Evaluation of *Agrilus planipennis* (Coleoptera: Buprestidae) Control Provided by Emamectin Benzoate and Two Neonicotinoid Insecticides, One and Two Seasons After Treatment

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J. Econ. Entomol. 104(5): 1599–1612 (2011); DOI: http://dx.doi.org/10.1603/EC11101

ABSTRACT Effective methods are needed to protect ash trees (*Fraxinus* spp.) from emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an invasive buprestid that has killed millions of North American ash (*Fraxinus* spp.) trees. We randomly assigned 175 ash trees (11.5–48.1 cm in diameter) in 25 blocks located in three study sites in Michigan to one of seven insecticide treatments in May 2007. Treatments included 1) trunk-injected emamectin benzoate; 2) trunk-injected imidacloprid; 3) basal trunk spray of dinotefuran with or 4) without Pentra-Bark, an agricultural surfactant; 5) basal trunk spray of imidacloprid with or 6) without Pentra-Bark; or (7) control. Foliar insecticide residues (enzyme-linked immunosorbent assay) and toxicity of leaves to adult *A. planipennis* (4-d bioassays) were quantified at 3–4-wk intervals posttreatment. Seven blocks of trees were felled and sampled in fall 2007 to quantify *A. planipennis* larval density. Half of the remaining blocks were retreated in spring 2008. Bioassays and residue analyses were repeated in summer 2008, and then all trees were sampled to assess larval density in winter. Foliage from emamectin benzoate-treated trees was highly toxic to adult *A. planipennis*, and larval density was <1% of that in comparable control trees, even two seasons posttreatment. Larval densities in trees treated with trunk-injected imidacloprid in 2007 + 2008 were similar to control trees. Dinotefuran and imidacloprid were effectively translocated within trees treated with the noninvasive basal trunk sprays; the surfactant did not appreciably enhance *A. planipennis* control. In 2008, larval densities were 57–68% lower in trees treated with dinotefuran or imidacloprid trunk sprays in 2007 + 2008 than on controls, but densities in trees treated only in 2007 were similar to controls. Highly effective control provided by emamectin benzoate for ≥2 yr may reduce costs or logistical issues associated with treatment.

KEY WORDS emerald ash borer, dinotefuran, imidacloprid, ash tree protection, invasive pest

Ash (*Fraxinus* spp.) trees have been planted in municipal and private landscapes across much of the continental United States for decades. Green ash (*Fraxinus pensylvanica* Marshall) and white ash (*Fraxinus americana* L.) cultivars are particularly common, comprising >25% of some urban forests (MacFarlane and Meyer 2005, Raupp et al. 2006). Ash trees tolerate a variety of soils and stressful conditions often associated with urban forests. They are prized for their attractive growth form and, until recently, the lack of major pest problems.

The nonindigenous emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), discovered in 2002 in southeast Michigan and Windsor, ON, Canada, now threatens ash trees in forests, landscapes, and riparian settings across North America. Newly infested trees with low larval densities exhibit virtually no external symptoms of infestation (Poland and McCullough 2006, McCullough et al. 2009a, Poland et al. 2011). As larval density builds over time, galleries disrupt nutrient and water transport, resulting in characteristic top-down thinning and dieback in the canopy and ultimately mortality (Cappaert et al. 2005). Tens of millions of ash trees, ranging from 2.5 cm to >1.5 m in diameter, have been killed by *A. planipennis* in southern Michigan and northern Ohio alone. Established populations of *A. planipennis* initiated by inadvertent transport of infested ash nursery stock, logs, or firewood had been found in at least 13 additional states and the Canadian province of Quebec as of June 2011 (http://www.emeraldashborer.info/).

Systemic insecticides are increasingly used to control pests on shade trees because they minimize problems associated with cover sprays such as drift, applicator exposure, and nontarget effects. Systemic

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products are typically injected into the base of the trunk or applied to the soil and then translocated in xylem up the trunk and into the canopy (Sur and Stork 2003, Mota-Sanchez et al. 2009, Tanis et al., unpublished data). Several neonicotinoid products with the active ingredient imidacloprid and one product containing dintoefuran are registered for *A. planipennis* control on landscape ash trees (Herms et al. 2009). Recent studies indicated dinotefuran and possibly imidacloprid products also could be applied as a basal trunk spray (McCullough et al. 2007, Anulewicz et al. 2010, Cowles 2010). In trials to control *A. planipennis* in Michigan and Ohio, efficacy of neonicotinoid products has varied, depending on factors such as product formulation, application rate or method, tree size and vigor, and *A. planipennis* pressure (McCullough et al. 2004, 2007; Herms et al. 2009; Anulewicz et al. 2010; Smitley et al. 2010).

Insecticides containing the active ingredient emamectin benzoate, an avermectin compound that is a macrocyclic lactone salt derivative, have been registered for pest control in veterinary medicine, fish farming, and agricultural commodities for several years (Leibee et al. 1995, White et al. 1997, Stone et al. 1999). Early water-soluble formulations of emamectin benzoate were developed for application as trunk injections (Takai et al. 2001) and evaluated for control of seed and cone insects, engraver beetles, nematodes, and longhorned beetles, primarily in conifers (Grossman et al. 2002, Takai et al. 2003, Grossman and Upton 2006, Poland et al. 2006). A new formulation of emamectin benzoate was recently developed and registered for *A. planipennis* in ash trees (Arborjet 2010), but few field evaluations have been conducted to date.

Systemic insecticides may act on different life stages of *A. planipennis.* Adults, for example, must feed on ash foliage for at least 7 d before mating begins, and females must feed for an additional 10–14 d before they begin to lay eggs. Emergence of *A. planipennis* begins at ≈450–500 accumulated degree-days base 10°C (DD_{10}), typically in late May in the Upper Midwest (MSU IPM 2010). Beetles remain active for several weeks with activity peaking at ≈1000 DD_{10} in late June or early July (McCullough et al. 2009a,b; Poland et al. 2011). This provides an important window to control beetles before oviposition begins.

A. planipennis larvae also may be controlled by systemic insecticides, although underlying mechanisms are not clear. Neonate larvae hatch from eggs laid beneath bark flaps or in bark crevices and tunnel through the outer bark and into the phloem to begin feeding in mid- to late summer. Larvae feed on phloem and cambium in serpentine galleries that often score the outer sapwood. Most larvae complete four instars by late autumn, overwinter as prepupal larvae in thick outer bark or in the outer sapwood, and then pupate the following spring (Cappaert et al. 2005). In relatively healthy, newly infested trees, some larvae overwinter as early instars and feed for a second summer, completing a 2-yr life cycle (Siegert et al. 2010, Tluzcek et al. 2011). Systemic insecticides could affect *A. planipennis* larvae if toxic compounds diffuse from xylem into phloem, or larvae may encounter toxic compounds if their feeding penetrates the outer sapwood.

We evaluated the ability of systemic insecticides, including dinotefuran, two imidacloprid products, and emamectin benzoate, to protect ash trees from *A. planipennis.* All trees were treated in spring 2007 and then exposed to wild *A. planipennis* colonization during the summer. Some trees were debarked during winter to evaluate larval density. Half of the remaining trees were retreated in spring 2008, and the rest remained untreated. This design enabled us to quantify *A. planipennis* control two seasons posttreatment. Evaluation included assessing toxicity of leaves to adult beetles, quantifying larval density, and monitoring insecticide residues in foliage. Our specific objectives were to 1) compare the relative efficacy of the neonicotinoid insecticides and emamectin benzoate, 2) assess noninvasive basal bark sprays of imidacloprid or dinotefuran with and without a surfactant, and 3) determine whether any of the products protected trees for more than one season posttreatment.

Materials and Methods

Study Sites. In May 2007, 25 blocks in total, each consisting of seven ash trees, were established using a randomized complete block design (n = 175 trees total). Blocks were laid out at three sites encompassing four counties in Michigan. Trees within blocks were similar in size, growing conditions, and exposure to sun. Although *A. planipennis* was established in all sites, tree canopies remained generally healthy and no epicormic shoots were present. We established 12 blocks of open-grown, green ash trees in the median of Interstate 96 between the cities of Brighton, Livingston Co., and Webberville, Ingham Co., MI (Interstate site). Seven blocks of white ash trees were selected in a wooded area in Seven Lakes State Recreation Area near Holly, Oakland Co., MI (Seven Lakes site). Canopies of the Seven Lakes trees were fully or partially exposed to sun. Six blocks of open-grown, green ash trees were established at Wolverine Campground in Columbiaville, Lapeer Co., MI (Wolverine site). Diameter at breast height (dbh; 1.3 m aboveground) was measured on all trees in May 2007.

Insecticide Applications. In May 2007, trees within each block were randomly assigned to one of seven treatments (25 trees per treatment). Treatments included 1) untreated controls; 2) basal trunk spray with dinotefuran (Safari 20 SG) mixed with Pentra-Bark (PB), an agricultural organosilicone surfactant and 3) without Pentra-Bark; 4) trunk-injected emamectin benzoate; 5) trunk-sprayed imidacloprid mixed with Pentra-Bark and 6) without Pentra-Bark; and 7) trunk-injected imidacloprid (Table 1).

