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Evaluation of Xylem Discoloration in Ash Trees Associated with Macroinjections of a Systemic Insecticide

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Abstract. Emerald ash borer (EAB) (*Agrilus planipennis*), first identified near Detroit, Michigan, U.S., in 2002, has killed millions of ash trees (*Fraxinus* spp.) in 28 states and two Canadian provinces to date. Trunk injections of insecticide products containing emamectin benzoate (EB) (e.g., TREE-äge®) are often used to protect ash trees in landscapes from EAB, but wounds and potential injury resulting from injections are a concern. Researchers examined 507 injection sites on 61 trees and recorded evidence of secondary wounding (e.g., external bark cracks, internal xylem necrosis and pathogen infection). Researchers assessed 233 injection sites on 22 green ash and 24 white ash trees macro-injected with a low or a medium-high rate of EB in 2008 only, or in both 2008 and 2009. Only 12 of 233 injection sites (5%) were associated with external bark cracks and there was no evidence of pathogen infection. On 39 of the 46 trees (85%), new xylem was growing over injection sites. Researchers assessed 274 injection sites on 15 green ash trees injected annually with EB from 2008 to 2013 or injected in 2008 and again in 2011. Bark cracks were associated with four injection sites on three trees, but no evidence of injury was found on the other 12 trees. All 15 trees had new xylem laid over injection sites. Confocal laser scanning and polarizing digital microscopy were used to assess the integrity of discolored xylem tissue removed from the immediate area surrounding 140 injection sites on 61 trees. Researchers found no evidence of decay associated with discoloration.

Key Words. *Agrilus planipennis*; Bark Cracks; Emamectin Benzoate; *Fraxinus americana*; *Fraxinus pennsylvanica*; Green Ash; Insecticide; Macroinjection; Trunk Injection; White Ash.

Emerald ash borer (EAB), (*Agrilus planipennis*) (Coleoptera: Buprestidae), a phloem-feeding insect native to Asia, has killed hundreds of millions of native ash (*Fraxinus* spp.) trees in landscapes and forests since it was identified in southeast Michigan, U.S., and Windsor, Ontario, Canada, in 2002 (Cappaert et al. 2005). To date, populations of EAB have been found in at least 28 states and two Canadian provinces (EAB Information Network 2016). Economic costs associated with removing dead or dying landscape ash trees in urban and suburban landscapes can overwhelm municipal forestry budgets, as well as homeowners (Kovacs et al. 2010; McCullough and Mercader 2012; Kovacs et al. 2014). Soon after this invasive pest was identified, researchers began evaluating systemic insecticide options to protect valuable landscape ash trees. Control efforts typically target adult beetles, who feed on ash leaves throughout their 3–6-week life span, and larvae, which feed on phloem and cambium in serpentine galleries that generally score the outer sapwood (Cappaert et al. 2005; Herms and McCullough 2014).

Several systemic insecticides are now available in the U.S. to protect landscape ash trees from EAB (Herms and McCullough 2014; Herms et al. 2014; McKenzie et al. 2010). Although some products can be applied as soil drenches or as a basal trunk spray, most systemic products can be directly injected into the base of the trunk, eliminating insecticide drift and minimizing applicator exposure, as well as non-target or environmental effects (Sur and Stork 2003; Sanchez-Zamora and Fernandez-Escobar 2004; Hahn et al. 2011; Herms et al. 2014). Systemic insecticides are translocated in xylem tissue up the trunk to canopy branches and leaves (Choat et al. 2007; Mota-Sanchez et al. 2009; Tanis et al. 2012). Ash trees are ring porous (Dickison 2000), and all, or nearly all, insecticide translocation occurs in the outer rings of sapwood.

Potential wounds or related injuries from trunk injections of systemic insecticides can be a concern for tree care professionals, municipal arborists, and homeowners. Systemic insecticides are applied to trees either as microinjections, with holes ≤0.5 cm in diameter, or as macroinjections, which require larger holes (Costonis 1981). Microinjections generally deliver 1 to 3 ml of product per injection site and have been used for many years (Costonis 1981). Macroinjection devices enable a greater volume of product to be applied, facilitating higher application rates and potentially improving within-tree distribution of the insecticide (Doccola and Wild 2012). Drilling through the outer bark and into the sapwood, however, could potentially sever conductive tissues and may provide an entry point for pathogens (Shigo 1977; Lawson and Dahlsten 2003; Smith and Lewis 2005). Tree damage can also occur if products are injected under high pressure. For example, a microinjection system (Wedgle Direct-Inject®, ArborSystems, Omaha, Nebraska, U.S.) caused bark on maple (*Acer saccharum*) and ash (*Fraxinus americana*) trees to physically separate from cambium, damaging tissue at and around the injection point (Smith and Lewis 2005).

