Research Article

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Spatial and temporal distribution of trunk-injected ^{14}C-imidacloprid in *Fraxinus* trees

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Abstract

BACKGROUND: Since the discovery of *Agrilus planipennis* Fairmaire (emerald ash borer) in 2002, researchers have tested several methods of chemical control. Soil drench or trunk injection products containing imidacloprid are commonly used to control adults. However, efficacy can be highly variable and may be due to uneven translocation of systemic insecticides. The purpose of this study was to determine whether sectored xylem anatomy might influence imidacloprid distribution in tree crowns.

RESULTS: Imidacloprid equivalent concentrations were higher in leaves from branches in the plane of the injection point (0°) than in leaves from branches on the opposite side of the injection point (180°). Leaves from branches 90° to the right of injection points had higher imidacloprid equivalent concentrations than leaves from branches 90° to the left of injection points. Leaves and shoots had higher imidacloprid equivalent concentrations than roots and trunk cores, indicating that imidacloprid moves primarily through the xylem.

CONCLUSION: Imidacloprid equivalent concentration in leaves varied over time and in relation to injection points. It is concluded that ash trees have sectored 'zigzag' xylem architecture patterns consistent with sectored flow distribution. This could lead to variable distribution of imidacloprid in tree crowns and therefore to variable control of *A. planipennis*.

Keywords: sectored flow; ash; *Agrilus planipennis*; emerald ash borer; systemic control

1 INTRODUCTION

Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a phloem-feeding insect native to Asia. It likely arrived in the United States near Detroit, Michigan, in the early 1990s and was first identified in 2002 as the cause of *Fraxinus* spp. (ash) tree decline and mortality.[1] *Agrilus planipennis* has killed tens of millions of ash trees in Michigan and is now found in at least 14 other states and two Canadian provinces.[2] If left unchecked, *A. planipennis* is expected to spread and may threaten ash species across North America.[3]

Since the discovery of *A. planipennis* in 2002, researchers have tested several methods of chemical control, including cover sprays, soil drenches, basal trunk sprays and trunk injections.[4–12] Products containing imidacloprid, a neonicotinoid compound that acts on the insect acetylcholine binding site on nicotinic acetylcholine receptors,[13] are the most commonly used insecticides for *A. planipennis* control. These products are applied as either a soil drench or trunk injection.[9–12,14] Imidacloprid is lethal to leaf-feeding EAB adults, but efficacy can vary substantially.[8,10]

One possible cause of variable insect control could be uneven translocation of systemic insecticides within trees. Depending upon tree species, sap flow in trees may be sectored, with specific xylem pathways supporting given branches, or integrated, with pathways supporting a wide array of branches.[15] Trees with greater degrees of sectoriality have specific translocation paths that move resources primarily through a longitudinal plane. In contrast, trees with greater degrees of integration have translocation paths that move resources in both longitudinal and radial planes.[16] Increased vessel-to-vessel contact and higher pit ratios in integrated species usually equate to higher degrees of integration, while sectored trees have less contact between vessels and lower bordered pit ratios.[17] The degree to which xylem flow is sectored or integrated may impact upon the distribution of xylem-mobile insecticides applied via trunk injection.[18,19] For example, Takai *et al*.[20] found that emamectin benzoate followed a spiral ascent pattern when injected into the trunks of *Pinus densiflora* trees.

Mota-Sanchez *et al*.[10] demonstrated that imidacloprid moves slowly and steadily through ash trees and accumulates in leaves following trunk injection. Their sampling protocol, however, did not assess spatial variability of within-tree imidacloprid

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distribution. If ash trees have highly sectored xylem anatomy, trunk-injected insecticides may move in a relatively narrow path to the canopy. Depending on injection location and spacing, there could be areas of the canopy with relatively low insecticide concentrations. A better understanding of translocation paths within trees could lead to improved efficacy of trunk-injected insecticides.

Persistence of trunk-injected imidacloprid may also vary, potentially affecting efficacy. Some research suggests that imidacloprid products can provide two years of *A. planipennis* control,[21] but other studies have shown that only one season of control can be achieved.[8,9,14,22,23] It is not yet clear whether an adequate amount of imidacloprid remains in the tree after litterfall to provide a second season of effective *A. planipennis* control, and, if so, where the compound is stored. The objectives of this study were to assess: (1) the spatial and temporal variation in imidacloprid in *Fraxinus* spp. leaves; (2) the variation in imidacloprid among tree tissues; (3) the residual imidacloprid in trunk tissue.