Basal trunk sprays of dinotefuran and imidacloprid were applied at a rate of 95 ml/2.5 cm dbh by using a 7.6-liter garden sprayer, with the nozzle set to conical spray, and low pressure to avoid any splash-back. When appropriate, 89 ml of Pentra-Bark was added per 3.8 liter of formulated dinotefuran or imidacloprid

Table 1. Insecticide products, distributors, and application rate (grams [AI] per 2.5 cm dbh), method and dates for *Fraxinus* sp. trees treated for *Agrilus planipennis* control

Treatment	Product	Distributor	Rate	Method	Date
Dinotefuran	Safari 20 SG	Valent USA Corp., Walnut Creek, CA	1.704	Basal trunk spray	31 May 2007, 30 May 2008
Dinotefuran + Pentra-Bark[a]	Safari 20 SG	Valent USA Corp.	1.704	Basal trunk spray	31 May 2007, 30 May 2008
Emamectin benzoate	TREE-Äge 4% ME	Arborjet, Inc., Woburn, MA; Syngenta Crop Protection, Inc., Greensboro, NC	0.10, 0.15, or 0.20[b]	Trunk injection; Arborjet Quik-Jet	22 May 2007, 20 May 2008
Imidacloprid	Macho 2F (21.4%)	Albaugh Inc./Agri Star, Ankeny, IA	1.704	Basal trunk spray	4 May 2007, 16 May 2008
Imidacloprid + Pentra-Bark[a]		Albaugh Inc./Agri Star, Ankeny, IA	1.704	Basal trunk spray	4 May 2007, 16 May 2008
Imidacloprid	Imicide (Hp 10%)	JJ Mauget Co., Arcadia, CA	0.06	Trunk injection; 3-ml capsules	22 May 2007, 20 May 2008

[a] Pentra-Bark is an agricultural organosilicone surfactant (Quest Products, Lindwood, KS); 89 ml was added per 3.8 liter of formulated dinotefuran or imidacloprid.

[b] Application rates for trees with dbh ≤15.7, 15.8–20.8, and > 20.8 cm, respectively.

(Table 1). We sprayed the circumference of the trunk from ≈10 to 1.6 m aboveground until the bark was thoroughly wet, and the appropriate amount of solution was applied.

Trunk injections of imidacloprid and emamectin benzoate were spaced evenly around the base of the tree, per label directions, avoiding any wounds, cankers, or dead tissue. Imidacloprid was injected using Mauget microinjection capsules at a rate equivalent to one 3-ml capsule per 5 cm dbh (Table 1). Capsules were checked ≈1 h after application and if liquid remained, the capsules were repressurized and left on trees until the next day. Emamectin was injected using the Arborjet Quik-Jet system. Number of injection sites (using #4 plugs) was based on dbh (one injection site per 5 cm dbh). Trees with a dbh ≤ 15.7 cm, 15.8–20.8, and >20.8 cm were treated at rates of 0.10, 0.15, or 0.20 g (AI)/2.5 cm dbh, respectively (Table 1).

Application timing was based on previous experience and weather conditions and was designed to ensure insecticides were likely to be present in canopy foliage when peak activity of adult *A. planipennis* was expected (e.g., 800-1200 DD_{10}; McCullough et al. 2009a,b; Poland et al. 2011; Tluczek et al. 2011). Noninvasive basal trunk sprays of imidacloprid were applied in early May, ≈2.5 wk before emamectin benzoate and imidacloprid were injected into trees (Table 1). Dinotefuran trunk sprays were applied 9 d after the trunk injections (Table 1).

In spring 2008, 18 blocks of trees remained (125 trees total), including nine blocks at the Interstate site, three blocks at Seven Lakes site, and six blocks at the Wolverine site. Nine blocks were randomly assigned to be retreated with the same insecticide product as in 2007, whereas the other nine blocks were left untreated. This resulted in 13 treatments including 1) untreated controls; 2) trees treated with the dinotefuran trunk spray in 2007 + 2008 (3), or in 2007 only; 4) trees treated with the dinotefuran + Pentra Bark trunk spray in 2007 + 2008, (5) or in 2007 only; 6) trees injected with emamectin benzoate in 2007 + 2008 (7) or in 2007 only; 8) trees treated with the imidacloprid trunk spray in 2007 + 2008 (9) or in 2007 only; 10) trees treated with the imidacloprid + Pentra Bark trunk spray in 2007 + 2008 (11) or in 2007 only; and 12) trees injected with imidacloprid in 2007 + 2008 (13) or in 2007 only. Treatments were applied using the same methods and similar timing as in 2007 (Table 1).

Canopy Condition. Visual estimates of canopy dieback (10% classes) on each tree were made after full leaf expansion and again in late summer before leaves senesced. Lower branches that had self-pruned were not considered in dieback estimates. At least two experienced observers examined all aspects of the canopy and if estimates diverged, the average of the estimates was recorded for the tree. Canopy condition was rated on 31 May and 21–22 August in 2007 and on 30 May and 4 September in 2008.

Adult Leaf-Feeding Bioassays. Bioassays to assess survival of adult *A. planipennis* caged with leaves from each study tree were conducted in 2007and 2008. Adult *A. planipennis* used in bioassays were reared from infested ash logs. Upon emergence, beetles were held in small cages in growth chambers (24°C, 75% RH, and a photoperiod of 18:6 [L:D] h), provided with fresh tropical ash, *Fraxinus uhdei* (Wenz.) Lingelsh., foliage and monitored for 3–4 d, to ensure healthy beetles were used for bioassays. Two intact shoots collected from the mid-canopy of opposite sides of each tree by using pole pruners were bagged, transported in coolers to the Michigan State University (MSU) Forest Entomology Laboratory, and refrigerated. Within 24 h of foliage collection, we removed one leaf from each of the two intact shoots, inserted the petioles into water pics and placed each leaf into a petri dish (15 cm in diameter). Three beetles (<7 d old) were placed into each petri dish (two dishes per tree; six beetles per tree). Equal numbers of male and female beetles were assigned to leaves from each tree. Beetles were allowed to feed undisturbed for 4 d. Beetle mortality was tallied 24 h (day 1) and 4 d (day 4) after beetles were placed on foliage. Bioassays began on 8 June, 8 July, and 1 August in 2007 and on 13 June and 10 July in 2008.

Larval Density. Between late September and early November 2007, we selected and felled four blocks of

trees (28 trees total) at the Interstate site and three blocks at the Seven Lakes site to evaluate larval density. One additional emamectin benzoate tree was felled at the Interstate site. Using drawknives and chisels, we carefully debarked 9–32 windows, each $\geq$500 cm^2, on the upper surface and sides of the trunk, main leader, and all primary branches (>5 cm in diameter) on each tree. We excavated 9–12 bark windows per tree on the 21 trees felled at the Seven Lakes site and a minimum of 32 windows per tree on the 29 trees felled at the Interstate site. We subsequently returned to both sites and completely debarked the trunk and primary branches on the eight trees treated with emamectin benzoate, from ≈15 cm above the base of the tree to the point where the leader or primary branches were ≈ 5 cm in diameter. Area of exposed phloem was measured and summed for each tree. Numbers and stage of larvae and adult *A. planipennis* emergence holes were counted and standardized per square meter of exposed phloem for each tree.

The remaining 18 blocks (124 trees) were exposed to *A. planipennis* colonization during summer 2008 and then were sampled from late September to December 2008. Trees at the Interstate and Seven Lakes sites were felled and the trunk, leader and primary branches were sawn into 1-m-long sections from the base until the diameter was ≈5 cm. Alternate 1-m-long sections were completely debarked and measured. At the Wolverine site, the landowner preferred that trees not be felled. These trees were sampled by excavating two bark windows, each 0.3 by 0.15 m), ≈1.5 m high on opposite sides of the trunk, and then climbing trees to excavate 10–12 additional bark windows on the main leader and primary branches. *A. planipennis* data were recorded and standardized per square meter of exposed phloem, as in 2007.

We quantified the density of old galleries when we peeled trees in 2007 to assess pretreatment infestation. Old galleries were present but not included in 2008 analyses because we could not differentiate between pre- and posttreatment gallery initiation.

Insecticide Residues in Foliage. Residues of imidacloprid, dinotefuran, and emamectin benzoate were assessed in composite samples of leaves collected at ≈2–4-wk intervals posttreatment from each tree. Pole pruners were used to access shoots from four to eight mid-canopy branches on four aspects of each tree. Leaves were stripped from shoots and foliage from each tree was individually bagged, transported in coolers with ice to the MSU Forest Entomology laboratory in East Lansing, MI, and then frozen. Frozen foliage was shipped by overnight mail to the USDA–APHIS laboratory in Buzzards Bay, MA, for residue analysis. Foliage samples were collected on 7 June, 7 July, 1 August, and 14 August in 2007 and on 12 June, 9 July, and 11 August in 2008.