In addition to wounds made by drilling, trunk-injected insecticides have also reportedly caused discoloration of internal tissues in multiple tree genera, including *Fraxinus* (Shigo et al. 1977; Smith and Lewis 2005; Mota-Sanchez et al. 2009; Doccola et al. 2011; Tanis et al. 2012). When maple (*Acer* spp.) and ash trees were injected with imidacloprid, internal discoloration around the injection points was assumed to be a wound compartmentalization response (Smith and Lewis 2005). If tissue discoloration is indicative of compartmentalization, insecticide distribution throughout the tree could be compromised because barrier zone tissues rarely translocate (Shigo 1977). In a study where ^{14}C-imidacloprid (Imicide™ 10%, J.J. Mauget Co., Arcadia, California, U.S.) was injected into small (≈ 6 cm diameter) ash trees, discolored sapwood adjacent to injection sites had 75 to 300 times higher imidacloprid equivalent concentrations than adjacent unstained tissues (Mota-Sanchez et al. 2009; Tanis et al. 2012). Whether discoloration is indicative of a wound response or of non-viable cells, however, remains unknown. Discoloration was previously observed in green ash (*Fraxinus pennsylvanica*) trees (21.5 to 36.3 cm DBH) treated with a formulation of 4% emamectin benzoate (TREE-äge®, Arborjet, Inc., Woburn, Massachusetts, U.S.), but the discolored areas remained "firm," and no symptoms of infection or deterioration were observed four years post-injection (Doccola et al. 2011). This study, however, included only 16 injection sites on four trees injected with emamectin benzoate, and the integrity of xylem cells were not evaluated.

A systemic insecticide with the active ingredient emamectin benzoate, sold as TREE-äge (4.0%, ArborJet, Inc., Woburn, Massachusetts, U.S.), is commonly used in many areas to protect valuable landscape trees from EAB (Smitley et al. 2010a; Doccola et al. 2011; McCullough et al. 2011; Herms et al. 2014) and in some areas, to slow EAB population growth (Mercader et al. 2011; McCullough et al. 2015; Mercader et al. 2015). Large-scale studies have shown trunk injections of TREE-äge in spring or early summer provide >99% EAB control for at least two years (Smitley et al. 2010a; McCullough et al. 2011; Lewis and Turcotte 2015). Systemic products with the active ingredients imidacloprid or dinotefuran can also be used for EAB control (Herms et al. 2014). These products can be applied as soil drenches, basal trunk sprays, or trunk injection, but require annual application and were less effective than the emamectin benzoate insecticide (TREE-äge) in side-by-side studies (Smitley et al. 2010a; Smitley et al. 2010b; McCullough et al. 2011).

Trunk injection of TREE-äge typically begins by inserting a plastic plug (Arborplugs®, ArborJet, Inc., Woburn, Massachusetts, U.S.) into a hole drilled with a 0.95 cm drill bit. The emamectin benzoate product is injected with a needle that penetrates a membrane in the plug at approximately 310 kPa or 1,379 kPa of pressure for the TREE IV® or QUIK-jet®, respectively (Doccola et al. 2011). Because TREE-äge does not require annual application, protecting ash trees requires fewer injection sites compared to other products that must be injected annually (Smitley et al. 2010a; Doccola et al. 2011; McCullough et al. 2011).

Although trunk injection systems and insecticide formulations continue to be refined, tree response to macroinjections remains relatively unevaluated. Understanding effects of macroinjections on ash trees is especially important given the increasingly wide use of emamectin benzoate (EB) and other trunk-injected systemic products for control of EAB and other pests. The objectives of the current study were to determine if 1) trunk injections of the TREE-äge product created external bark cracks or necrosis in green ash or white ash trees, 2) discoloration in xylem was indicative of injury to xylem, and 3) whether injury or response of trees to injections varied between ash species, application rates, or frequency of injections.

METHODS

Two-Year Study

Researchers evaluated injection sites on 46 ash trees growing in four locations in Ionia, Genesee, and Clinton Counties in central Michigan. The 22 green ash trees ranged in size from 10.7 to 29.2 cm DBH (mean ± SE = 18.1 ± 1.10 cm), and the 24 white ash trees ranged from 10.7 to 22.6 cm DBH (mean = 17.5 ± 0.61 cm). All trees were injected with TREE-äge (4.0%, ArborJet, Inc., Woburn, Massachusetts, U.S.) between 21 and 23 May 2008. A total of 24 trees were treated using the lowest label application rate (EB-low) (0.1 g a.i. per 2.5 cm DBH) applied with a QUIK-jet system (Arborjet, Inc. Woburn, Massachusetts, U.S.). The other 22 trees were treated with a medium-high rate (EB-high) (0.4 g a.i. per 2.5 cm DBH) plus an equal amount of water applied with a TREE IV Micro-Infusion® system (Arborjet, Inc. Woburn, Massachusetts, U.S.). The number of injection sites was based on individual tree DBH (one injection site for every 15.2 cm DBH). Injection sites were evenly spaced around the base of the trunk, avoiding areas with dead tissue or previous wounds. The insecticide was applied using a 0.95 cm drill bit, then inserting plastic plugs (Arborplugs #4, 0.95 cm, Arborjet, Inc., Woburn, Massachusetts, U.S.) with a set tool and plastic mallet, according to label directions.

From 02–04 June 2009, 22 trees, including 11 EB-high and 11 EB-low trees, were re-treated at the same rates and with the same methods as in 2008. Injection sites were approximately 5 cm above and offset from 2008 injections, according to label directions. The remaining 24 trees, including 11 EB-high and 13 EB-low trees, were not re-treated in 2009. All trees were felled between October and December 2009. After felling, the basal 50 cm of each bole was returned to the laboratory and cut into 5 cm thick cross sections. Cross sections were reassembled after cutting, and reference points were established to ensure proper orientation throughout the experiment.

