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Distribution of trunk-injected ^{14}C-imidacloprid in ash trees and effects on emerald ash borer (Coleoptera: Buprestidae) adults

David Mota-Sanchez [a,*], Bert M. Cregg [b,c], Deborah G. McCullough [a,c], Therese M. Poland [d], Robert M. Hollingworth [a]

[a] Department of Entomology, Michigan State University, 206 Center for Integrated Plant Systems, East Lansing, MI 48824, USA
[b] Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA
[c] Department of Forestry, Michigan State University, East Lansing, MI 48824, USA
[d] USDA Forest Service, Northern Research Station, East Lansing, MI 48823, USA

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae) is a destructive exotic pest of North American ash (*Fraxinus* sp.) trees. Trunk injection of imidacloprid is commonly used to protect landscape ash trees from *A. planipennis* damage. Efficacy can vary and little is known about the distribution, accumulation and persistence of this compound in trees. Green ash (*Fraxinus pennsylvanica*) and white ash (*Fraxinus americana*) trees were injected with 25 μCi of ^{14}C-imidacloprid plus non-labeled imidacloprid and were grown under water-sufficient and water stress conditions. Tree trunks, twigs, leaves and roots were sampled periodically for two years following injection. Imidacloprid concentrations did not vary ($P > 0.05$) between tree species or water treatments. Imidacloprid concentrations differed ($P < 0.001$) among plant tissue types, leaves had much greater concentrations (>30×) than any of the other plant tissues. Imidacloprid concentrations in leaves increased steadily throughout the first (2004) growing season, whereas in the year following injection (2005), little imidacloprid was detected in leaves. Samples from outer bark and phloem collected with a cork-borer at 1 m and 2 m above ground line had low levels of imidacloprid as did fine roots. This suggests that imidacloprid translocation occurred mainly in the xylem. When adult *A. planipennis* were fed leaves from trunk-injected trees, an average of 71% of beetles were killed or intoxicated in 2004 compared with an average of 24% in 2005. Ash species and water treatment had little effect on *A. planipennis* mortality in either year.

1. Introduction

Ash trees, primarily green ash (*Fraxinus pennsylvanica* Marsh.) and white ash (*Fraxinus americana* L.) are a major component of urban and community forests in the eastern United States (Poland and McCullough, 2006). The emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), is an Asian beetle that was first identified in 2002 as the cause of substantial ash decline and mortality in southeastern Michigan and Essex County, Ontario, Canada (Cappaert et al., 2005). Adults feed on foliage during the summer for maturation and energy, but cause little damage. *A. planipennis* beetles appear to require at least two weeks of leaf-feeding before oviposition begins (Cappaert et al., 2005). Eggs are laid from late June to early August and hatch within roughly 2 weeks. Larvae chew through the bark and feed on phloem until late autumn (Cappaert et al., 2005) thereby weakening the tree. Most *A. planipennis* overwinter as pre-pupal larvae and emerge the following summer. Tens of millions of ash trees in lower Michigan have been killed by *A. planipennis* and populations have now been found in at least seven other states and in areas of Ontario and Quebec (Emerald Ash Borer, 2008). Anulewicz et al. (2008) reported that North American ash species vary in their susceptibility to emerald ash borer (EAB), but none appear to be completely resistant. Green ash trees are generally colonized earlier and succumb more rapidly than white ash when both occur on the same site (Anulewicz et al., 2007, 2008). Prior to the discovery of *A. planipennis*, ashes comprised up to 29% of the urban and community forest canopies in Michigan cities (MacFarlane and Meyer, 2005). In Ohio, potential costs of municipal ash tree removal and replacement were estimated at $1.8–$7.6 billion (Sydnor et al., 2007). The ability to protect valuable ash trees in urban and community forests from *A. planipennis* requires the development of insecticide treatments that are safe, effective, and reliable.

* Corresponding author. Tel.: +1 517 432 2668; fax: +1 517 353 4354.
E-mail address: motasanc@msu.edu (D. Mota-Sanchez).