Residue analysis was conducted using enzyme-linked immunosorbent assays (ELISA) to quantify imidacloprid, dinotefuran, and emamectin benzoate residues. Leaf samples remained frozen until ready for analysis. Leaves from each sample were separated from the stems and petioles and stored in paper bags at room temperature for several days until dry and brittle. Leaves were initially compressed and broken by hand, then placed into a 1.9-liter stainless steel vessel atop a Waring 2-speed commercial blender. The blender was run on high speed for ≈30 s to homogenize the sample and break up the leaf tissue into a fine powder. Vessels were thoroughly cleaned after each use to avoid cross-contamination between samples.

Quantity of insecticides in the ground foliage was determined using commercially available 96-well plate ELISA kits. Assay kits for imidacloprid (EP 006) were purchased from EnviroLogix Inc. (Portland, ME). Assay kits for dinotefuran and emamectin benzoate (3100176146 and 3100176052, respectively) were purchased from Horiba, Ltd. (Kyoto, Japan). The assay kits are marketed for the determination of pesticide residues in aqueous samples. We slightly modified the manufacturers' methods and used a solvent to extract the insecticides from dried leaf material and quantify residues. For all analyses, a 0.5-g sample of the ground leaf material was weighed into a 50-ml plastic centrifuge tube and extracted in 10 ml of pure methanol for 3 h on a table-top shaker. Samples in tubes were spun down in a high-speed centrifuge (model 5810, Eppendorf, New York, NY) at 6,000 rpm for 10 min, and the supernatant diluted a minimum of 20× to avoid matrix effects from the kit due to the methanol. Samples were then run on the assay kits according to the manufacturer's specifications. Individual samples were run in duplicate and the sample was reassessed if the resulting value exceeded the standard curve or if an individual sample varied by >15% between the duplicate wells. Sample values were derived from the average number and adjusted to milligrams per liter to achieve a value in parts per million.

Statistical Analysis. Variables were tested for normality using the Shapiro–Wilk test (Shapiro and Wilk 1965) and residual plots. Several variables including tree dbh, live and dead larval density in 2007, canopy dieback in 2008, and density of dead larvae in 2008 were normalized by $\ln(x + 1)$ transformations (Ott and Longnecker 2001). Differences among sites, treatments, and months were tested as unplanned comparisons, and multiple comparison tests were applied only when overall analysis of variance (ANOVA) was significant ($P < 0.05$). Three-way repeated measures ANOVA was performed to assess effects of site, treatment, and month on foliage residues and adult mortality in leaf-feeding bioassays. Effects of site and treatment on larval density were evaluated using two-way ANOVA followed by the Tukey–Kramer least significant means test (Ott and Longnecker 2001) if significant differences occurred. Estimates of canopy dieback in 2007 were not normalized by transformations and nonparametric ANOVA (Friedman's F statistic; Kruskal–Wallis H statistic) was applied to assess differences among sites and treatments (Kruskal and Wallis 1952, PROC NPAR1WAY; SAS Institute 2003). When results were significant, nonparametric multiple comparisons were applied (Zar 1984). All analyses

were conducted at the $P < 0.05$ level of significance by using SAS statistical software (PROC GLM and PROC MIXED; SAS Institute 2003).

Results

Tree Size and Canopy Condition: 2007. Trees at the three sites ranged from 11.5 to 48.1 cm in dbh. Tree dbh differed among sites ($F = 121.1$; df = 2, 172; $P < 0.0001$) but not among treatments ($F = 0.49$; df = 6, 168; $P = 0.82$), and the interaction was not significant ($F = 0.52$; df = 12, 165; $P = 0.90$). Trees at the Seven Lakes site were smaller than trees at the other sites, averaging (mean ± SE) 16.8 ± 0.4 cm in dbh. Tree dbh at the Interstate and Wolverine sites also differed, averaging 26.8 ± 0.7 and 33.5 ± 0.6 cm, respectively.

Canopy dieback recorded in May 2007 ranged from 0 to 45% and was higher at the Interstate site than at the other two sites ($H = 58.7$; df = 2, 172; $P < 0.0001$). At the Interstate site, a few blocks of trees were flooded during spring and some branches on these trees had few or no leaves in late May. Once wet conditions subsided, most branches leafed out within a few weeks. Dieback was 15.5 ± 1.2% at the Interstate site, but at Seven Lakes (2.3 ± 1.2%) and Wolverine (0.0 ± 0.0%) dieback was minimal. Canopy dieback in May did not differ among trees assigned to different treatments (Friedman's $F = 0.35$; df = 6, 168; $P = 0.91$), nor was the interaction between the main effects of treatment and site significant (Friedman's $F = 0.41$; df = 12, 165; $P = 0.96$).

In late August 2007, trees at the Interstate site had recovered from the early flooding, but canopy dieback (7.6 ± 1.1%) was higher than at Wolverine (1.7 ± 0.9%), whereas Seven Lakes (6.2 ± 1.7%) was intermediate ($H = 11.8$; df = 2, 172; $P = 0.003$). Only six of the 50 trees felled in 2007 had dieback estimates ≥30%, and no tree had >40% dieback. Canopy dieback did not vary among treatments (Friedman's $F = 0.30$; df = 6, 168; $P = 0.94$), nor was the interaction between main effects significant (Friedman's $F = 0.68$; df = 12, 165; $P = 0.77$).

Adult Bioassays: 2007. In total, 1,050 *A. planipennis* beetles were caged with leaves from the study trees during each of the three bioassays in 2007. Mortality of beetles increased substantially from day 1 to day 4 for all treatments during all three bioassays (Fig. 1).

Mortality of beetles on day 1 varied among treatments ($F = 144.94$; df = 6, 54; $P < 0.0001$). Overall, day 1 mortality for beetles caged with leaves from emamectin benzoate-treated trees was 77.6 ± 2.97%, higher than that of beetles caged with foliage from any other trees (Fig. 1). Day 1 mortality for beetles on leaves from emamectin benzoate-treated trees was highest in June, when 92.7 ± 3.21% of beetles died. Mortality of beetles caged with leaves from trees treated with dinotefuran, with and without Pentra-Bark (19.1 ± 2.61 and 18.8 ± 3.03%, respectively), was higher on day 1 than mortality of beetles on trees treated with imidacloprid products and control trees. Average day 1 mortality did not differ among beetles on leaves from trees treated with imidacloprid trunk sprays or injection, ranging from 8 to 10%, which was similar to mortality of beetles on leaves from control trees (Fig. 1). Beetle mortality on day 1 was higher for leaves from trees at the Seven Lakes site (26.0 ± 2.49%), compared with leaves from trees at the Interstate (18.8 ± 1.87%) and Wolverine (20.3 ± 2.79%) sites ($F = 7.78$; df = 2, 56; $P = 0.001$), which did not differ. More beetles died by day 1 in the mid-June bioassay (27.4 ± 2.55%) than in the mid-July (18.1 ± 2.09%) and mid-August (18.1 ± 2.16%) bioassays ($F = 9.49$; df = 2, 208; $P < 0.0001$). Beetle mortality on day 1 was affected by two-way interactions between treatment and time, (e.g., the June, July, and August bioassays) ($F = 2.29$; df = 12, 107; $P = 0.012$), treatment and site ($F = 2.23$; df = 12, 56; $P = 0.022$), and site and time ($F = 6.33$; df = 4, 111; $P < 0.0001$). The three-way

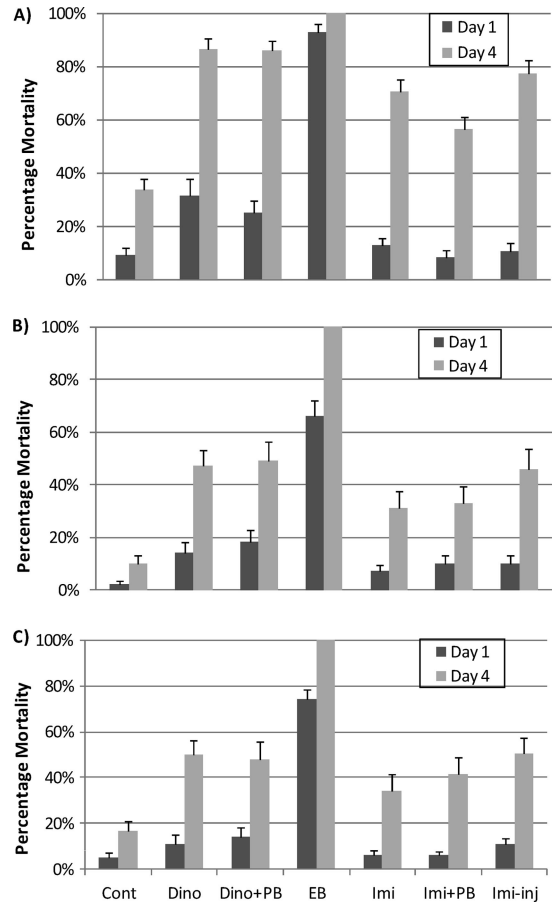

Fig. 1. Cumulative percentage mortality (average + SE) on day 1 and day 4 for *A. planipennis* beetles provided with *Fraxinus* sp. foliage in bioassays in June (A), July (B), and August (C) 2007 from untreated control trees (Cont) and trees treated with basal trunk sprays of dinotefuran (Dino) with or without Pentra-Bark (PB), trunk-injected emamectin benzoate (EB), basal trunk sprays of imidacloprid (Imi) with or without Pentra-Bark (PB), or trunk-injected imidacloprid (Imi-inj) ($N = 25$ trees per treatment). Main effects of treatment, site, month, and some two-way interactions significantly affected day 1 and day 4 mortality ($P < 0.05$).

interaction of treatment, time, and site was not significant ($F = 1.15$; df = 24, 111; $P = 0.30$).