A total of 239 injection sites were examined on 46 trees. Six injection sites had no associated discoloration (four sites on four 2008 and 2009 EB-high trees, one site on a 2008 EB-high tree, and one site on a 2008 EB-low tree) because the applicator determined plastic plugs were set improperly and no insecticide was injected. These six injection sites were excluded from further analyses. Of the 233 injection sites examined, 50 were from the 11 EB-high trees treated in 2008 only, 77 were from the 11 EB-high trees treated in both 2008 and 2009, 42 were from the 13 EB-low trees treated in 2008, and 64 were from the 11 EB-low trees treated in 2008 and 2009.

Researchers visually examined each cross section and recorded the presence of secondary wounds, including external bark cracks, internal xylem necrosis, and any evidence of pathogen infection (e.g., soft tissue, discoloration) around each injection site (Figure 1). The presence of "new xylem tissue" was also recorded, a term used to refer to latewood produced later in the summer following the injections, and when appropriate, the earlywood and latewood produced the year after injections (Figure 2) (Dickison 2000). Researchers also assessed discoloration height, length, and width, and calculated the area of discoloration around each injection site. Discoloration height (cm) was the vertical distance discoloration persisted above and below the basal cross sections. Discoloration length (mm) was the longest distance between the outer edge of discoloration (near the bark) and the inner edge (near the heartwood) (Figure 1). Discoloration width (mm) was the widest distance between left and right edges of the discoloration associated with a single site (Figure 1). Discoloration area (cm^2) was calculated using the formula for an ellipse: Area = $\pi(\text{length} \div 2) \times (\text{width} \div 2)$.

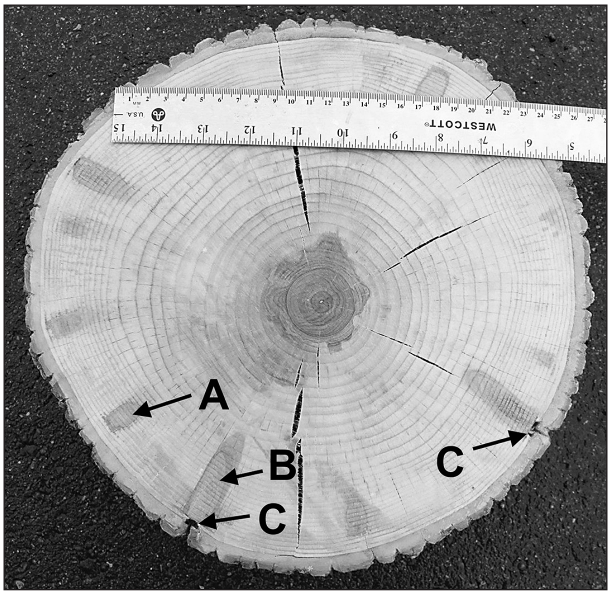

Figure 1. A cross section from a *Fraxinus pennsylvanica* tree, injected in May 2008 and 2009 with a low rate (0.1g a.i. per 2.54 cm at diameter at breast height) of emamectin benzoate (TREE-äge) applied with a QUIK-jet system. The cross section was cut 10 cm above 2008 injection sites. Discoloration from (A) 2008 and (B) 2009 injections and (C) 2009 injection sites are visible. Latewood is visible on the periphery of the 2009 injection sites. Cracking in the sapwood was caused by drying. Dark heartwood is present in the center for the cross section.

Six-Year Study

In 2014, researchers had the opportunity to assess 15 additional green ash trees used in an unrelated study at a site in Midland County, Michigan. Initial tree size ranged from 11.4 to 38.6 cm DBH (mean ± SE = 26.1 ± 1.99 cm). Trees were treated with either a low rate of emamectin benzoate (TREE-äge) applied with the QUIK-Jet (0.1 g a.i. per 2.5 cm DBH) or a high rate (0.4 g a.i. per 2.5 cm DBH) applied with the Tree IV, as previously described. Seven trees (4 EB-low, 3 EB-high) were treated annually from 2008 to 2013. The remaining eight trees (4 EB-low, 4 EB-high) were treated only twice over the six-year period, once in June 2008, and again in June 2011. All trees were felled between February and March 2014. Trees were cut approximately 60 cm aboveground because of deep snow and the remaining stump was left in place until August 2014, when researchers returned to the site and cut stumps into 5 cm thick cross sections down to ground level.