2 EXPERIMENTAL METHODS

2.1 Plant material

The authors acquired 16 *F. americana* ('Autumn Applause' or 'Junginger') and 16 *F. pennsylvanica* ('Patmore') trees for this study (J Frank Schmidt and Sons, Boring, OR) on 5 April 2006. Trees were bare-root liners, 4.0–5.0 cm in diameter and approximately 2.5–3.0 m tall, and were planted in sand in 95.0 L plastic containers at the Michigan State University Horticulture Teaching and Research Center (MSUHTRC) near Holt, Michigan. After planting, pots were placed inside plastic wading pools (1.2 m diameter, 0.3 m depth) and elevated on a layer of bricks to allow for drainage while containing leachate from planted containers. Trees were then fertilized once with Osmocote® Plus control release fertilizer (15-10-12; The Scott's Company, Marysville, OH) at a rate of 314 g container^{-1} and watered to runoff with drip irrigation 2–3 times per week. Trees were assigned to eight replicates, each of which consisted of two randomly selected trees of each species. Care was taken to ensure that all trees contained branch whorls with two opposite branches, and that the first three whorls were oriented at 90° angles to one another.

2.2 Trunk injection

On 28 June 2006, each tree was injected at a single point at the base of the tree with 25 µCi of ^{14}C imidacloprid (labeled at the imidazolidine ring) mixed with 3.1 mL of Imicide® (10% active ingredient; Mauget Co., Arcadia, CA) in a ratio of 1 : 1300 labeled to unlabeled compound. Weather conditions at the MSUHTRC at 14 : 00 were as follows: air temperature 25.4 °C, relative humidity 46.0%, wind southwest, 3.5 m s^{-1}. Of the 32 trees injected, 24 were injected at 0° relative to the first whorl of branches, i.e. the injection port was directly below one of the branches in the first whorl (Fig. 1). Eight *F. pennsylvanica* trees were injected at 90° to the first whorl of branches (Fig. 1). Injection holes were made using a cordless drill with an 8.0 mm (5/16″) drill bit, 10.0 cm above the graft union, to a depth of approximately 1.5 cm. Systemic tree injection tubes (STITs) were inserted and gently tapped into place with a hammer.[24] After insertion, the imidacloprid mixture was introduced with a syringe into the lower half, STITs were reassembled and 2.0 bar pressure was applied using a hand-operated bicycle pump.

Insecticide uptake was monitored, and STITs were reinflated as needed for up to 48 h or until all liquid was taken up by the tree. Tubes were then removed, and electrical tape was wrapped around the injection point to prevent leakage or leaching of radioactive material. Tape remained in place throughout the experiment. Three trees were eliminated from the experiment because insecticide leaked during injection.

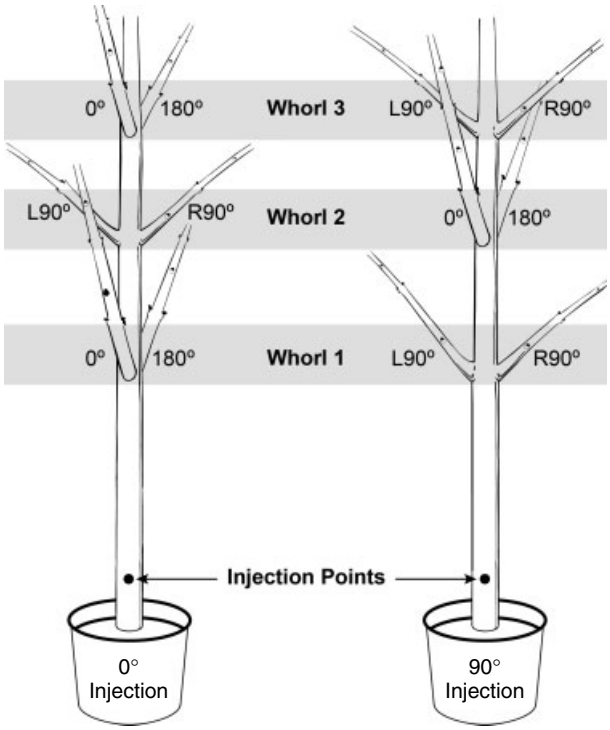

Figure 1. Sampling schematic of *Fraxinus americana* and *F. pennsylvanica* trees injected at 0° (left) or 90° (right) to the first whorl of branches. Branches of the first three whorls were labeled 0°, 180°, L90° or R90° in relation to the injection point.