By day 4 of the bioassays, insecticide treatments had obviously affected the beetles and differences among treatments were significant ($F = 75.49$; df = 6, 54; $P < 0.0001$) (Fig. 1). In all three bioassays, 100% of the beetles caged with leaves from the emamectin benzoate-treated trees died by day 4, and mortality was higher for emamectin benzoate-treated trees than for all other treatments. Overall beetle mortality (across months) on leaves from trees treated with dinotefuran trunk sprays with and without Pentra-Bark, averaged 61.0 ± 4.27 and 61.2 ± 3.84%, respectively, over the summer. More than 80% of the beetles caged with foliage from dinotefuran-treated trees were dead by day 4 in the mid-June bioassay, but average mortality was <60% in July and August. The imidacloprid trunk injection resulted in 58.0 ± 4.06% mortality over the summer, similar to that of the dinotefuran-treated trees, but higher than mortality on trees treated with the imidacloprid trunk sprays. On foliage from trees treated with imidacloprid trunk sprays, with and without Pentra-Bark, beetle mortality was 43.7 ± 3.83 and 45.2 ± 4.08%, respectively, over the summer. Beetle mortality was higher on foliage from all treated trees than on foliage from the untreated control trees, where an overall 20.2 ± 2.42% of the beetles died by day 4.

When we examined the petri dishes at the end of the bioassays, beetles caged with leaves from control trees typically consumed large areas of leaves, produced substantial amounts of frass and were active on day 4. Beetles caged with leaves from emamectin benzoate-treated trees produced virtually no frass and took only a few bites from leaves before they died. Many beetles caged with leaves from dinotefuran-treated trees also died after only one or a few bites, typically after regurgitating, which we did not observe on other treatments. This was most apparent in the June bioassay. Some beetles caged with leaves from imidacloprid trees died relatively quickly, but most fed, produced some frass and several beetles exhibited symptoms of insecticide intoxication such as moving only when prodded.

Beetle mortality on day 4 also varied significantly among sites, averaging 71.4 ± 2.71, 52.9 ± 3.44, and 47.8 ± 2.35% on leaves from Seven Lakes, Wolverine, and Interstate, respectively ($F = 50.06$; df = 2, 56; $P < 0.0001$). Overall beetle mortality (across treatments and sites) was highest in June (73.0 ± 2.11%), a value higher than mortality in July (45.1 ± 2.95%) and August (48.7 ± 2.95%) ($F = 57.91$; df = 2, 208; $P < 0.0001$). Mortality did not differ between the July and August bioassays. Beetle mortality on day 4 was significantly affected by two-way interactions between treatment and time ($F = 2.90$; df = 12, 108; $P = 0.003$) (Fig. 1), treatment and site ($F = 5.07$; df = 12, 56; $P < 0.0001$), and site and time ($F = 15.00$; df = 4, 112; $P < 0.0001$). The three-way interaction was not significant ($F = 1.29$; df = 24, 112; $P = 0.19$).

Larval Density: 2007. At Seven Lakes, three blocks of trees (21 trees) were felled. For the three trees treated with emamectin benzoate, 2.9 ± 0.28 (average ± SE) m^2 per tree was exposed to assess larval density (total of 8.6 m^2 of phloem). On the remaining trees, 0.5 ± 0.01 m^2 of phloem per tree was exposed (9.4 m^2 total). At the Interstate site, four complete blocks of trees plus one additional emamectin benzoate-treated tree were felled (29 trees). For the five emamectin benzoate-treated trees, 6.6 ± 1.31 m^2 of phloem was exposed per tree (33.0 m^2 total). On the remaining trees, 1.5 ± 0.01 m^2 of phloem per tree was exposed (34.8 m^2 total).

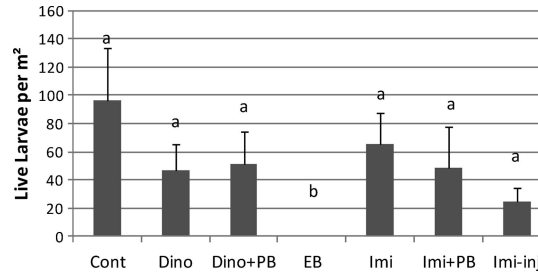

Fig. 2. *A. planipennis* larval density (average + SE) on *Fraxinus* sp. trees in fall 2007 for untreated controls (Cont) and trees treated in spring 2007 with basal trunk sprays of dinotefuran (Dino) with or without Pentra-Bark (PB), trunk-injected emamectin benzoate (EB), basal trunk sprays of imidacloprid (Imi) with or without Pentra-Bark (PB) or trunk-injected imidacloprid (Imi-inj) ($N = 7$ trees per treatment). Letters above bars indicate statistically significant differences among treatments ($P < 0.05$).

We recorded 570 *A. planipennis* galleries in total initiated by larvae that fed on the trees before our treatments were applied in spring 2007. Overall density of galleries from larvae that developed before 2007 was 9.2 ± 3.87 and 9.6 ± 1.67 per m^2 on the trees at Seven Lakes and Interstate, respectively. Density of pretreatment galleries did not differ among sites ($F = 3.37$; df = 1, 48; $P = 0.07$) or treatments ($F = 1.57$; df = 6, 43; $P = 0.19$), nor was the interaction between the main effects significant ($F = 0.55$; df = 6, 41; $P = 0.77$).

Density of current-year larvae was lower on the emamectin benzoate-treated trees than on all other trees ($F = 6.68$; df = 6, 43; $P < 0.0001$) (Fig. 2), but differences among other treatments were not significant. We identified a total of 2,629 live *A. planipennis* larvae in the 85.8 m^2 of phloem exposed on the 50 trees felled in 2007. Larval density on untreated control trees at the Seven Lakes and Interstate sites averaged 134.0 ± 80.47 and 67.6 ± 32.62 emerald ash borer per m^2, respectively. A total of only eight live larvae were present on the eight emamectin benzoate-treated trees that were completely debarked in 2007, equivalent to 0.19 larvae per m^2. Larval density was similar at the two sites, with overall values of 48.3 ± 14.75 and 45.4 ± 11.43 larvae per m^2 at Seven Lakes and Interstate, respectively ($F = 0.40$; df = 1, 48; $P = 0.53$). The interaction between site and treatment was not significant ($F = 0.81$; df = 6, 41; $P = 0.57$).

Larval development was not affected by treatment ($F = 1.20$; df = 6, 43; $P = 0.33$), site ($F = 0.27$; df = 1, 48; $P = 0.61$), or the interaction ($F = 0.54$; df = 6, 41;

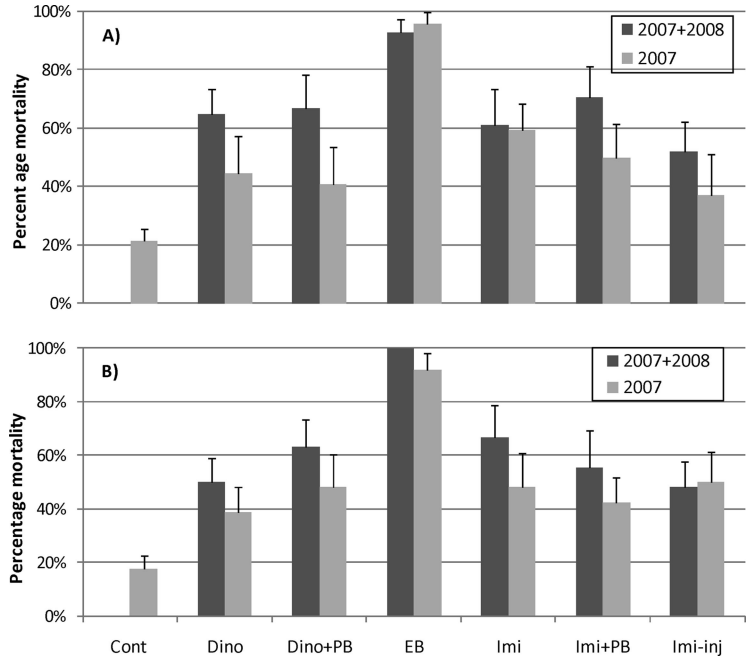

Fig. 3. Cumulative percentage mortality (average + SE) on day 4 for *A. planipennis* beetles provided with *Fraxinus* sp. foliage in bioassays in June (A) and July (B) 2008 from untreated control trees (Cont) and trees treated in 2007 + 2008 or 2007 only with basal trunk sprays of dinotefuran (Dino) with or without Pentra-Bark (PB), trunk-injected emamectin benzoate (EB), basal trunk sprays of imidacloprid (Imi) with or without Pentra-Bark (PB), or trunk-injected imidacloprid (Imi-inj) ($N = 9$ trees per treatment). Day 1 mortality was significantly affected by treatment, whereas day 4 mortality was significantly affected by treatment and site ($P < 0.05$).