A total of 274 injection sites were examined on the 15 trees, including 86 on the three EB-high trees treated annually, 35 sites on the four EB-high

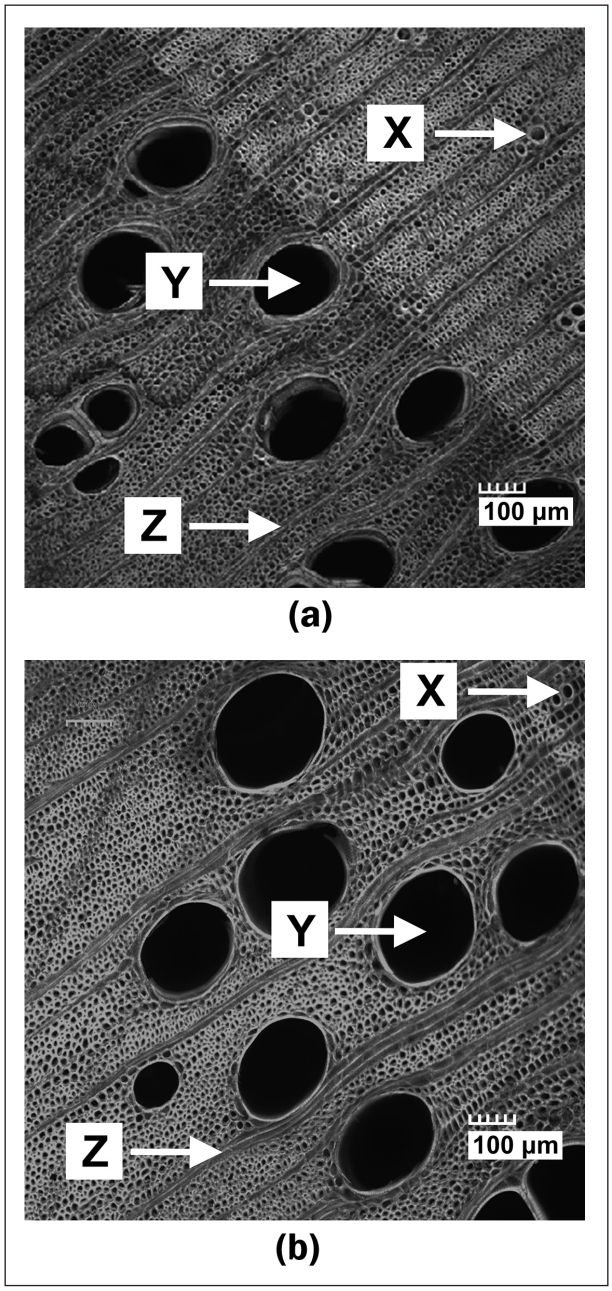

Figure 2. Transverse cross sections from a *Fraxinus pennsylvanica*, taken from tissues (a) discolored by trunk-injected emamectin benzoate and (b) unstained sapwood. The images were obtained using a confocal laser scanning microscope equipped with helium-neon (λ = 543 nm) and argon (λ = 488 nm) lasers. The three-dimensional images consists of (a) eight slices obtained 14.5 µm apart and (b) 27 slices obtained 10.3 µm apart. A 10× dry objective was used for magnification. Images show (X) latewood xylem vessels, (Y) earlywood xylem vessels, and (Z) xylem ray cells.

trees treated in 2008 and again in 2011, 116 sites on the four EB-low trees treated annually, and 37 sites on the four EB-low trees treated in both 2008 and 2011. Injection sites were visually examined on each cross section, and the presence of plastic plugs, external bark cracks, internal xylem necrosis, evidence of pathogen infection, and presence of xylem (earlywood, latewood) growing over injection sites was recorded (Figure 1).

Evaluation of Discolored Xylem Tissue

Transverse tissue samples (approximately 5 mm × 3 mm) were removed with a razor blade from the interface of the discolored and unstained xylem tissue associated with four randomly selected injection sites on 20 of the 46 trees (80 total injection sites) from the two-year study, and each of the 15 trees from the six-year study (60 total injection sites). Five to ten samples were removed from each injection site to ensure researchers would have quality samples. Samples were immediately placed into fixing solution (50% ethanol, glycerol, formaldehyde, 18:1:1) and were left undisturbed for at least 72 hours. After fixation, samples were placed in a solution of safranin orange dye (1% safranin, Carolina Biological Supply Company, Burlington, North Carolina, U.S.) for 15 minutes, dehydrated through 30%, 50%, 75%, 90%, and 100% ethanol (Decon Labs, Inc., King of Prussia, Pennsylvania, U.S.), and then rehydrated backward through the same solutions. Samples remained in each alcohol solution for 10 minutes. Solutions were changed multiple times during each cycle to remove excess dye. After rehydrating, samples were placed in deionized water for 15 minutes, then processed through 25%, 50%, and 75% glycerol solutions (1 hour each with frequent decanting), and finally left overnight in 100% glycerol.

Confocal laser scanning microscopy (CLSM) and/or polarizing digital microscopy was used to evaluate sapwood integrity. Images were viewed and recorded the day after samples were soaked in 100% glycerol. Xylem samples from trees used in the two-year study were viewed with a confocal laser scanning microscope (Olympus FluoView™ 1000, Olympus Corporation, Tokyo, Japan), while xylem samples from trees used in the six-year study were viewed with a handheld polarizing digital microscope (Dino-Lite AM4113ZT®, AnMo Electronics Corporation, New Taipei City, Taiwan).