All handling of radioactive material complied with Michigan State University's Office of Radiation, Chemical and Biological Safety (ORCBS) standards. The study area and containers were surveyed periodically using a Geiger counter (Survey Meter Model 44-9; Ludlums Measurement Inc., Sweetwater, TX) to ensure that no radioactive material leached into the environment.

2.3 Tissue sampling and analysis

Branches from the first three whorls of each tree were labeled 0°, 180°, Right 90° (R90°) or Left 90° (L90°), depending on the position of the branch in relation to the injection point (Fig. 1). Individual leaflets (*F. americana* and *F. pennsylvanica* trees have compound leaves composed of 5–7 leaflets) were collected from proximal, middle and distal portions of each of the six branches. Leaflet samples from terminal leader branches were removed with a pole pruner. Trunk cores were taken 1 m above the injection point at 0° and 180° (in relation to the injection point) using a No. 1 cork borer to remove samples of bark, phloem and sapwood approximately 2 mm deep. Fine root samples were obtained by exposing a portion of the root system and removing approximately 1 g (dry weight) of fine root material. Fine roots were rinsed with tap water to remove sand. Shoot samples, each approximately 2 cm in length, were taken from current-year stem growth of the first three whorls using hand pruners (two samples per whorl, six samples per tree). Samples were placed individually into small paper bags, labeled and placed in a 70 °C drying oven for at least

72 h. After drying, leaf samples were removed from the oven, ground to a fine powder using a mortar and pestle under a fume hood and placed into individually labeled paper envelopes. Root, core and shoot samples were left intact.

A 150–180 mg subsample of tissue was processed in a biological tissue oxidizer (OX300; RJ Harvey Instrument Corporation, Tappan, NY). Stem samples were burned on a 4 min cycle, leaf samples on a 3 min cycle and root and core samples on a 2 min cycle. Resulting $^{14}CO_2$ was trapped in scintillation cocktails and processed through a Packard Tri Carb liquid scintillation counter (LSC) (Packard Bioscience, currently PerkinElmer Life and Analytical Science, Waltham, MA) on a 1.5 min cycle. Counts per minute (CPM) were recorded for each sample and converted to imidacloprid equivalents after accounting for background activity and oxidizer and LSC efficiencies. Imidacloprid injected into trunks of *Fraxinus* trees may be metabolized into a series of compounds,[25,26] many of which exhibit insecticidal properties. In the present study, ^{14}C associated with the initial injection of ^{14}C imidacloprid was quantified, but parent compound and metabolites were not separated; therefore, the concentration of insecticide in the tree is referred to in terms of imidacloprid equivalent concentration, which was calculated as µg of total radioactivity plus µg of unlabeled material per gram dry weight of sample. For the 2006 growing season, leaves from the first three branch whorls and the terminal leader, trunk cores and fine roots were sampled 0, 2, 7, 21, 60 and 98 days after treatment (DAT). Shoot samples were taken from trees only at 60 DAT to prevent excessive tree damage. In late September 2006, all trees were enclosed with mesh netting to collect litterfall. After abscission, leaves were removed from each net, placed in paper bags and oven dried. Subsamples of 5–7 leaves from each tree were ground, weighed, oxidized and processed through the scintillation counter. Trees were overwintered in a holding area at the HTRC. In April 2007, trees were placed back in their original configuration. Tree fertilizer and irrigation rates in 2007 were the same as used in 2006.

In 2007, leaves and fine roots were sampled on 29 May, on 12 June (roughly corresponding to peak *A. planipennis* flight in the region[27]) and on 31 August. Trunk cores were not taken in 2007 to prevent excess tree damage. At the end of the 2007 growing season, all leaves were removed from trees by hand just prior to natural leaf abscission. Leaves from the first three branch whorls were dried, stored and processed separately from other leaves.