$P = 0.77$). Overall, the proportion of live larvae overwintering as fourth instars or prepupae averaged 64.8 ± 3.63%. On control trees, 75.6 ± 4.84% of larvae were late instars. For the trees treated with dinotefuran, with and without Pentra-Bark, 54.3 ± 10.99 and 78.5 ± 3.99% of larvae, respectively, were late instars. Of the larvae in trees that were trunk-sprayed with imidacloprid, with and without-Pentra Bark, 53.4 ± 8.53 and 69.3 ± 4.56%, respectively, were late instars. In trees injected with imidacloprid and on control trees, 62.9 ± 12.62 and 75.6 ± 4.84%, respectively, of larvae were late instars. Four of the eight larvae on the emamectin benzoate trees were late instars.

We recovered 229 dead larvae on the trees, including 81 cadavers (40% of all dead larvae) from the trees treated with emamectin benzoate. Density of dead larvae averaged 4.0 ± 1.69 per m^2. Density of dead larvae did not differ among treatments ($F = 1.38$; df = 6, 43; $P = 0.25$) or sites ($F = 0.01$; df = 1, 48; $P = 0.92$), and the interaction was not significant ($F = 1.19$; df = 6, 41; $P = 0.33$). Overall, 68% of the larval cadavers were first or second instars, 15% were third instars, and 17% were fourth instars. We found no dead prepupae.

Canopy Condition: 2008. In early September 2008, canopy dieback estimates ranged from 0 to 90%, with an overall average ± SE of 15 ± 2.0%. Average dieback was higher on control trees (21 ± 5%) and trees treated only in 2007 with imidacloprid + PB (26 ± 9%), than on trees treated with emamectin benzoate (<1 ± 0%), whereas dieback on the other trees was intermediate ($F = 2.82$; df = 12, 112; $P = 0.003$). Seven of the 18 control trees had ≥30% dieback whereas 16 of the 17 trees treated at least once with emamectin benzoate had no detectable dieback (one tree had 10% dieback). Of the 36 trees treated with dinotefuran (with or without PB), six trees had dieback ≥30%. Twelve of the 36 trees treated with an imidacloprid trunk spray (with or without PB) and two of the 18 trees injected with imidacloprid had ≥30% dieback. Trees at the Interstate site had more dieback (21 ± 4%) than trees at the Wolverine site (5 ± 1%) ($F = 5.35$; df = 2, 122; $P = 0.006$), whereas dieback on trees at Seven Lakes was intermediate (18 ± 5.0%). The interaction between the main effects of site and treatment was not significant ($F = 1.47$; df = 24, 109; $P = 0.10$).

Adult Bioassays: 2008. We evaluated survival of 750 adult *A. planipennis* in the June and July bioassays (total of 1,500 beetles). Day 1 mortality of beetles feeding on leaves from trees injected with emamectin benzoate in 2007 + 2008 averaged 69 ± 9% over the summer (across sites and months), which was higher than beetle mortality associated with all other treatments ($F = 14.70$; df = 12, 23; $P < 0.0001$) (Fig. 3). In contrast, day 1 mortality on trees injected with emamectin benzoate only in 2007 was 24 ± 6%, similar to that recorded for the dinotefuran + PB trees treated in 2007 + 2008 (24 ± 6%). For trees treated with dinotefuran (no PB) in 2007 + 2008 and in 2007 only, beetle mortality on day 1 was 13 ± 4 and 15 ± 8%,

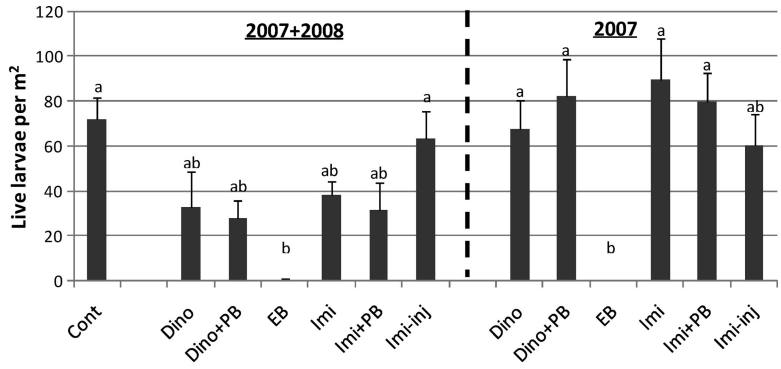

Fig. 4. *A. planipennis* larval density (average + SE) on *Fraxinus* sp. trees in fall 2008 for untreated controls (Cont) and trees treated in spring of 2007 + 2008 (A) or only 2007 (B) with basal trunk sprays of dinotefuran (Dino) with or without Pentra-Bark (PB), trunk-injected emamectin benzoate (EB), basal trunk sprays of imidacloprid (Imi) with or without Pentra-Bark (PB) or trunk-injected imidacloprid (Imi-inj) ($N = 9$ trees per treatment). Letters above bars indicate statistically significant differences among treatments ($P < 0.05$).

respectively. Average day 1 mortality was <6% for beetles feeding on leaves from the control trees and any of the imidacloprid-treated trees. The main effects of site ($F = 1.92$; df = 2, 9; $P = 0.20$) and time ($F = 2.62$; df = 1, 83; $P = 0.11$) did not affect day 1 mortality, nor were two- or three-way interactions significant, including site by treatment ($F = 1.66$; df = 24, 9; $P = 0.22$), treatment by time ($F = 0.76$; df = 12, 23; $P = 0.69$), site by time ($F = 0.49$; df = 2, 9; $P = 0.63$), or site by treatment by time ($F = 0.50$; df = 24, 9; $P = 0.91$).

Beetle mortality on day 4 of the bioassays (Fig. 3) was significantly affected by the main effects of treatment ($F = 12.14$; df = 12, 23; $P < 0.0001$) and site ($F = 21.97$; df = 2, 9; $P < 0.001$) but not by time ($F = 0.64$; df = 1, 83; $P = 0.42$). More than 90% of beetles feeding on leaves from emamectin benzoate-treated trees were dead by day 4, regardless of whether the trees were treated in both 2007 + 2008 or in 2007 only (Fig. 3). Beetle mortality on leaves from emamectin benzoate-treated trees was higher than that of beetles on leaves from all other trees. Only 19 ± 3% of beetles on leaves from control trees died by day 4, which was lower than mortality on trees treated with any of the insecticides. Beetle mortality on foliage from trees treated with the dinotefuran or imidacloprid products was intermediate, with average mortality ranging from 44 ± 9 to 65 ± 8% (Fig. 3). The addition of the surfactant (Pentra-Bark) to the dinotefuran and imidacloprid trunk sprays did not appreciably affect beetle mortality (Fig. 3). As in 2007, we again noted beetles that died on leaves from emamectin benzoate or dinotefuran-treated trees typically succumbed after a minimal amount of feeding, whereas numerous beetles caged with leaves from imidacloprid-treated trees did some feeding and produced frass, even when they succumbed by day 4. Significantly more beetles died feeding on foliage from trees at the Seven Lakes site (70 ± 5%), than from the Interstate (50 ± 3%) and Wolverine (48 ± 4%) sites. None of the two- or three-way interactions were significant, including site by treatment ($F = 1.75$; df = 24, 9; $P = 0.19$), treatment by time ($F = 0.29$; df = 12, 23; $P = 0.99$), site by time ($F = 0.26$; df = 2, 9; $P = 0.78$), or site by treatment by time ($F = 0.59$; df = 24, 9; $P = 0.86$).

Larval Density: 2008. In total, 22,041 live, current-year *A. planipennis* larvae were identified in 363.8 m^2 of phloem exposed on the 124 trees evaluated in 2008. We felled and debarked 1.9 ± 0.13 m^2 (mean ± SE) and 5.2 ± 0.47 m^2 per tree at the Seven Lakes and Interstate sites, respectively, and examined 0.5–0.6 m^2 of phloem per tree at the Wolverine site. There were 16 trees with zero live larvae; eight were treated with emamectin benzoate in 2007 + 2008 and six were treated with emamectin benzoate in 2007 only. One tree treated with imidacloprid in 2007 only and a tree treated with imidacloprid + PB in 2007 + 2008 also had zero larvae.

Density of live larvae differed among treatments ($F = 5.75$; df = 12, 112; $P < 0.0001$). Trees treated with emamectin benzoate in 2007 + 2008 had <1 larva per m^2, a value that was lower than larval density in the untreated controls from the same blocks with ≈88.4 ± 14.21 larvae per m^2 (Fig. 4). Larval densities in trees treated in 2007 + 2008 with dinotefuran or imidacloprid trunk sprays (with or without Pentra-Bark), were 57–68% lower than on control trees. Larval density in trees treated with emamectin benzoate only in 2007 also averaged <1 larva per m^2, whereas control trees in these blocks had an average of 56.1 ± 10.20 larvae per m^2 (Fig. 4). Larval densities in trees treated with any neonicotinoid product in 2007 only were similar to densities on untreated controls (Fig. 4). Overall larval density was significantly lower at the Seven Lakes site (30.5 ± 6.81 larvae per m^2) than at the Wolverine or Interstate sites (51.4 ± 0.94 and 62.7 ± 6.16, respectively) ($F = 8.40$; df = 2, 122; $P < 0.001$). The interaction between the main effects was not significant ($F = 1.57$; df = 24, 109; $P = 0.07$).

