TreeAzin® Systemic Insecticide: Evidence for biennial Emerald Ash Borer treatments (Agrilus planipennis Fairmaire)

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Introduction

Emerald ash borer (EAB) (*Agrilus planipennis*), a wood boring beetle introduced to North America, attacks and kills ash (*Fraxinus* sp.) trees. Native to eastern Asia and known to occur in China, Korea, Japan, Mongolia, the Russian Far East, and Taiwan (McCullough and Katovich 2004), EAB was first discovered in North America in the summer of 2002, near Detroit, Michigan and Windsor, Ontario (Poland and McCullough 2006). EAB was likely introduced to North America via solid wood packing material from cargo ships or airplanes originating in Asia.

On July 24, 2008, the Canadian Food Inspection Agency (CFIA) confirmed the presence of EAB in the North Iroquois Ridge Community in the Town of Oakville, Ontario (CFIA 2008). No further surveys were conducted by CFIA, but Town staff was alerted to look for EAB symptomatic ash trees. Figure 1 presents a map of Oakville that shows where Oakville Forestry staff suspected the presence of EAB based on sightings of symptomatic trees during a 2009 tree inventory project. The 2009 observations (Figure 1) prompted the Town to conduct formal EAB detection and delimitation surveys starting in February of 2010. These detection surveys were a collaborative effort between the Town and the Canadian Forest Service (CFS), whose scientists had been conducting research to provide methodologies for early detection of EAB. The result of this research effort is a new EAB detection methodology that significantly improves the likelihood (>80%) of early detection of EAB in an area (Ryall *et al.* 2010).

Results of the 2010 detection survey were surprising and showed that EAB was more widely dispersed in Oakville than previously suspected. Branch samples showed that EAB populations were highest in the area of the original 2008 detection, but low populations were dispersed more widely than the 2009 surveys of symptomatic trees suggested.

In response to the detection of EAB in Oakville in 2008, the Town treated some 83 trees in the vicinity of the detection site with TreeAzin® Systemic Insecticide (5 ml/cm dbh) (dbh equals diameter at breast height or 4.5 ft/1.3 m above ground) to reduce EAB populations and protect trees. The objective was to protect high value ash trees along streets and in parks near the epicenter of the infestation and to reduce dispersal of EAB into uninfested areas of the Town. Research has since demonstrated that this was likely the best response at the time in terms of population management (Mercader *et al*. 2011).

TreeAzin is a biopesticide derived from seed kernel extracts of the neem tree (*Azadiractchta indica*: Meliaceae). Isman (1999) reported that azadirachtin, the active ingredient in TreeAzin, "functions primarily as in insect growth regulator, but also as a behavior-modifying substance, deterring feeding and/or oviposition in certain pest species." Isman (1999) also noted that "of equal importance neem has minimal toxicity to vertebrates, is soft on natural enemies and pollinators, and degrades rapidly in the environment."