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Trunk or soil injection of systemic insecticides is increasingly preferred as a method for controlling insect pests in urban and suburban landscapes because of minimal risks of applicator exposure, drift and impacts on non-target organisms. Imidacloprid products are commonly used to protect ash trees from *A. planipennis* (Emerald Ash Borer, 2008). This compound acts at the nicotinic acetylcholine receptors (nAChRs) in the insect nervous system (Schroeder and Flattum, 1984; Bai et al., 1991; Matsuda et al., 2001). Imidacloprid treatment is primarily targeted to adult stage EAB which feed on leaves rather than larvae, which feed on the phloem. Imidacloprid insecticides effectively control many groups of insects, including sap-feeders and beetles (Jeschke and Nauen, 2008). Differences in binding potency between insect and mammalian acetylcholine receptors, however, make these products relatively safe for humans (Thyssen and Machemer, 1999). Imidacloprid has been used successfully in the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), eradication program (Markham, 2004) and for control of related phloem-borers such as the bronze birch borer (*Agrilus anxius* Gory) (USDA APHIS, 2000). Imidacloprid-treated ash leaves have been observed to have a strong antifeeding effect on adult *A. planipennis* (McCullough et al., 2003), and on Asian longhorned beetle (Poland et al., 2006) and sucking insects such as aphids and whiteflies (Nauen, 1995; Nauen et al., 1998a,b).

An improved understanding of the movement and persistence of trunk-injected imidacloprid in ash trees is needed to develop appropriate recommendations and effective protocols for *A. planipennis* control. Arborists commonly inject trunks with imidacloprid insecticides in spring or occasionally in autumn. Ideally, these treatments result in uptake and distribution of imidacloprid in foliage before adult *A. planipennis* begin to feed or oviposit. In Michigan, however, results from several studies involving imidacloprid applied to soil or as a trunk injection show that efficacy can vary substantially among and within sites (McCullough et al., 2003, 2005, 2008). The cause of the variation in imidacloprid efficacy is unknown but is likely related to site factors (insect density, ash tree density) and movement of imidacloprid within injected trees. Presently relatively little is known about imidacloprid translocation and persistence within ash trees once uptake has occurred.

To be effective, systemic insecticides must be translocated from the roots or injection site to the site of insect feeding. Once in the xylem, chemicals are dependent upon the transpiration stream to move upward to the leaves. Recovery of soil-applied ^{14}C-imidacloprid in a trial with 13 herbaceous plant species indicated that movement was almost entirely via the xylem (Sur and Stork, 2003). Almost all of the activity was recovered from leaves; negligible amounts were recovered from reproductive organs. Recovery rates of ^{14}C-imidacloprid applied as a spray to the trunks of orange trees were higher than those reported by Sur and Stork (2003) for soil applications and recovery was higher for younger trees than for older trees (Mendel, 1998). Mendel (1998) also observed that drought stress increased overall recovery despite slower translocation.

In this study, we monitored the distribution of radiolabeled (^{14}C) imidacloprid in leaves, twigs, trunks and roots of green and white ash trees for two years following trunk injection. We also conducted bioassays on adult *A. planipennis* beetles using leaves from treated trees.

2. Materials and methods

2.1. Ash trees and insecticide injection

Twenty green ash (*F. pennsylvanica*) (mean diameter 7.6 ± 0.2 cm) and white ash (*F. americana*) (10.1 ± 0.3 cm) (40 trees total) were donated by a local nursery (Discount Trees, Inc., Mason, MI). Trees were dug with a 100 cm mechanical tree spade on April 15, 2004 and placed in wire baskets lined with burlap and transported to the Michigan State University Horticulture Teaching and Research Center (HTRC) near East Lansing, MI. In early May, the trees were planted in large (90 cm × 90 cm × 80 cm: $W \times L \times H$) individual planter boxes filled with soil. All trees were inspected by the Michigan Department of Agriculture and appeared healthy at the time of planting. The trees were arranged in 10 rows (replications) with four trees (2 species × 2 water stress treatments) arranged at random within a row.

Each tree was injected on June 15, 2004 with 6 ml of Imicide® (10% imidacloprid a.i., J.J. Mauget, Arcadia, CA), including 25 μCi of ^{14}C-imidacloprid, at 15 cm above ground level via two injection ports on opposite sides of the tree using Systemic Tree Injection Tubes (STITs) (Helson et al., 2001). We used ^{14}C-imidacloprid labeled at the imidazolidine ring (Institute of Isotopes Co., Ltd.1121 Budapest, Hungary), and with a specific activity of 125.5 μCi/mg, 32.1 mCi/mmol for the study. Purity of the compound was confirmed by spotting on TLC plates (250 mm thick silica gel plates Whatman LK5F, Clifton, NJ) and using a mobile phase of methylene chloride + methanol (186 + 14). The ^{14}C-imidacloprid was localized by phosphorimaging (Biorad, Personal Fx) and removed from the TLC plates by scraping the areas where the compound was localized. The scraped material was placed in liquid scintillation vials to which liquid cocktail was added. Vials were counted in a liquid scintillation counter. The imidacloprid was found to have a radiochemical purity of 98%. Labeled imidacloprid was mixed with unlabeled imidacloprid (Imicide® 10%; J.J. Mauget Co, 100 g of a.i./L) in a proportion of 1:2400 (labeled:non-labeled imidacloprid).

