and 25 states across the United States resulting in the widespread decline of ash trees (Siebert et al., 2007; Wang et al., 2010; Flower et al., 2013a). The serpentine galleries created in the cambium of ash trees by EAB larvae sever conductive tissue between roots and shoots resulting in tree mortality in as little as two years, although typically mortality occurs in 2–5 years (Flower et al., 2013b). At the tree level, the presence of EAB can be identified by characteristic D-shaped exit holes, woodpecker holes, epicormic growth, and canopy decline (Cappaert et al., 2005; Smith, 2006; Flower et al., 2013b, 2014). In EAB-infested regions, tree-level canopy health condition (or tree decline) is well correlated with tree-level EAB densities (Flower et al., 2013b), however ash trees with low EAB densities seldom exhibit symptoms, making them difficult to distinguish from non-infested trees and treat with insecticides before infestation takes place.

In the conterminous United States, urban counties (metropolitan areas) contain over 74.4 billion trees which cover ~33 percent of these areas (Dwyer et al., 2000). Many of these urban and residential trees are ash trees (Kovacs et al., 2009), which were intentionally planted due to their tolerance of high pH, salinity, drought and compacted soils (MacFarlane and Meyer, 2005). Prior to the EAB outbreak in Chicago, for example, ash trees ranked as the second most common species across the Cook and DuPage County study area (19.4% of trees) (Nowak, 1994). The widespread distribution of ash trees and their high abundance in urban areas highlights the magnitude of potential economic, environmental, and emotional costs associated with EAB induced ash mortality. In light of the EAB outbreak, landowners and managers in urban and residential areas have three options regarding tree management: (1) repeatedly treat infested tree with insecticide, (2) remove and perhaps replace the infested tree with a non-ash species, and (3) allow the tree to succumb to EAB and remain in place. While a variety of effective insecticide treatments have been deployed to maintain tree health during an EAB outbreak (Table 1), it is commonly thought that because of treatment costs and mixed success, trees with declining canopies should not be treated (Herms et al., 2014;

McKenney and Pedlar, 2012). However, as long as plant vasculature remains intact insecticide treatment has the potential to slow the senescence of trees in later stages of decline and may reduce or delay capital expenditures associated with removal and replacement. Furthermore, due to the risks to people and property in urban and residential areas, leaving standing dead trees across the landscape is not plausible. If effective, insecticide treatment of infested trees can provide alternatives to managers and citizens by delaying or reducing ash mortality in areas of interest.

The objectives of this research were to investigate the response of EAB infested ash trees in different stages of EAB infestation to biennial emamectin benzoate insecticide injections over four years from 2010 to 2013. Information regarding the survival of emamectin benzoate injected ash trees across different canopy condition classes will allow for informed cost-effective decisions based on the value placed at the individual tree or stand level by the local communities.

Methods

Site description

This study was initiated in May 2010 in an ash plantation with a silty loam soil characterization located at the United States Department of Agriculture (USDA) Forest Service Northern Research Station near Delaware, Ohio (40°23'N, 83°02'W). The plantation was established on an agricultural field and subsequently left unmanaged. At the start of the experiment canopy trees were on average ~35 years old (determined dendrochronologically). The forest is at an elevation of 290 m above sea level and its average precipitation from 2006 to 2010 was 92.3 cm per year. Upon initial inspection of the forest in 2010, the presence of D-shaped exit holes on ash trees (>80% of ash trees exhibiting exit holes) and the positive trapping of EAB with purple panel traps hung in the canopy (28 EAB captured from May–August 2010) indicated that EAB was present. The forest canopy is dominated by *Fraxinus americana* L. (white ash) and *F. pennsylvanica* Marshall (green ash), which together represent ~80% of tree basal area, as well as *Ulmus americana* L. (American elm), *Tilia americana* L. (basswood), and *Morus rubra* L. (mulberry). The understory is dominated by *Lonicera mackii* (amur honeysuckle), *Multiflora rosea* (multiflora rose), *Alliaria petiolata* (garlic mustard), *Poa* spp. and saplings of the canopy species.