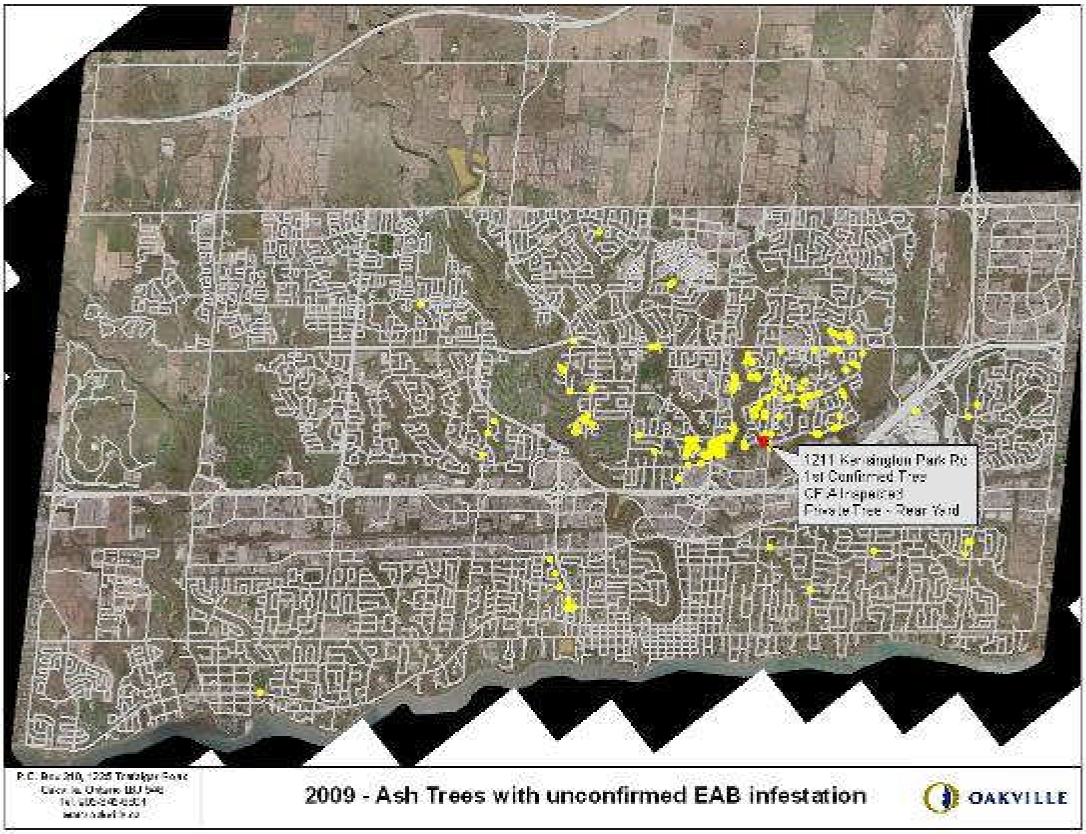

Figure 1. Emerald ash borer symptomatic trees in the Town of Oakville as of 2009.

Early studies conducted by CFS had shown that azadirachtin-based formulations were very effective against EAB when injected into the trunks of small diameter (Avg dbh = 2.2 cm) green ash infested with EAB (McKenzie *et al*. unpublished report). Analysis of azadirachtin residues in leaves from trees treated with the highest rate (54.5 mg a.i./cm dbh) were highest at seven days after treatment, the first post treatment sample date, and declined over the following 48 days. The authors reported that adult EAB that fed on leaflets of treated trees showed no outward negative adverse effects due to azadirachtin. McKenzie e*t al*. concluded that levels and/or location of azadirachtin inside the leaves were not sufficient to inhibit EAB adults from feeding. In terms of larval effects, they reported that newly colonizing EAB larvae were completely stopped from developing beyond the second instar and that control was effective even at the very low dose of 1.7 mg a.i./cm dbh.

Helson *et al.* (2006) repeated experiments with TreeAzin using larger diameter ash trees. Azadirachtin residues were assessed from foliage collected from green ash averaging 22 cm dbh and injected with 25 mg a.i./cm dbh. Results indicated:

- rapid uptake and translocation;

- mean foliar residues were similar to residues typically observed for other insecticides and injection techniques;
- total residue levels declined in a slow exponential fashion.

Efficacy assessments were conducted on small trees (mean dbh = 8 cm) and large trees (mean dbh = 37 cm) using two treatments: 10 mg a.i./cm dbh and 25 mg a.i./cm dbh. Results showed:

- significantly reduced (82% to 100%) numbers of emerged adults and exit holes in branches from TreeAzin-treated small and large diameter trees at both dosages;
- significantly reduced number of new exit holes on trunks for both dosages of TreeAzin on smaller trees, but only for the higher dose on larger trees;
- significantly higher crown densities for TreeAzin-treated small trees, but not for TreeAzin-treated large trees, as these trees were already under heavy EAB attack at the time of treatment.

TreeAzin treatments had no effect on adult male or female survival and did not reduce the numbers of females laying eggs. Helson *et al.* (2006) did report that fecundity in females fed treated leaves was reduced by 66% and that fertility was reduced by 98%.