After injection, half of the trees were kept well-watered (38 mm irrigation week^{-1}) and half were subjected to water stress (10 mm irrigation week^{-1}). The surface of the soil was covered with white heavy-gauge plastic to prevent rainfall from reaching the surface. Imposition of water stress was verified with periodic measurements of midday leaf gas exchange with a portable photosynthesis system (LI-6400, LiCor, Inc. Lincoln, NE).

2.2. Ash tree sampling

Samples of leaves, twigs, trunk cores, and roots were collected 0, 2, 7, 21, 60,105, and 150 days after treatment and again on June 15 and July 15 in 2005, a year after injection. Samples of leaves and twigs were taken from the lower, mid and upper canopy of each tree with a pole pruner. The leaves were removed from the twigs. Samples of the trunk were collected using a 0.5 cm diameter cork-borer to remove a 0.4 cm thick disk of bark and wood at 1 m and 2 m above ground line at a 90° angle to the injection point. The trunk core samples were comprised mainly of bark and phloem, but also contained a small amount (generally <10%) of xylem material. Fine roots were collected by carefully digging through the soil with a small trowel. Three samples (approximately 20 g fresh weight) were collected from each tree, gently rinsed in distilled water, and composited for drying and analysis. In early October 2004, the canopy of each tree was enclosed in netting to collect leaf litterfall. All the leaves were collected after leaf-fall and a 10 g (fresh weight) subsample was collected for analysis.

Prior to budbreak in 2005 we destructively harvested two replications (four trees of each species) by cutting trees down just above ground line, approximately 15 cm below the injection point. The trunks of the trees were cut into 0.5 m sections and 3 cm thick disks were cut from the bottom end of each section. Localized staining from the imidacloprid was visible in stem sections up to 1.5 m above the injection point. The stained sections were excised from the disks using a hammer and chisel and activity was

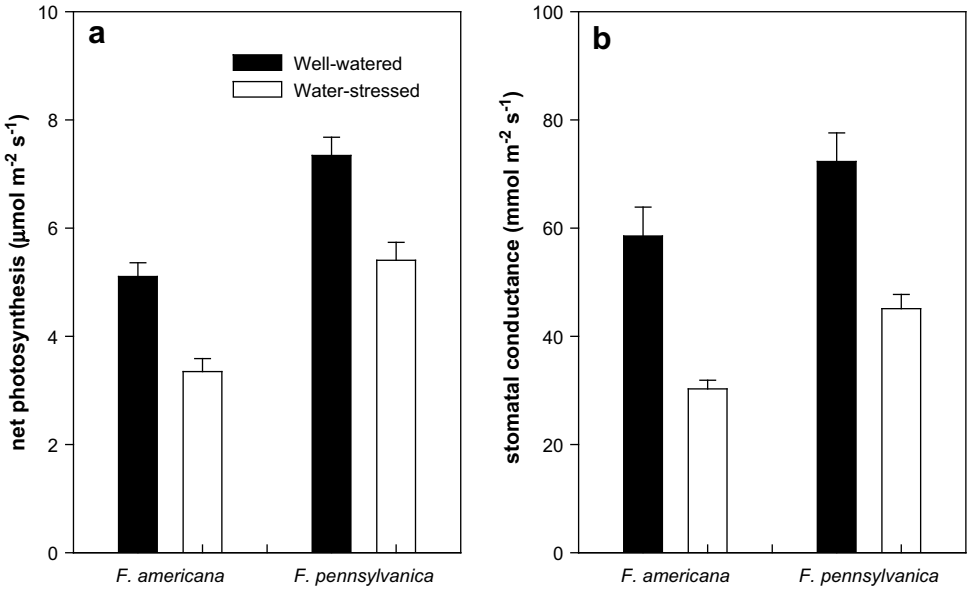

Fig. 1. (a) Mean (±SE) net photosynthesis and (b) mean (±SE) stomatal conductance of *Fraxinus americana* and *Fraxinus pennsylvanica* trees receiving either 38 mm of irrigation week^{-1} (well-watered) or 10 mm of water week^{-1} (water stressed) during the 2004 growing season. Species and water stress effects were significant ($P < 0.0001$); species × water stress interaction effect was not significant. $N = 10$ trees per species × water stress 1 combination.

determined separately for stained and non-stained material. In spring 2005, stumps from five of the harvested trees produced adventitious shoots providing a serendipitous opportunity to sample leaves from branches that originated below the point of imidacloprid injection. All the plant tissue material involved in the research during and at the end of the experiments was disposed following Michigan State University Office of Radiation, Chemical and Biological Safety regulations for use of ^{14}C.