Table 1

Insecticide products, application methods, timing and effectiveness for treating *Fraxinus* sp. trees for emerald ash borer control.

Active ingredient	Insecticide formulation	Application method	Application timing	Effectiveness
Acephate	Orthene	Trunk Spray	Spring-Early Summer	Low to Moderate ^d
Bidrin®	Inject-A-Cide B®	Trunk Injection	Spring-Early Summer	Low to Moderate ^{a,f}
Carbaryl	Sevin®	Trunk Spray		
Dinotefuran	Safari™	Trunk Spray	Spring-Early Summer	Moderate ^c
Emamectin Benzoate	TREE-äge™	Trunk Injection	Spring-Early Summer	High ^{b,c,e}
Imidacloprid	Bayer Advanced Tree & Shrub Insect Control [†]	Soil Drench	Fall-Spring	Mixed ^d
Imidacloprid	IMA-jet®	Trunk Injection	Spring-Early Summer	High ^d
Imidacloprid	Imicide®	Trunk Injection	Spring-Early Summer	Moderate ^{c,f}
Imidacloprid	Merit®	Soil Injection/Drench	Spring-Early Summer	Mixed ^{d,e,f}
Imidacloprid	Xytect™	Soil Injection/Drench	Spring-Early Summer	Mixed ^d
Imidacloprid	Macho 2F	Trunk Spray	Spring-Early Summer	Moderate ^c
Pyrethroid (Bifenthrin)	Onyx	Trunk/Foliar Spray	Biannual Application (spring and summer)	Moderate ^d
Pyrethroid (Cyfluthrin)	Tempo SC Ultra	Whole Tree spray	Spring-Early Summer	Moderate ^d
Pyrethroid (Cyfluthrin)	Pointer	Trunk Injections	Spring-Summer	Moderate ^{d,f}

[†] Appropriate for homeowner application.

^a McCullough et al. (2004).

^b Flower et al. (in this issue).

^c McCullough et al. (2011).

^d Herms et al. (2014).

^e Smitley et al. (2010).

^f McCullough et al. (2003).

The experimental plots were located >10 m from the edge of the forest to reduce edge effects. The forest was divided into 12 m × 12 m plots and insecticide ($n=4$ plots, containing 32 ash trees) and control ($n=3$, containing 21 ash trees) treatments were randomly assigned to plots. All ash trees >7 cm diameter at breast height (DBH) were measured. Pre-treatment DBH (control = 16.43 ± 1.37 and insecticide = 17.21 ± 1.28 ; mean cm $\pm$ SE) and ash canopy condition (AC; control = 2.23 ± 0.22 and insecticide = 1.94 ± 0.18) were not different between control and insecticide treated plots (t -test; $P > 0.05$). Ash trees >7 cm DBH residing in the insecticide treatment plots were treated in the spring (May 2010 and 2012) with trunk injections of emamectin benzoate (TREE-äge™, Arborjet Inc, Woburn, MA, USA) in quantities recommended by the manufacturer (0.2 g active ingredient for every 2.5 cm DBH). Injection plugs (Arborplug # 4 for trees <10 cm DBH and # 5 for trees >10 cm DBH) were installed evenly around the base every 5 cm at a height of no more than 30 cm from the soil. Areas exhibiting decay were avoided. Injections were conducted using the Arborjet Quik-Jet system (Arborjet Inc.). Ash trees within the control plots were not exposed to any experimental manipulation, but were exposed to moderate natural EAB densities.