An additional study on the effects of systemic TreeAzin treatments on EAB adult fecundity was conducted by Dr. D. Thompson (pers. comm.). Studies by McKenzie *et al*. (2010) and Grimalt *et al*. (2011) showed strong correlations between azadirachtin residues in treated tree foliage and significant inhibition of larval growth, development and emergence. Thompson *et al*. examined the biological significance of these residue levels in relation to maturation-feeding of adult EAB beetles. Their study showed that foliar azadirachtin concentrations of 10 mg/kg f.w. (i.e., fresh weight) consistently resulted in >95% reduction in fecundity and that azadirachtin concentrations of >2.5 mg/kg f.w. resulted in >70% reduction. The authors noted that these concentrations are routinely observed in foliage of white or green ash trees systemically injected with TreeAzin at rates of 20 mg a.i./cm dbh or higher.

Early field observations suggested that a single treatment with TreeAzin could provide two years of protection against the EAB. The mechanisms for two-year control were not completely understood, but laboratory and field data showed significant impacts to EAB fecundity and egg viability, as well as EAB larval mortality in treated trees.

Operational treatments using TreeAzin began in Oakville in 2008. Despite earlier observations and studies supporting biennial treatments, BioForest Technologies Inc. and the Town of Oakville conducted assessments in 2010 to assess EAB populations in trees first treated in August 2008. The objective of the study was to compare 2009 EAB population densities in trees treated with TreeAzin in 2008 to untreated control trees. Several surveys were conducted in February/March 2010 to assess the effectiveness of the TreeAzin treatments on the ash trees treated in 2008.

Methods

From August 19 to 28, 2008, the Town of Oakville treated some 83 ash trees within the infested area with TreeAzin: 19 in the parking lot area of Town Hall and 64 in the Golden Meadow Trail area. TreeAzin was injected into the base of the ash trees at a dose of 5ml/cm dbh using the tree injection system developed by BioForest known as the EcoJect® System. The trees were not treated again until the summer of 2010.

EAB population assessments were not conducted at the time of treatment in 2008, as survey methods were still under development. Visual examinations of the treated and control trees indicated that most were under attack by varying EAB populations in 2008. It is likely that EAB had been active in the area for several years prior to the initial detection. Trees with greater than 30% crown dieback were not treated.

In February and March 2010, several surveys were conducted by the Town and BioForest to assess EAB activity on trees treated with TreeAzin in August, 2008. All trees assessed were located within the area delineated as infested in 2008 and included both treated and control trees. Trees ranged in diameter (dbh) from 20 cm to 40 cm and in height from 6 m to 17 m. All were municipal trees adjacent to roads.

Survey 1: Early detection of EAB infestations has been difficult, but the presence of woodpecker feeding activity on ash has been reported as an effective method for detecting infestations and especially identifying specific trees under attack (OMNR, Cornell University Cooperative Extension). In February 2010, a total of 209 ash trees were assessed for signs of woodpecker feeding damage. This included the 83 trees treated with TreeAzin in 2008 and 126 untreated trees. Trees were simply rated for the presence or absence of woodpecker feeding damage.

Survey 2: A second more detailed survey was conducted on a subset of 25 treated and 25 untreated ash trees in the area around White Oak Drive, Golden Meadow Trail, and Ivy and Holly courts. Trees were more closely examined and rated for presence and relative abundance of woodpecker damage (0, 1-5, >5 feeding sites per tree).

A one-sample Chi-square test was used to determine whether the frequency of woodpecker feeding differed between treated and untreated trees.

Survey 3: In March 2010, two mid-canopy branches (approximately 1 m each) were collected from 15 treated and 15 nearby untreated ash trees (Ryall *et al*. 2011). To expose current EAB galleries, 25 cm of bark were removed from each end of each branch for a total of 60 treated branch samples and 60 untreated branch samples. New galleries were counted and the total length of each gallery was measured (cm). Branch diameter was recorded and used to calculate the number of EAB galleries per 100 cm^2 to standardize comparisons between treatments.

Results

Survey 1: Of the 83 treated trees assessed, woodpecker feeding damage was observed on two trees (Table 1). Woodpecker feeding sites were observed on 80 of the untreated trees and the remaining 46 showed no signs of woodpecker feeding.