All tissue samples were taken immediately to the laboratory, dried in an oven at 70 °C, ground and oxidized in a biological tissue oxidizer (OX-300, R.J. Harvey Instrument Corp., Tappan, NJ). The resulting $^{14}CO_2$ was trapped in a scintillation vial with 15 ml of cocktail fluid (Safety Solve, Research Product International Corp., Mount Prospect, IL). Radioactivity was determined by scintillation counting (LKB Wallac) and values corrected for the efficiency of the oxidizer and liquid scintillation counter (LSC). Values were transformed to specific activity; microgram of total radioactivity plus microgram of unlabeled material per gram of dried plant tissue. Since labeled imidacloprid and unlabeled imidacloprid (proportion of 1:2400, labeled:non-labeled imidacloprid) were mixed, we expected to get similar proportions of both types in the samples. Another important consideration is that total radioactivity includes the parent compound (imidacloprid) as well as potential metabolites that contain the ^{14}C in their structure. Therefore we refer to all activity as *imidacloprid equivalent*, but we also use simply 'imidacloprid' to refer to imidacloprid equivalent as well.

Table 1
Summary of analysis of variance (*F* values) for effects of species, water stress, and days after treatment (DAT) on imidacloprid equivalent concentration of various tissue types of *Fraxinus americana* and *Fraxinus pennsylvanica* trees following trunk injection.

Source	df	*F*
Between subject effects		
Tissue type	4	93.46***
Species (spp.)	1	1.26
Tissue × spp.	4	1.08
Stress	1	1.65
Tissue × stress	4	1.41
Spp. × stress	1	0.81
Tissue × spp. × stress	4	1.14
Within subject effects		
Days after treatment (DAT)	5	19.67***
Tissue × DAT	14	23.23***
Spp. × DAT	5	0.75
Tissue × spp. × DAT	14	0.74
Stress × DAT	5	0.80
Tissue × stress × DAT	14	1.20
Spp. × stress × DAT	5	0.99
Tissue × spp. × stress × DAT	14	1.27

Note: significant effects are indicated by asterisks; ***$P = 0.001$.
Tissue types: leaves, roots, twigs, trunk core samples collected at 1 m or 2 m above injection port. $N = 10$.

2.3. Bioassays with adult A. planipennis beetles

Leaves were collected from each tree 21 and 45 days after treatment in 2004 and July 15, 2005 for bioassays with adult *A. planipennis* beetles reared from field-collected ash logs. To collect beetles for bioassays, native ash trees infested with *A. planipennis* were felled and bucked into 90-cm long bolts and stored at 4 °C. As beetles were required for experiments, they were allowed to emerge in rearing tubes at 25 °C. They were separated by sex and kept in 295 ml plastic beverage containers with an evergreen ash, *Fraxinus uhdei* (Wenzig) Linglesh, leaf in a vial of water for feeding. Containers with beetles were stored in growth chambers at 25 °C and >70% relative humidity with fresh food that was replaced twice a week, until they were used in bioassay experiments. For the bioassay, one leaf from each ^{14}C-imidacloprid injected tree, with the petiole inserted into a vial of distilled water, was placed in a clear plastic cup with a lid. Four beetles (10 days old) were introduced into each cup. The caged beetles were maintained in a growth chamber at 28 °C, 50% RH and 16:8 (L:D). Beetle mortality and knockdown were recorded 24, 48 and 72 h later. Beetles that were alive but unable to stand and walk a distance equal to their own body length were counted as knocked down. The sum of dead beetles and knocked down beetles was scored as affected beetles and most of the knocked down beetles died within two days after treatment.

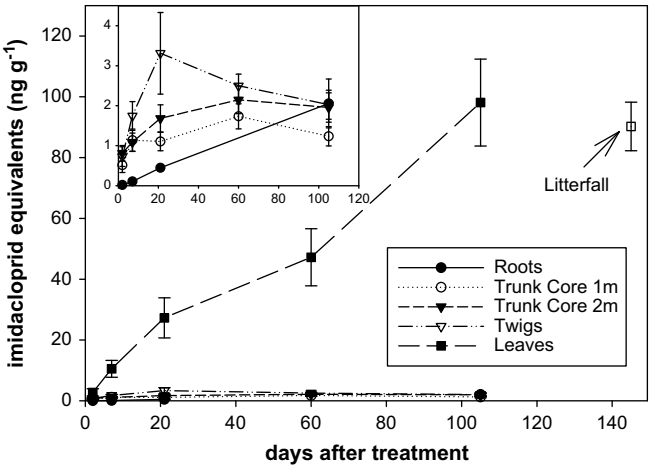

Fig. 2. Mean (±S.E.) imidacloprid equivalent concentration of tissue types from *Fraxinus americana* and *Fraxinus pennsylvanica* trees following trunk injection in June 2004. Values were averaged across ash species due to lack of significant species effect. Inset: tissue type means (±S.E.) re-scaled without leaves. $N = 40$ trees.