When trees were debarked, 54.2 ± 2.87% of live larvae were late instars (fourth instar or prepupae). On control trees, 59.1 ± 6.65% of the larvae were late instars. For trees treated in 2007 + 2008, the proportion of late instars ranged from 39.3 ± 9.83% in trees

Table 2. Average ± SE residues (ppm) by sampling date and site in composite samples of leaves collected in 2007 from *Fraxinus* sp. trees treated with basal trunk sprays of dinotefuran (Dino) with or without Pentra-Bark (PB), trunk-injected emamectin benzoate (EB), basal trunk sprays of imidacloprid (Imi) with or without Pentra-Bark (PB) or imidacloprid applied as a trunk injection (Imi-inj) ($N = 25$ trees per treatment)

	Date	Dino	Dino + PB	Em Ben	Imi	Imi + PB	Imi-inj
Seven Lakes ($n = 7$)	7 June	3.0 ± 1.0	4.4 ± 1.3	7.8 ± 3.2	1.1 ± 0.2	1.5 ± 0.6	8.5 ± 3.2
	7 July	3.8 ± 1.1	6.5 ± 2.1	3.6 ± 0.9	3.8 ± 1.6	3.7 ± 1.1	6.5 ± 3.1
	1 Aug.	3.4 ± 1.0	3.5 ± 0.8	3.5 ± 0.7	2.5 ± 1.1	2.3 ± 0.6	5.8 ± 2.7
	14 Aug.	2.9 ± 1.0	3.5 ± 1.2	2.2 ± 0.6	2.4 ± 0.7	3.0 ± 0.8	5.5 ± 2.3
Interstate ($n = 12$)	7 June	2.0 ± 0.3	1.9 ± 0.5	11.1 ± 3.9	0.7 ± 0.4	0.5 ± 0.1	7.2 ± 1.9
	7 July	1.4 ± 0.2	1.5 ± 0.3	7.2 ± 3.3	1.0 ± 0.3	0.9 ± 0.3	5.4 ± 1.9
	1 Aug.	1.0 ± 0.1	0.7 ± 0.1	6.5 ± 0.9	0.7 ± 0.3	0.8 ± 0.2	4.2 ± 1.0
	14 Aug.	1.1 ± 0.2	0.9 ± 0.1	7.2 ± 2.0	0.6 ± 0.4	0.9 ± 0.3	2.9 ± 0.7
Wolverine ($n = 6$)	7 June	3.0 ± 0.7	3.6 ± 0.6	4.7 ± 0.7	1.4 ± 0.4	0.9 ± 0.2	7.4 ± 1.9
	7 July	2.3 ± 0.6	2.0 ± 0.7	4.8 ± 0.5	1.8 ± 0.5	1.2 ± 0.2	8.5 ± 1.8
	1 Aug.	1.4 ± 0.7	0.8 ± 0.2	3.9 ± 0.5	1.3 ± 0.5	0.6 ± 0.2	5.9 ± 1.4
	14 Aug.	3.8 ± 1.2	2.6 ± 0.5	2.9 ± 0.6	1.3 ± 0.5	1.0 ± 0.3	4.6 ± 1.5

treated with dinotefuran + PB to 68.2 ± 6.85% in trees treated with the imidacloprid trunk spray. On the single emamectin benzoate tree treated in 2007 + 2008 that had larvae, three of the five larvae were late instars. For trees treated in 2007 only, the proportion of larvae that were late instars ranged from 44.2 ± 10.95% on the imidacloprid-injected trees to 72.2 ± 6.67% in trees treated with the imidacloprid trunk spray. The three larvae found on the two trees treated in 2007 only with emamectin benzoate were early instars.

We recovered 851 dead larvae in total, nearly 70% of which were first or second instars, whereas 19, 11, and <1% were third instars, fourth instars, and prepupae, respectively. For trees treated in both 2007 + 2008, average density of dead larvae on control trees was 2.6 ± 1.15 larvae per m^2. Density of dead larvae on treated trees was similar, ranging from 2.3 ± 0.90–0.9 ± 0.27 in trees treated with dinotefuran or imidacloprid trunk sprays (with or without Pentra-Bark), 4.8 ± 3.30 on emamectin benzoate-treated trees, and 6.9 ± 3.65 larvae per m^2 in trees injected with imidacloprid. For trees treated in 2007 only, average density of dead larvae on control trees was 3.6 ± 1.22. Average density of dead larvae on treated trees ranged from 1.7 ± 0.73–3.4 ± 1.31. More dead larvae were found in trees at Wolverine (4.1 ± 0.94 larvae per m^2) than at Seven Lakes (1.3 ± 0.38), whereas the density of dead larvae at the Interstate site was intermediate (2.5 ± 0.58) ($F = 3.89$; df = 2, 122; $P = 0.024$). Differences among treatments and the interaction between site and treatment were not significant ($F = 1.47$; df = 12, 112; $P = 0.15$ and $F = 1.30$; df = 24, 109; $P = 0.19$, respectively).

Foliage Residues: 2007. Average foliar residues by month and site in 2007 for each insecticide are shown in Table 2. Across sites, average ± SE monthly residue levels in foliage from trees treated with a basal trunk spray of either dinotefuran or dinotefuran + PB ranged from 3.0 ± 0.49 ppm (mid-June, mid-July) to 1.5 ± 0.33 ppm (early August), but differences among months were not significant ($F = 2.50$; df = 3, 135; $P = 0.06$). Across months, average residues of dinotefuran with and without PB, respectively, ranged from 5.1 ± 1.19–3.2 ± 0.74 ppm at the Seven Lakes site and 2.0 ± 0.26–0.9 ± 0.10 ppm at the Interstate site. Residues at both sites were significantly higher than those of trees at the Wolverine site ($F = 27.44$; df = 2, 2; $P = 0.035$), where average residues for dinotefuran and dinotefuran + PB treated-trees ranged from 3.3 ± 0.44 to 1.1 ± 0.34 ppm. Addition of the Pentra-Bark surfactant did not increase dinotefuran residue levels ($F = 0.37$; df = 1, 1; $P = 0.65$). The interaction between the main effects (treatment × time) was not significant ($F = 0.55$, df = 3, 3; $P = 0.68$). Foliage residues of emamectin benzoate were highest in trees at all sites in June (Table 2), but differences among months were not significant ($F = 1.13$; df = 3, 45; $P = 0.35$). Across months, residue levels were 3.9 ± 0.73, 7.6 ± 1.09, and 3.8 ± 0.32 ppm at the Seven Lakes, Interstate, and Wolverine sites, respectively, but they did not vary significantly among sites ($F = 3.46$; df = 2, 1; $P = 0.36$).

Foliar residues of imidacloprid were significantly higher in trees that were trunk-injected than in trees where imidacloprid was applied as a basal trunk spray ($F = 55.36$; df = 2, 7; $P < 0.0001$) (Table 2). Across sites and months, imidacloprid residue levels over the summer were 5.8 ± 0.54 ppm in trees that were injected, and only 1.4 ± 0.19 and 1.3 ± 0.15 ppm for trees with bark sprays of imidacloprid and imidacloprid + PB trees, respectively. For trees treated with the imidacloprid products, residues (across months) were higher in trees at Seven Lakes (3.7 ± 0.50 ppm) than at the Interstate site (2.2 ± 0.30 ppm) and intermediate (3.0 ± 0.42 ppm) in trees at the Wolverine site ($F = 7.05$; df = 2, 6; $P = 0.027$). For trees treated with a basal trunk spray of imidacloprid, with or without PB, average foliar residues (across months) ranged from 3.8 ± 1.63 to 1.1 ± 0.22 ppm at Seven Lakes, from 1.0 ± 0.31 to 0.5 ± 0.1 ppm at Interstate, and from 1.8 ± 0.52 to 0.6 ± 0.2 ppm at the Wolverine site. Addition of the Pentra-Bark surfactant did not increase imidacloprid residue levels ($t = 0.25$, df = 7, $P = 0.81$). Overall residue levels of imidacloprid did not differ significantly among months ($F = 1.80$; df = 3, 183; $P = 0.15$). Average residue levels over the summer (across months) in trees treated with imidacloprid + PB ranged from 3.7 ± 1.13 to 1.5 ± 0.55 ppm at the Seven Lakes site, from 0.9 ± 0.28 to 0.5 ± 0.12 ppm at Interstate, and from 1.2 ± 0.22 to 0.6 ± 0.17 ppm at the

Table 3. Average ± SE residues (ppm) by sampling date (across sites) in composite samples of leaves collected in 2008 from *Fraxinus* sp. trees treated in 2007 + 2008 or 2007 only with basal trunk sprays of dinotefuran (Dino) with or without Pentra-Bark (PB), trunk-injected emamectin benzoate (EB), basal trunk sprays of imidacloprid (Imi) with or without Pentra-Bark (PB) or imidacloprid applied as a trunk injection (Imi-inj) (N = 9 trees per treatment)