Emamectin benzoate

Emamectin benzoate is a systemic insecticide from the avermectin family of macrocyclic lactones which acts as a chlorine channel agonist (Lasota and Dybas, 1991). This insecticide was selected for the study as it has been shown to be highly effective at tree level protection from bark beetles, including mountain pine beetle, spruce beetle and western pine beetle (Herms et al., 2014; Grosman et al., 2009; Smitley et al., 2010; Fettig et al., 2013). Emamectin benzoate is thought to provide two levels of protection against EAB. First, larvae will be exposed if compounds diffuse from the xylem to the phloem or if larvae penetrate the xylem during feeding. Second, as emamectin benzoate is transported in the xylem to the canopy adult EAB feeding on leaf tissue will be exposed. A study by (McCullough et al., 2011) found emamectin benzoate trunk injections reduced EAB larval densities by almost 100% in treated trees compared to non-treated trees and resulted in near 100% mortality of adults in bioassay feeding trials. Additionally, a study by (Herms, 2011) indicated that while emamectin benzoate injections provided effective control for 2 years (<10% decline) at several application rates, efficacy past 2 years was less successful. Despite these findings, it remains unknown whether biennial emamectin benzoate application can provide control to infested trees over a wide range of tree diameters and canopy condition classes for extended time periods (>2 years), particularly those in later stages of decline >50% (AC4).

Ash canopy condition monitoring

Annually (2010–2013), following leaf expansion, each ash tree from the study was evaluated for EAB infestation using the visual assessment of canopy health called ash canopy condition (AC) developed by the USDA (Knight et al., 2014; adopted in part from Ball and Simmons, 1980; Fig. 1). Ash canopy condition exhibits a significant relationship with tree-level EAB densities (larvae/adult) and EAB gallery cover (determined via quantification of EAB larval gallery cover in 2–100 cm² grids on the north and south side of 1 m long debarked logs) representing a visual proxy for EAB infestation levels in infested regions not exposed to ash yellows or other tree stress (Flower et al., 2011, 2013b). Briefly, AC is graded on a 1 (healthy) to 5 (dead) scale: (1) healthy/full canopy—a healthy ash canopy is full and exhibits no defoliation; (2) thinning canopy—slight reduction in leaf area, all top branches exposed to sunlight have leaves; (3) dieback—canopy is thinning and some top

branches exposed to sunlight are defoliated, lower branches which exhibit natural thinning are not considered; (4) >50% dieback—the canopy has less than 50% of the leaves of a class 1 tree and/or over half of the top branches are defoliated; and (5) dead canopy—no leaves remain in the canopy portion of the tree, regardless of epicormic sprouts (Knight et al., 2014). This canopy condition rating system was utilized in contrast to the 10% canopy decline system used by (Smitley et al., 2008), because it was developed for easy visual canopy classification by untrained individuals (such as homeowners and land managers) and thus minimizes observer bias (in excess of 20% in the middle range in the Smitley scheme). Furthermore, the proposed scheme is established and in widespread use by the USDA Forest Service and has been linked to EAB densities and backed by neutral tree physiological characteristics such as water use, sapflux, and foliar carbon isotope ratios (Flower et al., 2011, 2013b; Knight et al., 2014). Therefore our scheme and the results of the study are directly applicable to practitioners, planners and the scientific community.

Statistical analyses

To determine whether pre-treatment mean tree diameter (DBH) or initial ash canopy condition (AC) differed between ash trees in the control and insecticide treatment groups, we used two-tailed t -tests ($P < 0.05$) with homogeneity of variance between treatments. No significant differences were observed between treatments for DBH or initial AC ($P=0.579$ and $P=0.205$ respectively) permitting further analysis between treatments. To test for differences in the change in ash tree canopy condition (ΔAC ; $AC_{2010}-AC_{2013}$) between treatments, we used an analysis of covariance (ANCOVA; $\alpha=0.05$) with DBH treated as a covariate. An a priori test of the mean ΔAC of trees in the insecticide treatment group and zero was conducted to determine if there was significant decline in the mean canopy condition ($\alpha=0.05$). We also conducted a repeated measures analysis of variance (RM ANOVA) test in which treatments (control v. insecticide) were treated as the between subjects effect and annual ash canopy conditions as the repeated measure. While the ΔAC approach only considers shifts in tree health between the beginning and end of the experiment, the RM ANOVA examines the changes in tree condition each year throughout the treatment period. Because significant differences were found in the canopy decline between insecticide treated trees and control trees, we conducted a follow-up analysis excluding the control treatment to test our hypotheses regarding the differential decline in ash trees with different initial canopy conditions. Here we conducted an ANCOVA with ΔAC as the dependent variable but the with initial ash canopy condition as a categorical effect and with DBH as a covariate. Post hoc Tukey's HSD pairwise comparisons ($P < 0.05$) were used to test differences between canopy decline ash trees with different initial canopy conditions. All statistics were analyzed using SYSTAT v. 12 statistical software (SYSTAT, 2007).