Xylem tissues examined using CLSM were illuminated with argon (λ = 543 nm) and helium-neon (λ = 488 nm) lasers. Single channel z-scan collections were recorded (Hutzler et al. 1998). A 10× dry objective was used to magnify transverse samples removed from the discolored-unstained interface of trees trunk-injected with TREE-äge. Total slice depth ranged from 10.2 to 14.5 μm; 7 to 21 slices per sample were obtained, depending on optimal image acquisition parameters. Xylem samples assessed with the handheld polarizing microscope were examined under 10× and 50× magnification. Polarization was adjusted as necessary to reduce image glare.

Statistical Analysis

Data were analyzed using SAS statistical software (SAS Institute, Inc. 2008). Assumption of normality was tested with residual plots and the Shapiro-Wilk test (Shapiro and Wilk 1965). Width and length of discolored tissue on cross sections from treated trees of the two-year study were normalized using log transformations (Ott and Longnecker 2001), while all other variables met assumptions of normality. Three-way ANOVA was performed to assess effects of ash species (green or white), EB rate (EB-low or EB-high), and number of applications (2008 only, or 2008 and 2009) on the presence of xylem laid over injection sites, bark cracks or internal tissue necrosis, and discoloration length, width, and height (stumps from the two-year study). When ANOVA results were significant ($\alpha \leq 0.05$), Fisher's protected least significant difference (LSD) multi-comparison test was applied with Tukey's adjustment for unbalanced data sets (Ott and Longnecker 2001) to determine differences between means.

RESULTS

Two-Year Study

Green ash and white ash tree size did not vary among treatments (EB-low or EB-high trees treated in 2008 or in 2008 and 2009) (P = 0.65). The number of injection sites on EB-high and EB-low trees treated in 2008 only was similar (P = 0.36), averaging 4.6 ± 0.53 and 3.3 ± 0.24 injections per tree, respectively (Table 1). On average, EB-low and EB-high trees treated in 2008 and 2009 had 7.4 ± 0.54 and 5.8 ± 0.54 injection sites per tree, respectively, but differences between the EB-low and EB-high trees were not significant (P = 0.66) (Table 1). The number of injections did not vary between ash species (P = 0.75) nor was the species × insecticide rate interaction significant (P = 0.37).

Presence of new xylem tissue over the injection sites did not vary between tree species (P = 0.30), insecticide application rates (P = 0.21), injection frequency (P = 0.17), nor were the interactions significant. Xylem was growing over 134 (58%) of the injection sites. Among the 46 trees examined in this study, 39 (85%) had new xylem growing over at least one injection site, and 37 (80%) had new tissue growing over multiple injection sites (Table 1).

Presence of secondary wounds, such as external bark cracks or internal necrosis, was not affected by ash species (P = 0.29) or insecticide rate (P = 0.23) nor were interactions significant. Only 12 of the 233 injection sites (five on white ash trees, seven on green ash trees) were associated with vertical cracks in the outer bark. Three of the 13 EB-low trees treated only in 2008 had one to two bark cracks associated with injection sites, but no internal cracking or necrosis were found on these trees, nor were they found on the other 10 EB-low trees. None of the 11 EB-low trees treated in both 2008 and 2009 had internal cracks or necrosis, and only one injection site (on a white ash) was associated with a crack in the outer bark. Among the 11 EB-high trees treated only in 2008, one tree had three cracks in the outer bark, but none of the trees had internal cracks or necrosis. One of the 11 EB-high trees treated in both 2008 and 2009 had two cracks in the outer bark, but none of the trees had internal cracks or necrosis. The number of bark cracks was higher in trees treated only in 2008 (nine bark cracks) (F = 5.57; df = 1, 38; P = 0.024) than in trees treated in 2008 and 2009 (three bark cracks) (Table 1).

Length and width of discolored xylem did not vary between ash species (P = 0.57 and P = 0.28, respectively), application rates (EB-low, P = 0.93; and EB-high, P = 0.09), or number of applications (2008, P = 0.45; 2008 and 2009, P = 0.82), nor were the interactions significant. Discoloration length averaged (±SE) 31.6 ± 3.25 mm while discoloration width averaged 13.7 ± 1.22 mm. Area of discolored xylem around injection sites averaged 9.2 ± 0.86 cm^2 and 8.3 ± 0.91 cm^2 per site for EB-high trees treated in 2008 or in both 2008 and 2009, respectively, and 6.4 ± 1.01 cm^2 and 5.9 ± 0.85 cm^2 per site for EB-low trees treated in 2008 or in both 2008 and 2009, respectively. Discoloration extended 63% higher (F = 61.66; df = 1, 38; P < 0.001) in the trunk of EB-high trees (32.3 ± 2.4 cm) than in EB-low trees (12.1 ± 0.96 cm) but was similar between ash species (P = 0.61), between trees treated only in 2008 or in both 2008 and 2009 (P = 0.62), and none of the interactions were significant. At a height of 15.0 cm above injection sites, area of discoloration averaged 4.4 ± 0.93 cm^2 and 3.9 ± 0.55 cm^2 per site for EB-high trees treated in 2008 and in both 2008 and 2009, respectively, and 1.0 ± 0.33 cm^2 and 2.9 ± 1.83 for EB-low trees treated in 2008 and in both 2008 and 2009, respectively.