	June	July	Aug.
2007 + 2008			
Dino	3.2 ± 0.40	3.0 ± 0.45	2.1 ± 0.33
Dino + PB	4.8 ± 0.91	3.6 ± 0.62	3.1 ± 0.44
EB	9.1 ± 2.30	7.8 ± 1.49	5.5 ± 1.12
Imi	0.3 ± 0.10	1.1 ± 0.25	0.9 ± 0.28
Imi + PB	1.3 ± 0.66	1.7 ± 0.85	3.8 ± 2.26
Imi-inj	0.7 ± 0.32	0.7 ± 0.44	1.2 ± 0.93
2007 only			
Dino	1.9 ± 0.95	2.2 ± 1.58	0.7 ± 0.30
Dino+PB	1.6 ± 0.77	0.9 ± 0.24	0.8 ± 0.34
EB	0.7 ± 0.27	1.5 ± 1.00	0.8 ± 0.37
Imi	0.5 ± 0.44	1.4 ± 1.24	2.0 ± 1.78
Imi+PB	0.2 ± 0.08	0.4 ± 0.34	0.5 ± 0.29
Imi-inj	0.3 ± 0.16	0.4 ± 0.24	0.4 ± 0.31

Wolverine site. None of the two- or three-way interactions significantly affected imidacloprid residues, including site by treatment type (F = 0.55; df = 4, 6; P = 0.71), treatment type by time (F = 1.83; df = 6, 21; P = 0.14), site by time (F = 0.27; df = 6, 18; P = 0.94), and site by treatment type by time (F = 0.24; df = 12, 18; P = 0.99).

Foliage Residues: 2008. Foliar residues (across sites) averaged by month in 2008 for trees treated with each insecticide in 2007 + 2008 or only in 2007 are shown in Table 3. Trees treated with the basal trunk spray of dinotefuran + PB in 2007 + 2008 had significantly higher residue levels (3.8 ± 0.41 ppm) than trees treated only in 2007 (1.1 ± 0.29 ppm) (F = 7.98; df = 3, 24; P < 0.001). Residues in trees treated with dinotefuran (no PB) did not differ between trees treated in 2007 + 2008 (2.8 ± 0.24 ppm) and those treated in 2007 only (1.6 ± 0.61 ppm) (t = 1.26; df = 1, 24; P = 0.22). Addition of Pentra-Bark did not increase foliar residue levels in trees treated in 2007 + 2008 (t = 2.03, df = 24, P = 0.053) nor in trees treated in 2007 only (t = 1.42; df = 1, 24; P = 0.17). Overall residues of dinotefuran (across months), with or without PB, were higher at Seven Lakes (3.3 ± 0.67 ppm) and Wolverine (2.5 ± 0.38 ppm) than at the Interstate site (1.7 ± 0.23 ppm) (F = 6.70; df = 2, 24; P = 0.005). The interaction between site and treatment was significant (F = 5.02; df = 6, 24; P = 0.002). At the Interstate and Wolverine sites, dinotefuran residues in trees treated in 2007 + 2008 were four- to eight-fold higher than residues in trees treated in 2007 only, but at the Seven Lakes site, average residue levels were either lower in the trees treated in 2007 + 2008 or similar to levels in trees treated in 2007 only. Across sites, dinotefuran residues were higher in June (2.9 ± 0.43 ppm) and July (2.4 ± 0.46 ppm) than in August (1.7 ± 0.24 ppm) (F = 4.47; df = 2, 48; P = 0.017). Other two- and three-way interactions were not significant, including treatment by time (F = 0.59; df = 6, 48; P = 0.74), site by time (F = 0.75; 4, 48; P = 0.56), and site by treatment by time (F = 0.84; df = 12, 48; P = 0.61).

Emamectin benzoate residues over the summer (across sites and months) was 7.5 ± 0.10 ppm in trees treated in 2007 + 2008, which was 7 times higher than residues in trees treated in 2007 only, which averaged 1.0 ± 0.35 ppm (F = 39.31; df = 1, 11; P < 0.0001). Trees treated with emamectin benzoate at the Interstate site (6.8 ± 1.42 ppm) had higher residues than trees at the Seven Lakes (2.7 ± 1.01 ppm) and Wolverine (2.8 ± 0.64 ppm) sites (F = 6.93; df = 2, 11; P = 0.011). The interaction between treatment and site was significant (F = 11.21; df = 2, 11; P = 0.002). Residues in trees injected in 2007 + 2008 were nearly 18 and 12 times higher than residues in trees injected in 2007 at the Interstate and Wolverine sites, respectively. At Seven Lakes, however, trees injected with emamectin benzoate in 2007 + 2008 averaged 3.1 ± 1.63 ppm, only slightly higher than trees treated in 2007 only (2.3 ± 1.33 ppm) (Table 3). Although residues in trees treated in 2007 + 2008 declined over the summer, residues in trees treated in 2007 did not and overall differences among months were not significant (F = 1.37; df = 2, 22; P = 0.27). The remaining two- and three-way interactions were not significant, including treatment by time (F = 1.08; df = 2, 22; P = 0.36), site by time (F = 0.93; df = 4, 22; P = 0.46), and site by treatment by time (F = 0.33; df = 4, 22; P = 0.86).

Overall imidacloprid residues increased gradually over the summer for trees treated with the trunk sprays (with and without Pentra-Bark) in 2007 + 2008 or in 2007 only (Table 3). Imidacloprid residues were below detectable levels throughout the summer in 12 trees treated only in 2007 (five imidacloprid + PB, two imidacloprid [no PB] and five trunk-injected imidacloprid trees). Imidacloprid residues (across sites) were significantly higher in August than in June, whereas July was intermediate (F = 3.77; df = 2, 91; P = 0.027). Across months, imidacloprid residues were significantly higher in trees at the Seven Lakes site (3.1 ± 0.78 ppm) than in trees at the Wolverine (0.5 ± 0.11 ppm) and Interstate (0.3 ± 0.06 ppm) sites (F = 20.66; df = 2, 5; P = 0.004). Although average residues were somewhat higher in trees treated with the imidacloprid + PB trunk spray in 2007 + 2008 (2.2 ± 0.82 ppm) than in trees treated with imidacloprid (no PB) (0.8 ± 0.14 ppm) or injected with imidacloprid (0.9 ± 0.36 ppm) in 2007 + 2008, within-treatment variability was also high. The treatment effect was not significant (F = 2.17; df = 5, 2; P = 0.35) nor was the interaction between treatment and month (F = 0.38; df = 10, 4; P = 0.90).

Discussion

Our study encompassed a total of 175 trees in four counties representing a range of size classes and growing conditions. Pretreatment larval densities, which represent *A. planipennis* that developed before our study began in spring 2007, were <10 larvae per m^2 and there was little evidence of canopy decline or injury related to *A. planipennis.* Previous studies have

shown that on average, ≈89 *A. planipennis* beetles can develop and emerge from a square meter of ash phloem (McCullough and Siegert 2007) and significant canopy dieback is usually associated with densities of at least 30 *A. planipennis* per m^2 (Anulewicz et al. 2007). Low pretreatment larval densities and generally healthy canopies indicate all trees were capable of translocating insecticides to the canopy in 2007. Effective translocation and within-tree distribution of systemic insecticides is especially important in the *A. planipennis*–ash system. Like other buprestids such as the native *Agrilus bilineatus* (Weber), *A. planipennis* beetles generally colonize the canopy of host trees before the trunk, leading to a progressive, top-down canopy decline and dieback (Haack and Benjamin 1982, Cappaert et al. 2005, Tluzcek et al. 2011).

Bioassays with adult *A. planipennis* provide insight into the relative toxicity and persistence of insecticides in ash foliage. The nearly complete mortality of adult *A. planipennis* caged with leaves from emamectin benzoate trees, even for beetles caged in 2008 with leaves from trees treated only in 2007, was remarkable. Any wild female beetles that fed on emamectin benzoate-treated trees at our sites in the 20-d period preceding maturation would have died before they were able to lay eggs. Controlling *A. planipennis* beetles before they can oviposit is ideal because it prevents larval feeding that would otherwise injure vascular tissue.

The toxicity of foliage from emamectin benzoate-treated trees to adult beetles may also have implications for enhancing *A. planipennis* control in a given neighborhood or localized outlier site. Dispersal studies have shown beetles lay a high proportion of their eggs within 100 m of the tree from which they emerged (Mercader et al. 2009, Siegert et al. 2010), which indicates many beetles must feed on ash trees in the same area. If the insecticide is present and well distributed within the canopy by the time most adult *A. planipennis* have emerged and are actively feeding in early summer, a substantial portion of females may be killed before laying eggs. This obviously does not preclude the possibility that beetles may feed on nontreated trees but lay at least some eggs on treated trees in the same locale. It does, however, suggest that overall *A. planipennis* control may improve if most ash trees in a given area are treated and a high proportion of female beetles are killed before substantial oviposition occurs.