Results

Effectiveness of emamectin benzoate injections

The EAB-induced ash canopy decline (ΔAC) was significantly different between the control (untreated) and insecticide treatments (ANCOVA, $F_{1,50}=93.001$; $P < 0.001$), with canopies in the control treatment declining ($\Delta AC=-2.38$) more than the insecticide treatment ($\Delta AC=-0.41$) (Fig. 2). Canopies of all ash trees in the control group declined to AC 5 (dead) while only ~16% (5 trees) of trees in the insecticide treatment group declined to AC 5. The marginal but non-significant decline in mean ΔAC of insecticide treated trees over the course of the experiment indicates general

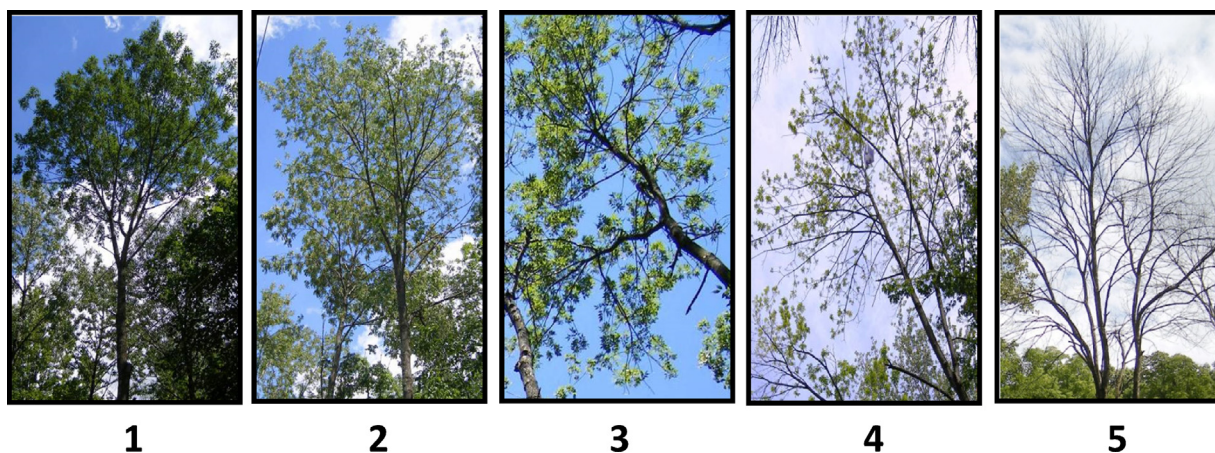


Fig. 1. Visual classification scheme denoting ash canopy condition class (AC) 1–5 (Knight et al., 2014).

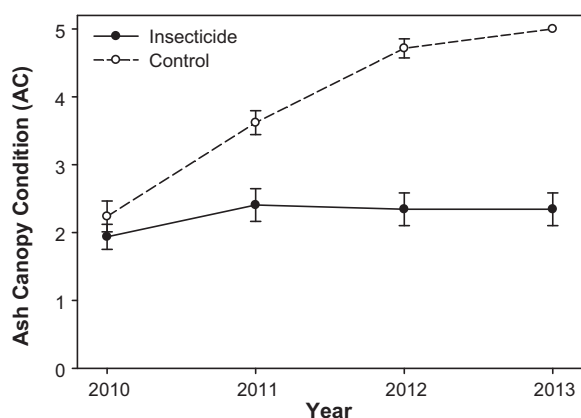


Fig. 2. Ash canopy condition class (AC) in the control and insecticide treated ash trees from 2010 to 2013. Values represent means $\pm$ SE.