2.4. Data analysis

Water stress, species, and water stress × species interaction effects on gas exchange and imidacloprid equivalent concentrations of leaves, twigs, trunk cores, and roots during the 2004 growing season and in 2005 were analyzed by repeated measures analysis of variance (PROC MIXED, SAS Institute, Cary, NC) (SAS, 2003). Normality of data was determined by inspection of normal probability plots. When necessary, data were normalized by either square root or logarithmic transformation.

Abbott's formula was used to correct the data for control mortality in the insects (Abbott, 1925). Statistical analysis was conducted using SAS software (SAS Institute, Cary, NC) (SAS, 2003). Mortality was analyzed by species (green ash versus white ash) and water stress (well-watered and water stress). The best model to fit the doses of total imidacloprid equivalents and mortality was selected. The equation to fit this model was $Y = 110.3(1 - e^{-0.0018x})^{0.126}$.

3. Results

3.1. Water stress

Imposition of water stress by varying irrigation reduced ($P < 0.0001$) midday rates of leaf gas exchange for green ash and white ash trees (Fig. 1), indicating that water stress caused partial stomatal closure. Compared with the well-watered trees, water stress reduced stomatal conductance to water vapor (g_{wv}) by 48% and 37% for the green and white ash, respectively. Gas exchange rates were higher ($P < 0.0001$) for green ash than for white ash (Fig. 1).

3.2. Imidacloprid equivalent concentrations

Imidacloprid equivalent concentrations differed significantly among the plant tissue types sampled (Table 1). Leaves had much greater concentrations (>30×) than any other plant tissue during the growing season. Imidacloprid concentrations in leaves increased steadily throughout the 2004 growing season (Fig. 2). Water stress and ash species effects on imidacloprid equivalent concentrations were small relative to effects of tissue type and days after treatment (Table 1). The interaction of tissue type and days after treatment was significant, which reflects the continued increase in imidacloprid equivalent concentration in leaves throughout the 2004 growing season, while concentrations in twigs and trunk core samples reach a plateau or declined by mid-season (Fig. 2, inset). Other interactions among tissue type, species, water stress and days after treatment were not significant (Table 1). The species effect on imidacloprid equivalent concentration in trunk cores (Table 2) reflects a lower concentration in white ash trees, which was slightly largely than the green ash trees.

Destructive sampling of a subset of trees at the beginning of the 2005 growing season indicated that a significant proportion of the imidacloprid injected into the tree remained in the trunk near the point of injection. The imidacloprid injected the previous year was readily visible, appearing as spots or stains in 2004 annual rings in cross-sections cut from the trunk (Fig. 3). The stains are likely caused by imidacloprid or ingredients in the pesticide formulation. Staining was visible in some trees up to 1.5 m above the injection point. Imidacloprid equivalent concentrations in the stained areas of the trunk cross-sections were up to 300× greater than imidacloprid concentrations in the remainder of the trunk cross-section (Fig. 4).

Imidacloprid equivalent concentrations of leaves sampled in June and July 2005, a year after injection, were 91% and 87% lower than in leaves sampled on 21 days after treatment in 2004, respectively (Fig. 5). We selected 21 days after treatment in 2004 as a point of comparison because it corresponded to approximately the same period sampled in 2005 and it coincided with the point when most *A. planipennis* beetles in mid-Michigan have emerged and are leaf-feeding. Imidacloprid concentrations in twig samples collected in June 2005 were similar to twigs sampled in 2004, suggesting that little imidacloprid moved into twigs over the year.

Adventitious shoots that formed below the original injection ports provided an opportunity to observe potential re-translocation of imidacloprid from the root system. Mean concentrations of imidacloprid in leaves from the adventitious shoots (Fig. 5) were very low (0.52 ng g^{-1}) and not different from zero ($P > 0.05$) based on *t*-tests.

3.3. Bioassays with A. planipennis beetles

Mortality of adult *A. planipennis* was not significantly affected by water stress treatments, ash species or the interaction of the two factors on any date in 2004 or 2005 (Table 3). At 21 days after treatment and 45 days after treatment, we observed knockdown of 40% and 36%, respectively, of the *A. planipennis* beetles 24 h after the bioassay began (data not shown). Mortality of *A. planipennis* after 24 h was relatively low, with 11% and 17% of the beetles dying

Table 2
Summary of analysis of variance (*F* values) for effects of ash species (*Fraxinus pennsylvanica* versus *Fraxinus americana*), water condition (water stressed versus irrigated) and days after treatment (DAT) on imidacloprid equivalent concentrations in plant tissues from green and white ash trees.