Table 1. Observed ash trees with woodpecker feeding damage in February 2010 on trees treated with TreeAzin® Systemic Insecticide in August 2008 and untreated ash trees.

	Number of trees		
	TreeAzin	**No Treatment**	**Total**
Woodpecker feeding damage	2	80	82
No woodpecker feeding damage	81	46	127
Total	83	126	209

Survey 2: When ash trees were rated for presence and relative abundance of woodpecker activity, the obtained $X^2 = 26.298$, df = 1, was significant at the .01 level. There were significantly more untreated trees than TreeAzin-treated trees with woodpecker feeding damage. Also, the density of woodpecker feeding sites was greater on untreated control trees than on trees treated with TreeAzin (Figure 2).

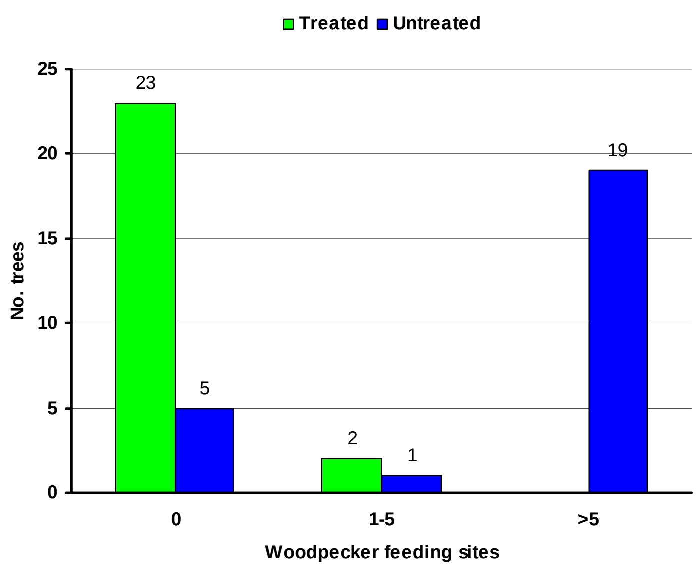

Figure 2. Comparison of woodpecker feeding site density in February 2010 on ash treated with TreeAzin® Systemic Insecticide in August 2008 and untreated ash.

Survey 3: When branches were removed from treated and untreated ash trees, and examined for 2009 EAB galleries, the number of ash branch samples with new EAB galleries was significantly ($p < .01$) lower on TreeAzin-treated trees than in untreated trees ($X^2 = 13.71$, df = 1) (Table 2). T-test analysis showed mean EAB galleries per 100

cm^2 were significantly ($p < .01$) lower in treated trees (Avg. = 0.09, SD = 0.27) than in the untreated controls (Avg. = 0.47, SD = 0.56).

Table 2. Frequency of emerald ash borer galleries in March 2010 in ash trees treated with TreeAzin® Systemic Insecticide (5 ml/cm dbh) in August 2008 and in untreated controls.

	New EAB Galleries		
	Treated	**Controls**	**Total**
Negative	45	25	70
Positive	15	35	50
Total branches	60	60	120

When the 2009 EAB galleries were measured, mean gallery lengths were significantly ($p < .01$) shorter on the treated trees (2.4 cm, SD = 1.7) than on the untreated trees (10.2 cm, SD = 5.5). The results showed that 2009 EAB did not complete development in trees treated with TreeAzin in 2008 (Figure 3).