Six-Year Study:

Green ash tree diameter did not differ between trees treated with high or low insecticide application rates (P = 0.51), trees injected annually from 2008–2013

Table 1. Number of green ash and white ash trees injected with a low rate (0.1 g a.i. per 2.5 cm DBH) or medium-high rate (0.4 g a.i. per 2.5 cm DBH) of emamectin benzoate (TREE-äge, 4.0%, ArborJet, Inc., Woburn, Massachusetts, U.S.), the total number of injection sites for those trees, the number and percentage of injection sites overlaid with new xylem tissue, and injection sites with associated wounds (external bark cracks, internal xylem necrosis, and evidence of pathogen infection). Trees in the two-year study were treated once in 2008 or in both 2008 and 2009. Trees in the six-year study were treated in 2008 and again in 2011, or annually from 2008 to 2013.

	Treatment	No. of trees	Years treated	Total no. injection sites	No. overlaid with new xylem	No. with wounds
Two-year study	EB High	11	2008	50	30 (60%)	3 (6%)
	EB Low	13	2008	42	29 (69%)	9 (21%)
	EB High	11	2008 and 2009	77	44 (57%)	2 (3%)
	EB Low	11	2008 and 2009	64	31 (48%)	1 (2%)
Six-year study	EB High	4	2008 and 2011	35	35 (100%)	1 (3%)
	EB Low	4	2008 and 2011	37	37 (100%)	1 (3%)
	EB High	3	2008–2013	86	86 (100%)	2 (2%)
	EB Low	4	2008–2013	116	116 (100%)	0 (0%)

or injected only in 2008 and 2011 ($P = 0.27$), nor was the interaction of application rate and frequency significant ($P = 0.13$). The number of injections on EB-high and EB-low trees treated in 2008 and 2011 was similar ($P = 0.31$) and averaged 8.8 ± 1.25 and 9.3 ± 1.25 injections per tree, respectively (Table 1). The number of injection sites on EB-high and EB-low trees treated annually from 2008 to 2013 was also similar ($P = 0.27$), averaging 28.7 ± 4.26 and 29.0 ± 6.24 injections per tree, respectively (Table 1).

Of the 274 injection sites assessed, only four sites on three trees (1 EB-high 2008 and 2011, 1 EB-low 2008 and 2011, and 1 EB-high annual treatment) resulted in a crack in the outer bark and none of the injection sites were associated with internal cracks or necrosis. The remaining 12 trees had no evidence of cracks in the outer bark or any other injury (Table 1).

Researchers observed that at least two injection sites in each tree no longer contained the plastic plugs used during injection (i.e., plugs were forced out of trees during the healing process). One EB-high tree treated annually from 2008 to 2013 forced 16 of the 23 plugs out of the xylem. Overall, 46% of the 274 injection sites still contained the plastic plugs inserted during the injection process. All trees had new xylem tissue growing over the injection sites.

Evaluation of Discolored Xylem Tissue

There was no evidence that xylem discoloration was associated with injury, necrosis, or decay in the 140 samples examined with microscopy (Figure 2). Cell lysis was not present in any sample and there were no visible symptoms of tissue damage or infection. The interface where discolored and unstained tissue were well-defined were scrutinized, as these areas might be indicative of a barrier zone (Shigo 1984), but we saw no evidence that trees had attempted to compartmentalize around the discolored tissue (Figure 1). Moreover, when tissues were prepared for examination, much if not all of the discoloration disappeared from the xylem. In addition, discoloration was often continuous from one year's growth to the next. For example, discoloration associated with 2008 injection sites overlapped discoloration associated with 2009 injection sites. Discoloration was continuous through the cross sections, indicating tissues discolored in 2008 were effectively translocated the product in 2009.

DISCUSSION

Projections suggest EAB populations will threaten at least 38 million ash trees planted in urban landscapes in the U.S. by 2020 (Kovacs et al. 2010). Although millions of ash trees have been killed by EAB to date, advances in the knowledge of EAB biology, new systemic insecticides, and better application technology have substantially improved practitioners' ability to protect valuable trees from EAB (Herms and McCullough 2014; Herms et al. 2014). Registration of TREE-äge in 2010, and mounting evidence of the ability of this product to provide highly effective, multiple-year control of EAB (Smitley et al 2010a; Doccola et al. 2011; McCullough et al. 2011), have reduced the costs and logistical constraints associated with treating landscape ash trees on municipal and private property. Economic analyses have shown costs of treating and protecting landscape ash trees with TREE-äge are consistently and substantially lower than costs of removing those trees (McCullough and Mercader 2012; Vanatta et al. 2012; Kovacs et al. 2011; Kovacs et al. 2014) and ecosystem services provided by mature landscape trees in urban areas are increasingly recognized (e.g., Dwyer et al. 1992; McPherson et al. 2005). Given the continued expansion of EAB, it appears likely that the number of trees treated with TREE-äge or other systemic products will increase.

Evaluating potential injury resulting from trunk injections of systemic insecticides, therefore, is a concern for many arborists and landscapers. The results of the current study indicate ash trees were rarely injured as a result of TREE-äge injections made using Arborplugs and either the QUIIK-jet or TREE IV Micro-Infusion systems. Overall, of the 544 injection sites on the 61 white ash and green ash trees assessed in this study, only 3% of injection sites had evidence of external bark cracks and none had disease symptoms. These results are consistent with those of Doccola et al. (2011), who observed no "cracking, oozing, or decay" on four green ash trees (21.5 to 36.3 cm DBH) injected with TREE-äge via the TREE IV Micro-Infusion system in 2005, or on two of the trees that were re-treated in 2008.