success of the treatment; this decline was driven most strongly by trees in AC 3 and AC 4 (Fig. 3a and b). There was no relationship between initial DBH and tree decline across treatments (ANCOVA, $F_{1,50} = 1.213$; $P = 0.276$). Similarly, the RM ANOVA also revealed the significant treatment effect (RM ANOVA $F_{1,51} = 32.762$; $P < 0.001$) and time effect (RM ANOVA, $F_{3,49} = 46.686$; $P < 0.001$, Wilks's Lambda $P < 0.001$) (Fig. 2). The significant time effect indicates that there is a change in AC over time, an increase in AC this case, when averaged over treatment levels. There was a significant treatment $\times$ time interaction indicating a rapid decline in control trees over time relative to the insecticide treated trees (RM ANOVA, $F_{3,153} = 72.324$; $P < 0.001$).

Importance of initial canopy conditions on treatment effectiveness

In insecticide treated trees, ash canopy decline varied significantly among initial canopy condition classes (initial AC) (ANOVA, $F_{3,27} = 5.514$; $P = 0.004$; Fig. 3). Trees with lightly impacted canopies (AC 1 and 2) maintained canopy health relative to the other canopy condition classes (Fig. 3). While the average canopy condition in trees initially ranked AC 1 declined marginally, the shift was not significantly different from zero, and because these trees could not possibly improve in canopy condition this change was not surprising. Insecticide treated trees initially AC 2 showed the greatest improvement ($\Delta AC = 0.36$) relative to AC 3 and AC 4 which declined significantly over the course of the experiment despite treatment (Fig. 3).

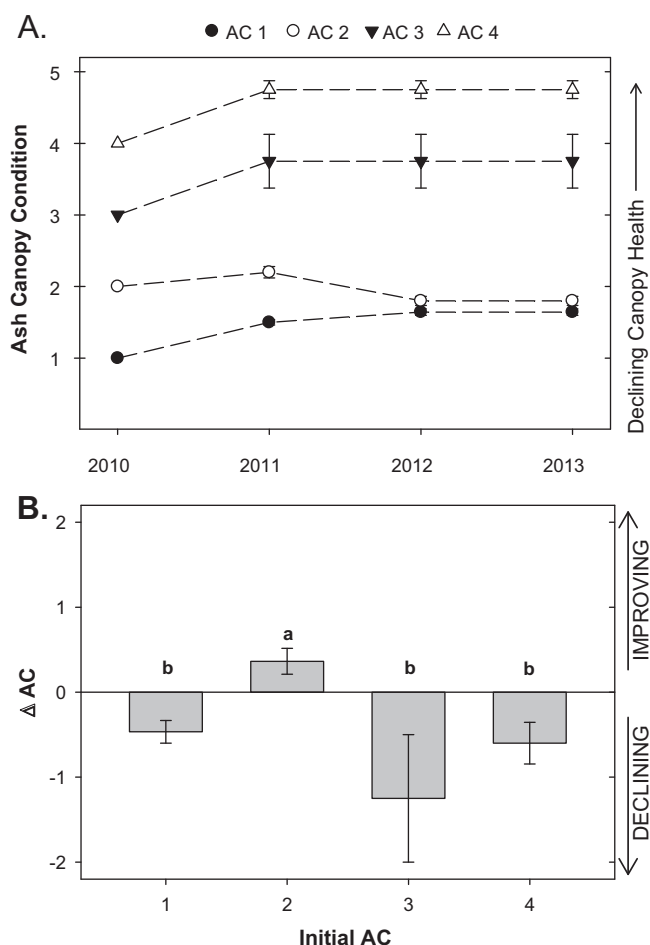


Fig. 3. (A) The relationship between initial ash canopy condition and the timeseries progression of each canopy condition class of insecticide treated trees over the course of the experiment and (B) the relationship between initial ash tree canopy condition (initial AC) and ash canopy decline over the course of the experiment (ΔAC ; $AC_{2010} - AC_{2013}$) (ANCOVA; $P < 0.001$). Error bars denote ± 1 SE. In panel B, letters represent significance levels determined by Post Hoc Tukey's HSD pairwise comparisons ($P < 0.05$).