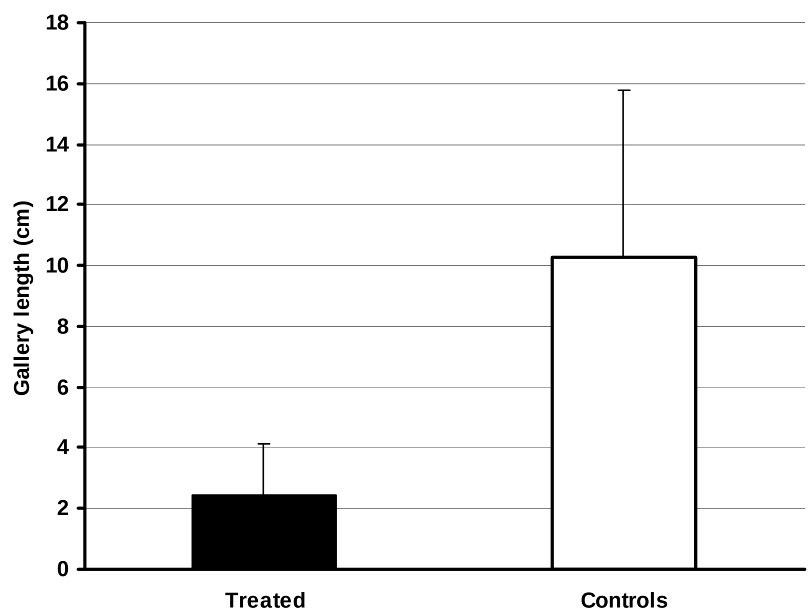

Figure 3. Average emerald ash borer gallery length in the year following treatment with TreeAzin® Systemic Insecticide (5 ml/cm dbh).

Conclusions

Ash trees treated with TreeAzin in Oakville in August 2008 were evaluated in February and March 2010 for evidence of EAB population control in year two. Results of the surveys conducted in 2010 showed:

- significantly lower incidence of woodpecker attack on treated trees than on untreated controls;
- on ash with woodpecker feeding damage, significantly lower density of feeding sites on treated trees than on controls;
- significantly fewer current EAB galleries per m^2 on treated trees than on untreated trees;
- significantly shorter mean EAB gallery length on treated trees than on untreated trees.

Previous studies showed that tree injections with TreeAzin at doses ranging from 1.7 mg a.i./cm dbh to 54.5 mg a.i./cm dbh effectively reduced larval survivorship in the year of treatment on small and large ash trees (McKenzie *et al.* unpublished, Helson *et al.* 2006 and McKenzie *et al*. 2010). Studies have also shown that treatment of trees with TreeAzin will significantly reduce adult female fecundity and egg viability. The result is that EAB populations are significantly reduced in treated trees in the year of treatment.

Results from the Oakville study showed that in 2009, the second summer following treatment, EAB larval densities were significantly lower on trees that had been treated in 2008 than on untreated trees. The surveys also showed that in 2009, EAB gallery lengths were significantly shorter on treated trees than on controls, indicating that larvae on treated trees failed to complete development in 2009. Early studies showed that EAB larvae were susceptible to very low doses of azadirachtin. We suggest that low concentrations of azadirachtin remained in the cambial tissues into the second year following treatment, thus preventing larval development and buildup of EAB populations to damaging levels in year two.

Results of these surveys show that injections of TreeAzin at the remedial dose (5 ml/cm dbh) in 2008 provided protection to EAB infested ash through 2009. These trees were re-treated in 2010 and remain in good health. Examples of treated and untreated ash trees are shown in Figure 4.

Discussion

Based on experimental results, Thompson *et al*. postulated that a plausible explanation for observations of two years of protection in trees treated with TreeAzin is that, in the year of treatment, there is:

- a substantial reduction in adult female fecundity and egg viability in females that feed on trees treated with TreeAzin (D. Thompson, pers. comm.);
- a significant reduction in larval development and adult emergence in trees treated with TreeAzin (McKenzie *et al*. 2010).

These effects would significantly reduce EAB populations and this reduction would carry over into year two. Populations would likely begin to recover by year three, so biennial injections with TreeAzin were recommended.

Figure 4. Examples of ash trees treated with TreeAzin® Systemic Insecticide (5 ml/cm dbh) in 2008 and 2010 to control emerald ash borer. Dead trees in foreground were not treated.

Results of the Oakville study support Thompson's hypothesis: EAB populations in year two were observed to be significantly lower on trees treated with TreeAzin. The observed failure of EAB larvae to complete development in year two ensures that EAB populations and damage to the tree will remain low in year two, and further strengthens the biennial treatment strategy.

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