The current study included trees treated once every two years, once every three years, annually for two years, and annually for six years. Operationally, ash trees are typically treated at two-year intervals with TREE-äge (Doccola et al. 2011), and unlike neonicotinoid products that require annual application, emamectin benzoate is efficacious for up to three years (Smitley et al. 2010a; McCullough et al. 2011, DGM, unpublished data). Reduced application frequency means fewer opportunities for wounding. Although practicing arborists would rarely, if ever, treat trees with TREE-äge on an annual basis, researchers were able to assess trees injected annually for six consecutive years as part of a long-term research project on EAB dynamics. On average, the seven trees in the six-year study group had 29 injection sites per tree, yet only one tree in this group had damage associated with two of its 23 injection sites. The majority of trees in the six-year study had at least four years of wood growing over sites injected in 2008 and 2009.

Many studies have reported the existence of tissue discoloration adjacent to trunk injection sites (Smith and Lewis 2005; Mota-Sanchez et al. 2009; Doccola et al. 2010; Tanis et al. 2012), but the integrity of discolored tissue has not been previously assessed. In this study, multiple lines of evidence indicate discoloration surrounding injection sites was simply a stain and not indicative of tissue damage. Microscopy results indicated the integrity of xylem tissues immediately surrounding injection sites was sound, regardless of ash species, insecticide application rate, or the number of injections. In addition, when samples were prepared for microscopic examination, much, if not all of the discoloration dissolved when samples were soaked in ethanol. If discoloration was indicative of wounding, it would presumably persist despite immersion in ethanol. Researchers also found discolored tissues surrounding injection sites effectively translocated insecticide applied in subsequent years, which would not have been possible if discoloration represented barrier zone formation (Shigo 1977). The six injection sites that were drilled and plugged but not injected with insecticide had no associated discoloration, further suggesting the formulated insecticide product was the source of discoloration. In hindsight, it would have been advantageous to inject water to assess the wound response exclusively associated with drilling, insertion of the plastic plug, and injection pressure. In other macroinjected ash trees, concentration of imidacloprid in discolored tissues was much higher than in adjacent unstained tissues (Mota-Sanchez et al. 2009; Tanis et al. 2012), and a previous study on green ash trees reported discolored tissues were sound even four years after injection (Doccola et al. 2011).

In this study and others, tissue discoloration diminished with tree height above the injection site (Mota-Sanchez et al. 2009; Tanis et al. 2012). Following the discoloration upward through the cross sections cut from the trees, discolored areas became smaller and paler, indicating the volume of translocated insecticide decreased with height. Previous studies with high concentrations of imidacloprid, a compound with relatively low solubility, showed a reservoir of insecticide can persist for multiple years around injection sites, suggesting some product remains in xylem near the injection sites (Mota-Sanchez et al. 2009; Tanis et al. 2012; Aćimović et al. 2014; Aćimović et al. 2015). In the current study, EB-high trees received at least twice as much product (mixed with an equal amount of water) as the EB-low trees and the height of discolored tissue was at least twice that observed in the EB-low trees, even though EB-low trees were injected under greater pressure. This suggests vertical discoloration was a function of the volume of insecticide applied rather than an indicator of application pressure. In contrast, discoloration width and length did not vary between EB-high and EB-low trees. This indicates lateral movement of insecticides in the lower 50 cm of the trees was unaffected by volume or the pressure applied during application.

Given the continued spread of EAB across North America and the increased use of systemic insecticides for EAB and other pests, understanding how trunk injection systems and insecticide products affect tree health over time will be important. This study showed green ash and white ash trees were rarely harmed by the trunk injection process. While the cause of internal discoloration is not known, it seems likely that it could result from inert dyes used in the emamectin benzoate formulation. Perhaps most importantly, discoloration was not indicative of an ash tree compartmentalization response that could impede translocation. Other systemic insecticides, including emamectin-benzoate-based products, along with an array of trunk injection devices, are available for landscape trees. Whether these results extend to other systemic insecticide products or injection tools will require additional evaluation.