Although adult bioassays are informative, density of *A. planipennis* larvae, the life stage that injures the tree, is critically important when assessing insecticide efficacy. Ash trees are highly sectorial (Tanis et al., unpublished data) and seem relatively tolerant of low densities of *A. planipennis* larvae. Canopy decline is generally not apparent until densities build to at least moderate levels (Anulewicz et al. 2007; McCullough and Siegert 2007; McCullough et al. 2009a,b).

Density of *A. planipennis* larvae on the emamectin benzoate-treated trees was <1% of that in comparable control trees in 2007 and again in 2008, regardless of whether trees were treated in 2007 only or in both 2007 and 2008. In 2007, we literally debarked eight trees from top to bottom, yet recovered only eight live larvae, whereas each of the untreated control trees typically hosted at least a few hundred live larvae. Perhaps the most striking result of our study was the near absence of live *A. planipennis* larvae in 2008, even in trees treated with emamectin benzoate only in 2007. Residues in 2008 foliage samples from trees treated with emamectin benzoate only in 2007 were detectable but orders of magnitude lower compared with 2007 residues and 2008 residues in trees treated in both 2007 + 2008. These data suggest that even low concentrations of emamectin benzoate provided highly effective *A. planipennis* control. This is further supported by rapid mortality of adult beetles in bioassays. Beetles died after consuming only one or a few bites from leaves of emamectin benzoate-treated trees, indicating that the lethal dose is very low. Although we did recover some larval cadavers when trees were debarked, they were relatively sparse. The scarcity of dead larvae, particularly dead late instars, indicates emamectin benzoate acted primarily by killing adult emerald ash borer or neonates, which meant that trees sustained little injury.

These results indicate ash trees can be effectively treated with emamectin benzoate at intervals of 2 yr and perhaps longer. Smitley et al. (2010) treated landscape ash trees with a relatively high rate of emamectin benzoate (0.4 g [AI]/2.5 cm dbh) and reported less canopy thinning in treated trees compared with control trees, 4 yr posttreatment. Treatment efficacy may have been somewhat enhanced in their study because three small branches (2–12 cm in diameter) were removed annually from trees to sample larval density. Tree dbh determines treatment rate and annual pruning in their study effectively decreased the ratio of canopy area relative to dbh. Nevertheless, they found no larvae on small branches from treated trees at least 2 yr posttreatment, whereas comparable branches sampled on control trees had >24 larvae per m^2.

Highly effective, multi-year control of *A. planipennis* with emamectin benzoate may substantially reduce costs and logistical issues associated with annual treatments, particularly for municipalities where large numbers of ash trees are at risk. Multiyear protection provided by emamectin benzoate also could be important in localized outlier sites where objectives may include reducing *A. planipennis* population growth and slowing the onset or progression of ash mortality (Poland and McCullough 2010, http://www.slameab.info/). Simulation models indicated highly effective insecticides were more effective at slowing *A. planipennis* population growth than using girdled ash trees as population sinks or targeted ash removal (Mercader et al. 2011a,b).

Noninvasive basal trunk sprays for neonicotinoid application, particularly dinotefuran, may be an attractive option for arborists because they are relatively quick and require no drilling or specialized injection equipment. We detected dinotefuran and imidacloprid in foliar residues within 3–4 wk posttreatment in both 2007 and 2008, indicating at least some portion of

the insecticide had moved into xylem and was translocated to the canopies. Dinotefuran is ≈80 times more soluble than imidacloprid (USEPA 2004, Extoxnet 2010), and we expected it would be translocated more rapidly within the tree than imidacloprid. Residue data were consistent with this hypothesis; dinotefuran residues in June 2007, for example, were 2–3 times higher than imidacloprid residues. Generally, imidacloprid residues tended to increase over the summer as translocation continued, whereas dinotefuran residues usually declined by late summer, consistent with previous studies (McCullough et al. 2007). Detection of dinotefuran in foliage in 2008, 12–14 mo posttreatment, was somewhat unexpected and suggests that additional work to assess dinotefuran persistence and within-tree translocation is warranted. Addition of the Pentra-Bark surfactant to either the formulated dinotefuran or imidacloprid did not consistently affect adult *A. planipennis* mortality, larval density, or residue levels.

Residues in trunk-sprayed trees were usually higher at the Seven Lakes site, where trees were smaller, than at the other two sites. Lower residues at the Wolverine and Interstate sites may reflect lower penetration of the products applied as basal bark sprays on larger trees with thicker bark. It is more likely, however, that the generally lower residues on large trees are a function of the nonlinear relationship between dbh and canopy area (McCullough and Siegert 2007, Cowles 2010). In contrast to label application rates for the neonicotinoid trunk-sprays, the label rate of active ingredient applied for injection of emamectin benzoate was adjusted depending on tree size (dbh), a practice that probably account for the relatively high residues in the large trees at the Interstate site. Similarly adjusting application rates based on tree size would probably improve efficacy of dinotefuran and imidacloprid basal trunk sprays and warrants further study.

Results clearly indicated the neonicotinoid products must be applied annually for *A. planipennis* control. Control trees and trees treated only in 2007 with dinotefuran or the imidacloprid products averaged >60 larvae per m^2, densities consistent with moderate to heavy infestations (Anulewicz et al. 2007, McCullough and Siegert 2007). Canopy decline and dieback was apparent on many of these trees in late summer 2008. Herms et al. (2009) suggested *A. planipennis* control with neonicotincoid products may improve when trees are treated for two or more consecutive years. Our results provide some support for this pattern, with the exception of the trunk-injected imidacloprid. If we compare average larval densities in 2007 on control versus treated trees, larval density was 47–51, 32–49, and 74% lower in trees treated with dinotefuran trunk sprays (with or without PB), imidacloprid trunk sprays (with or without PB) and imidacloprid trunk injection, respectively. In 2008, differences in larval density between control trees and those treated with trunk sprays in 2007 + 2008 were more pronounced. Larval density was 62–68, 57–64, and 28% lower in trees treated with dinotefuran trunk sprays (with or without PB), imidacloprid trunk sprays (with or without PB), and imidacloprid trunk injection, respectively.

Whether the 50–70% control provided by the neonicotinoid trunk sprays is adequate to effectively protect trees from serious *A. planipennis* injury over multiple years remains unknown. It may depend largely on the proportion of trees in the area that are treated with an insecticide and the local *A. planipennis* population, which determine the number of beetles that could potentially oviposit on treated trees.

It is difficult to assess the efficacy of injecting imidacloprid with Mauget capsules for *A. planipennis* control. This product seemed promising in 2007. Mortality of adult *A. planipennis* was particularly high in the mid-June bioassay. Foliar residues were also notably high, especially in June and July 2007, when they were ≈3 times higher than residues in trees treated with imidacloprid trunk sprays. Average larval density in fall 2007 was substantially lower in trees injected with imidacloprid than on the controls, although differences were not statistically significant. In trees injected with imidacloprid in 2007 + 2008, however, we did not observe high beetle mortality in the bioassays, larval density was comparable with controls and foliar residue levels were considerably lower than in 2007, especially in the large trees at the Interstate and Wolverine sites. Our application and evaluation methods were the same in 2007 and 2008, and the disappointing results in 2008 were unexpected. Grossman and Upton (2006) reported Mauget injection capsules did not work well on conifers because the pressure produced by priming the injector was often insufficient to overcome the tree's resin pressure. Although ash trees do not produce resin as conifers do, insufficient pressure or poor uptake and translocation in 2008 may have limited effectiveness of this treatment. These results serve to illustrate the considerable variability in the efficacy of different imidacloprid formulations and the importance of multiyear evaluations of control.

Municipalities, resource agencies and property owners will need to consider multiple factors, including insecticide efficacy, treatment costs, and logistical issues as they develop plans to cope with *A. planipennis.* Our results, which indicate viable options are available to protect landscape ash trees in the United States from *A. planipennis,* represent substantial progress since this invader was identified in 2002. Ultimately, insecticides will probably be one component of integrated *A. planipennis* management strategies that also may include sanitation cuts to remove overaged or unhealthy ash trees, biological control, and regulatory activities to limit artificial spread.

Acknowledgments

We thank Yan Xie (Center for Statistical Training and Consulting, MSU) for direction and assistance with statistical analyses. We are grateful to the many technicians and students who assisted with aspects of this project from 2007 to 2009, including Jake Baker, Joann Bloese, Jacob Bournay, Tara Dell, Nicholas Gooch, Chenin Limback, Ben Schmidt,

Sarah Smith, Andrew Tluczek, and James Wieferich, (all at MSU); Tina Ciaramitaro, Devin Foote, Stephen Burr (all at U.S. Forest Service); and John Molongoski, Hanna Wingard, and Erin O'Brien (all at USDA–APHIS). Joe Aiken and Joe Doccola (both at Arborjet), David Cox (Syngenta), Jason Fausey (Valent USA), Jim Rollins (JJ Mauget Co.), and Bill Stringfellow (Agri-Chem) provided insecticide products and application advice. The Michigan Department of Natural Resources, the Michigan Department of Transportation, and Genesee County Parks provided access to study sites. This work was funded by a grant from the USDA Forest Service, Northeastern Area, State & Private Forestry, Forest Health Protection (to D.G.M. and T.M.P.).

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Received 31 March 2011; accepted 19 July 2011.