Source	df	Leaves	Trunk cores 1 m	Trunk cores 2 m	Roots	Twigs
Between subject effects						
Species	1	1.53	6.79*	5.01*	0.16	0.01
Stress	1	1.62	0.27	3.72	1.28	0.07
Species × stress	1	0.12	0.41	0.03	1.29	0.01
Within subject effects						
DAT	4	78.12***	1.45	8.08***	27.50***	13.34***
Species × DAT	4	0.53	0.72	0.56	0.49	0.50
Stress × DAT	4	0.25	2.31	0.58	1.75	0.58
Species × stress × DAT	4	0.30	0.52	0.26	1.77	0.80

Note: trunk cores 1 m = trunk cores collected 1 m above stem injection port; trunk cores 2 m = trunk cores collected 2 m above injection port; DAT = days after treatment; ***$P < 0.0001$, **$P < 0.01$, *$P < 0.05$. $N = 10$.

Fig. 3. Staining visible in stem cross-section cut from ash trees harvested one year after 3 trunk injection. Stem section cut approximately 0.5 m above injection point.

at 21 days after treatment and at 45 days after treatment, respectively. Effects of the imidacloprid increased, however, as the bioassays continued. After three days of feeding, 44–79% of beetles caged with foliage collected at 21 days after treatment, were recorded as affected (dead beetles + knocked down) (Fig. 6). The percentage of affected beetles ranged from 70% to 81% on the third day of the bioassays with leaves collected at 45 days after treatment (Fig. 6). In the bioassays in 2005, a year after injection, the proportion of affected beetles decreased sharply (Fig. 6). Overall *A. planipennis* mortality in the June and July 2005 bioassays ranged from 17% to 30%. When *A. planipennis* mortality (affected beetles) was plotted against foliar imidacloprid equivalent levels ($\mu g\, g^{-1}$), we found that 0.55–60 $\mu g\, g^{-1}$ imidacloprid was associated with mortality of 11–100% of the beetles (Fig. 7). When imidacloprid equivalent concentrations exceeded 64 $\mu g\, g^{-1}$, *A. planipennis* mortality ranged from 70 to 100%.

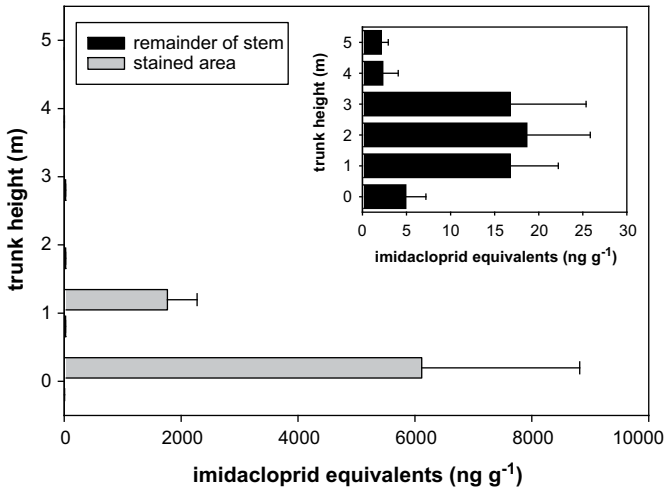

Fig. 4. Imidacloprid concentrations of trunk cross-sections of *Fraxinus americana* and *Fraxinus pennsylvanica* trees harvested in 2005. Cross-sections from lower samples (1 m and below) were separated into stained and non-stained regions. Inset: non-stained cross-section samples re-scaled without stained areas. $N = 8$.

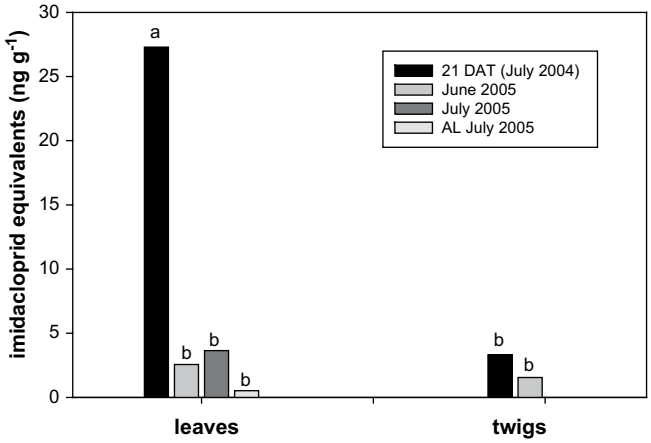

Fig. 5. Mean imidacloprid equivalent concentration of leaves and twigs of *Fraxinus americana* and *Fraxinus pennsylvanica* trees 21 days after trunk injection (21 DAT) and the year after injection (June and July 2005). Leaves refer to leaves sampled from tree canopies. AL refers to leaves collected from adventitious shoots originating below the original injection point on a subsample of trees harvested in June 2005. Means with the same letter as not different at $P = 0.05$ based on Tukey's range test.