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Résumé. L'agrile du frêne (*Agrilus planipennis*), identifié pour la première fois en 2002 près de Détroit au Michigan, États-Unis, a tué à ce jour des millions de frênes (*Fraxinus spp.*) dans 25 états et 2 provinces canadiennes. Des injections au niveau du tronc de produits insecticides contenant de l'émamectine benzoate (EB) (par exemple, *TREE-Age*®) sont souvent utilisées pour protéger les frênes de l'agrile du frêne dans les aménagements paysagers, mais les plaies et les blessures potentielles résultant de ces injections sont préoccupantes. Des chercheurs ont examiné 507 sites d'injection sur 61 arbres et ont constaté des preuves manifestes de blessures secondaires (par exemple, des fissures de l'écorce externe, de la nécrose du xylème interne et des infections par des agents pathogènes). Les chercheurs ont évalué 233 sites d'injection sur 22 frênes de Pennsylvanie et 24 frênes d'Amérique qui ont fait l'objet de macro-injections avec un taux faible ou moyen de EB en 2008 uniquement ou en 2008 et 2009. Seuls 12 des 233 sites d'injection (5%) ont été associés à des fissures de l'écorce externe et il n'y avait aucune preuve d'infection par un agent pathogène. Sur 39 des 46 arbres (85%), du nouveau xylème s'est développé sur les sites d'injection. Les chercheurs ont évalué 274 sites d'injection sur 15 frênes de Pennsylvanie injectés chaque année avec de l'EB de 2008 à 2013 ou injectés en 2008 et à nouveau en 2011. Des fissures de l'écorce ont été associées à quatre sites d'injection sur trois arbres, mais aucune trace de blessure n'a été constatée sur les 12 autres arbres. Les 15 arbres avaient développé du nouveau xylème recouvrant les sites d'injection. La microscopie confocale à balayage laser et la microscopie numérique de polarisation ont été utilisées afin d'évaluer l'intégrité de tissus décolorés du xylème retirés de la zone immédiate entourant 140 sites d'injection sur 61 arbres. Les chercheurs n'ont observé aucune carie associée à ces décolorations.

Zusammenfassung. Der Asiatische Eschenprachtkäfer, der in der Nähe von Detroit, Michigan erstmals in 2002 identifiziert wurde, tötete bis heute Millionen von Eschen in 25 Staaten und zwei kanadischen Provinzen. Stamminjektionen mit Insektiziden, die Emamectin-Benzoate (EB)(z.B. TREE-ägeR) werden oft zum Schutz von Eschen in der Landschaft gegen EAB verwendet, aber Wunden und potentielle Verletzungen, die aus der Injektion resultieren, geben Grund zur Besorgnis. Forscher untersuchten 507 Injektionen an 61 Bäumen und zeichnete alle Beweise von Baumverletzungen (z.B. äußere Rindenrisse, interne Xylem-Nekrose und Infektionen durch Pathogene). Die Forscher untersuchten 233 Injektionsstellen an 22 Grünen Eschen und zwei Weißen Eschen, die per Makro-Injektion mit einer niedrigen oder halbhohen Rate von EB in 2008 oder in 2008 und 2009. Nur 12 der 233 Injektionen (5%) waren verbunden mit externen Rissen in der Rinde und es gab keine Anzeichen von Infektionen durch Pathogene. An 39 der 46 Bäume (85%) war über der Infektionsfläche neues Xylem gewachsen. Die Forscher untersuchten 274 Injektionen an 15 Grünen Eschen, die jährlich von 2008 bis 2013, oder zuerst in 2008 und dann wieder in 2011 mit EB injiziert wurden. Rindenrisse waren assoziiert mit vier Injektionsstellen an drei Bäumen, aber es wurden keine Anzeichen von Verletzungen an den anderen 12 Bäumen gefunden. Alle 15 Bäume hatten neues Xylem über der Injektionsstelle. Konfokale Laser Scanner und polarizierende Digitalmikroskopie wurden eingesetzt, um die Integrität der verfärbten Xylemgewebe, die von der unmittelbaren Umgebung der Injektionsstellen an 61 Bäumen zu untersuchen. Die Forscher fanden keinen Hinweis auf Fäule in Assoziation mit der Verfärbung.

Resumen. El barrenador esmeralda del fresno (EAB, por sus siglas en inglés) (*Agrilus planipennis*), identificado por primera vez cerca de Detroit, Michigan, EE.UU., en 2002, ha matado hasta la fecha a millones de fresnos (*Fraxinus* spp.) en 25 estados y dos provincias de Canadá. Las inyecciones al tronco de productos insecticidas que contienen benzoato de emamectina (EB) (por ejemplo, TREE-age®) a menudo se utilizan para proteger los fresnos en los paisajes de EAB, pero las heridas y lesiones potenciales como resultado de las inyecciones son una preocupación. Los investigadores examinaron 507 puntos de inyección en 61 árboles y registraron evidencia de lesiones secundarias (por ejemplo, grietas en la corteza externa, necrosis del xilema interno e infección por patógenos). En 2008 y 2009 los investigadores evaluaron 233 sitios de macro inyección, en 22 fresnos verdes y 24 fresnos blancos con una baja o una velocidad media-alta de EB. Sólo 12 de 233 puntos de inyección (5%) estaban asociados con grietas externas en la corteza y no hubo evidencia de infección por patógenos. En 39 de 46 árboles (85%), nuevo xilema estaba creciendo sobre sitios de inyección. Entre 2008 y 2013 los investigadores evaluaron 274 sitios de inyección en 15 fresnos verdes inyectados anualmente con EB o inyectados en 2008 y nuevamente en 2011. Las grietas de la corteza estaban asociadas con cuatro puntos de inyección en tres árboles, pero no se encontró ninguna evidencia de daño en los otros 12 árboles. Todos los 15 árboles tenían nuevo xilema sobre los puntos de inyección. El escaneo láser y la microscopía de polarización digital se utilizaron para evaluar la integridad de los tejidos del xilema decolorados removidos del área inmediata que rodea 140 sitios de inyección en 61 árboles. Los investigadores no encontraron evidencia de deterioro asociado a la decoloración.