Discussion

The results of our research indicate that biennial emamectin benzoate treatment of ash trees can successfully maintain or improve tree health in lightly and moderately EAB infested trees

(AC 1 and 2 respectively) over short to moderate periods. Overall, the mean canopy health of emamectin benzoate treated trees did not significantly change over the four-year course of the experiment. These findings are consistent with other studies which highlight the effectiveness of emamectin benzoate tree injections on EAB adults and larvae which exhibited nearly 99% mortality feeding on treated tissue (Herms et al., 2014; Smitley et al., 2010; McCullough et al., 2011). No relationship between treated ash tree size and canopy senescence was observed, indicating that trees ranging from 7 to 27.6 cm DBH (as studied herein) exhibited similar beneficial responses to the emamectin benzoate treatment. This result also suggests that systemic emamectin benzoate injections may be equally effective on larger trees than utilized in the study. Higher application rates of emamectin benzoate (0.4–0.6 g active ingredient for every 2.5 cm DBH) have been approved and are recommended for larger trees, although results indicate that treatment at these higher levels may not confer added protection (Smitley et al., 2010; Herms, 2011).

While previous research has indicated that emamectin benzoate can maintain ash tree health at low pretreatment EAB densities (McCullough et al., 2011), its effectiveness on trees in later stages of decline (AC4 or >50% canopy decline) was previously unknown. Our results confirm that emamectin benzoate injections can be used to stabilize canopy health in lightly impacted trees (AC 1) and highlight that it can improve trees exhibiting light to moderate EAB infestations (AC 2). Additionally, our results indicate the mixed success of spring emamectin benzoate injections on trees in later stages of decline (AC 3 and 4), with nearly 63% of treated trees in later stages of decline dying over the course of the experiment (Fig. 3). Our data reveals success in treating trees exhibiting canopy thinning and lends support to the theory that insecticide treatment of trees with >50% canopy decline may not be as successful as it is for trees with healthy canopies (Herms et al., 2014); yet 37% of the heavily impacted trees treated were able to recover or stabilize so it may be worth the investment to treat valuable landscape trees. Furthermore, the lower effectiveness of emamectin benzoate on trees with high EAB densities and in later stages of canopy decline is likely be attributed to their inability to distribute the compound from the injection site to the rest of the canopy because of reduced sapflux rates caused by EAB damage to tree vascular tissues (Flower et al., 2011).

Although the results of this study highlight the effectiveness of emamectin benzoate treatments at controlling EAB induced tree decline after a tree is infested, we reiterate current recommendations and suggest that trees should be treated before symptoms are evident for maximum efficacy (Herms et al., 2014). Spring treatment of vulnerable ash trees with emamectin benzoate and other insecticides (Table 1) has been demonstrated to be most effective at controlling EAB as the insecticide is distributed throughout the cambium and transmitted to the leaf tissue prior to adult emergence (Herms et al., 2014; McCullough et al., 2011). In EAB impacted areas, insecticide treatments of ash trees in residential areas will maintain tree health and help foster wellbeing of citizens and of the urban ecosystem (Hunter, 2011). To maintain ash across the landscape, homeowners and municipalities should recognize and plan for sustained biennial emamectin benzoate injections throughout the duration of the EAB outbreak to prevent future infestation.

Although treatment of ash trees may help maintain biogeochemical and hydrological cycling in EAB impacted regions (Fissore et al., 2012), insecticide use should be minimized as there can be negative consequences for non-target insects. Non-target insects may be impacted through three primary sources of exposure: (1) direct insecticide contact, although this is virtually eliminated with applications via tree injection systems, (2) residual uptake from another surface and (3) food chain uptake (Croft, 1990). Applications of insecticides via soil drenching and broadcast spraying

likely have the highest non-target impacts relative to injections. Soil drenches of imidacloprid have been shown to negatively impact beneficial soil-dwelling arthropods (Kreutzweiser et al., 2008a, 2008b). Although of minor concern because of the current recommended use patterns, its plausible that long-term application of emamectin benzoate for ash tree protection from EAB could result in the evolution of resistant EAB or other non-target insects, as has been observed in reduced sensitivity of salmon lice to emamectin benzoate (Lees et al., 2008; Espedal et al., 2013).

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