4. Discussion

4.1. Movement and distribution of imidacloprid

Like other studies, our results show that imidacloprid translocation occurs primarily in xylem (Mendel, 1998; Harrell, 2006). Aside from extremely high concentrations in the stained regions of the trunk cross-sections, imidacloprid equivalent concentrations were highest in leaf tissues. Although not assessed in this study, imidacloprid concentrations of leaves may have also varied within tree crowns, since only two injection ports were used due to the relatively small circumference of the experimental trees. Very little imidacloprid occurred in fine roots, which are dependent upon translocation from the phloem for growth. Moreover, trunk core samples, which included phloem tissue but very little xylem, had very little imidacloprid activity.

Recent studies have used the amount of imidacloprid assessed by ELISA methods in xylem sap to predict the efficacy of insecticide treatments used to protect trees from *A. planipennis*. Harrell (2006) compared imidacloprid concentrations in the xylem sap of green ash trees that were injected with two imidacloprid formulations. For both products, imidacloprid concentrations in sap peaked within 30 days of injection and then declined. Sap concentrations of imidacloprid were highly

Table 3
Summary of analysis of variance (*F* values) for effects of species, and water stress, on the mortality of adults of EAB exposed to ash foliage following trunk injection.

Source	df	*F* values
21DAT 2004		
Species	1	1.28
Stress	1	0.77
Species × stress	1	3.63
45DAT 2004		
Species	1	0.35
Stress	1	0.00
Species × stress	1	0.09
DAT 2005		
Species	1	0.00
Stress	1	0.40
Species × stress	1	0.41

Note: DAT = days after treatment; $^{***}P < 0.0001$, $^{**}P < 0.01$, $^{*}P < 0.05$. $N = 40$.

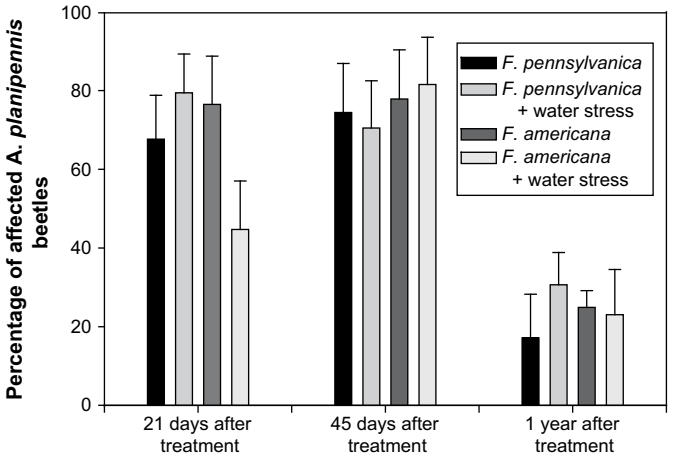

Fig. 6. Mean (±S.E.) control-corrected mortality of *A. planipennis* adults after 72.5 h caged with leaves from ash trees that had been trunk-injected with imidacloprid. $N = 40$.

variable, however, and differed from untreated controls on only one of five sample dates. Similarly sap concentrations of imidacloprid in hemlock (*Tsuga* spp.) were not significantly different from the untreated check (Cowles et al., 2006) following trunk injection. In the present study, we collected sap samples to detect the presence of ^{14}C-imidacloprid (data not shown). However, the levels detected were similar to background levels. In theory, xylem sap should be a good indicator of imidacloprid concentration since sap is responsible for the movement of imidacloprid to the leaves. However, the concentrations were too low to provide accurate measures, and also detection of imidacloprid in sap lacks the precision that is possible when measuring imidacloprid in leaves, where adults, the main target of control, would feed. In this study we demonstrated that the fate of imidacloprid is continuous accumulation in the leaves. While total radioactivity is accumulated in the leaves this does not mean that all detected radioactivity belonged to the parent compound. Metabolism of imidacloprid occurs in plants (Sur and Stork, 2003) and there is some evidence of the presence of 5-OH-imidacloprid, 4,5-OH-imidacloprid, and urea metabolites in ash leaves (unpublished results). Neither the total radioactivity method used here nor the ELISA assay provides specific data on

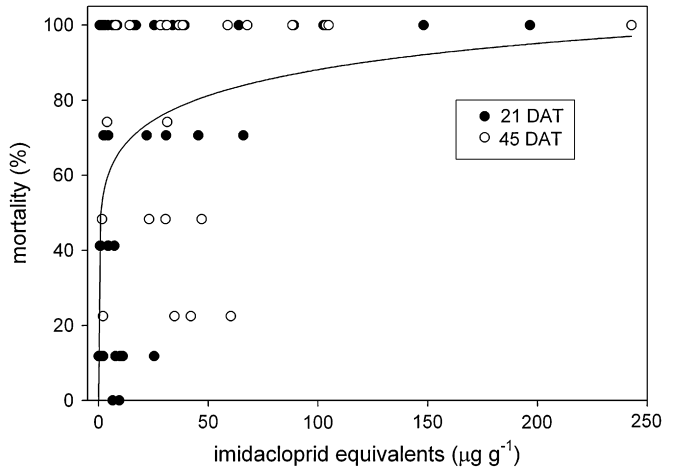

Fig. 7. Mortality (dead + knocked down) of emerald ash borer adults in relation to imidacloprid equivalent concentration of ash leaves collected 21 days or 45 days after treatment ($P < 0.01$, $R^2 = 0.17$).

the level of imidacloprid since in both cases metabolites may be included in the assay results. Despite this situation, these studies provide good insight into the movement of imidacloprid in ash trees.

Low amounts of imidacloprid equivalent levels were detected in leaves in 2005, the year following injection. We hypothesize that imidacloprid detected in leaves the year following injection reflect continued diffusion of imidacloprid from reservoirs of imidacloprid in xylem near the injection points. In ring-porous trees, such as ash, conduction of xylem sap decreases rapidly in sapwood from the cambium inward, with the majority of hydro-active sapwood occurring in the outer annual rings (Gebauer et al., 2008). One year after application, most of the imidacloprid was located in the inner rings rather than the outer annual ring, which may explain why low levels of imidacloprid were detected in leaves in 2005. This 'reservoir hypothesis' is supported by the extremely high levels of imidacloprid remaining in the trunk near the injection port a year after injection. Moreover, imidacloprid levels of leaves sampled in 2005 from adventitious shoots arising from the trunk below the injection point accumulated virtually no imidacloprid, indicating that the compound is not redistributed from below the injection point.

4.2. Bioassays with *A. planipennis* beetles

Translocation of trunk-injected imidacloprid effectively controlled *A. planipennis* beetles in green and white ash trees, regardless of water conditions in 2004. The percent of affected beetles (knockdown plus dead beetles) was above 71 at 20 and 45 DAT. Moreover, we consistently observed sublethal effects of imidacloprid, including reduced feeding and slowed movement. Similar effects of imidacloprid have been reported for insect pests of cotton (Nauen et al., 1998a,b, 1999). Presumably, the combined effects of imidacloprid on *A. planipennis* mating, oviposition, feeding, and activity may severely affect the fitness of any beetles that are not killed outright. Those combined effects would then prevent females from laying eggs on the trunks of the trees reducing the possibility of larval infestation. It is important to note that we used relatively small trees for this study due to the constraints associated with using radiolabeled material. Imidacloprid movement may be affected by the size of the trees as it has been found that residues of imidacloprid increase as tree size decreases (McCullough et al., 2008).

In 2005, low *A. planipennis* control was consistent with a low amount of imidacloprid in leaves. In this study, we measured total radioactivity, which includes imidacloprid metabolites as well as the parent compound. Many of these metabolites are toxic to other insects (Nauen et al., 1998a,b) and may also affect *A. planipennis*. We cannot determine whether the *A. planipennis* mortality that we observed in 2005 was caused by imidacloprid or other metabolites. In any case, the low *A. planipennis* mortality recorded in 2005 indicates that our trunk injection of imidacloprid would not have provided two years of effective control.

5. Conclusions

Imidacloprid accumulation was similar across two ash tree species and two levels of water stress. The largest accumulation of imidacloprid occurred in the leaves, where a steady increase was observed after injection until the end of the season following injection. *A. planipennis* beetles were controlled in both green and white ash trees grown under high and low water availability in the year of injection, but foliar imidacloprid levels were greatly reduced a year after injection and had relatively little effect on *A. planipennis* adults. Little imidacloprid was detected in the trunk and roots of

the trees, indicating that imidacloprid translocation occurred mainly in the xylem.

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