2.4 Leachate tests

Pour-through leachate tests[28] were performed on all treated trees 7, 21, 98, 350 and 392 DAT. Sand was saturated to runoff with water using a garden hose and allowed to drain for 10 min. After drainage, 2 L of water was added and allowed to drain. Leachate was captured from each of the container's four drainage holes and mixed. A 25 mL portion of the mixed sample was poured into a scintillation vial and sealed. All excess water was captured in wading pools, siphoned out and disposed of using proper ORCBS disposal methods. Upon completion, all samples were transported to the laboratory, where 300 µL of leachate was combined with 15 mL of scintillation cocktail (Carbon 14 Cocktail®; RJ Harvey Instrument Corp., Hillsdale, NJ) in a scintillation vial under a fume hood. Samples were sealed and shaken to ensure an even distribution of cocktail and immediately processed through the LSC on a 3 min cycle. Resulting CPMs were recorded for each sample and the background was subtracted.

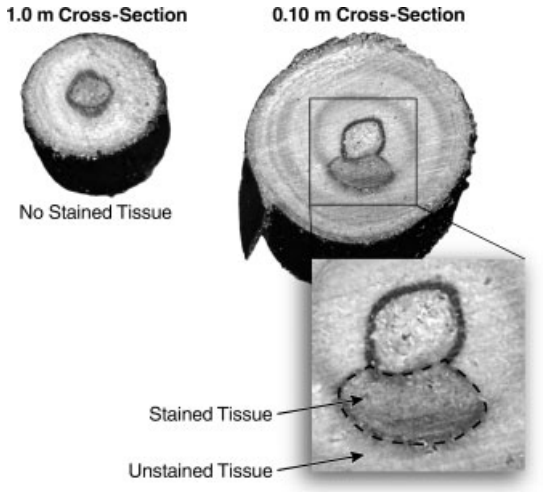

Figure 2. Cross-sections from a 0° (in relation to the first whorl of branches) ^{14}C-imidacloprid trunk-injected *Fraxinus americana*. Sections were removed from the bole of the tree 0.10 m (right) and 1.0 m (left) above the center of the injection scar. Stained tissue is outlined in black.

2.5 Destructive harvest

Fifteen trees (five *F. americana*/ 0° injection, five *F. pennsylvanica*/ 0° injection and five *F. pennsylvanica*/ 90° injection) were destructively harvested in October and November 2007. Trees were lifted out of pots using a skid steer and chain. Sand was rinsed off roots using a garden hose and spray nozzle. Roots were removed from trunks using hand pruners and loppers and classified as either coarse (>5.0 mm) or fine (<5.0 mm). Branches from whorls 1 to 6 (where applicable) were labeled 0°, 180°, R90° or L90°, based on their orientation to injection points, and removed from trees with loppers. Stem sections were removed from tips of branches and separated into 2006 and 2007 growth sections, as determined by the position of bud scale scars. Subsamples of 5–7 leaflets each from terminal leader and branches, from fine and coarse roots and from stems of harvested trees were dried, ground (leaves only), weighed, oxidized and processed through the LSC. Tree trunks were cut into sections at 10.0 cm, 1.0 m and 2.0 m above the center of the injection scar, and a 2.0 cm thick cross-section was removed at each height. Cross-sections were split into quarters using a hammer and chisel. One quarter section was randomly selected and further divided into three pieces. A single piece was dried, weighed, oxidized and processed through the LSC. Cross-sections from 10.0 cm above the injection scar contained portions of xylem that were stained by the initial imidacloprid injection (Fig. 2); a portion of this stained area was excised, dried, weighed, oxidized and processed through the LSC. All branches, fine and coarse roots and tree boles were cut into manageable sections, dried and weighed.

2.6 Statistical analysis

Data were tested for normality using residual plots. Where necessary to meet the assumptions of normality, imidacloprid equivalent concentrations were log transformed. Data were analyzed separately for trees injected at 0° and trees injected at 90° to the first whorl of branches using the Mixed procedure for mixed models (SAS Institute, 1996) with whorl nested in tree [Whorl(Tree)], branch position nested in whorl [Branch Position(Whorl)], tree species and day as fixed effects and tree number as a random effect (Table 1). Models for repeated measures

Table 1. Analysis of variance of imidacloprid equivalent concentration in *Fraxinus americana* and *F. pennsylvanica* trees trunk injected at 0° or 90° to the first whorl of branches with ^{14}C-imidacloprid on 28 June 2006 (* indicates significant effects, $\alpha = 0.05$)

	Trees injected at 0° below first whorl		Trees injected at 90° below first whorl	
Source	df	*F*-value	df	*F*-value
Between-subject effects				
Fraxinus Species	1	0.00	N/A	N/A
Whorl(Tree)	9	0.66	9	0.87
Whorl(Tree) × Species	9	1.40	N/A	N/A
Within-subject effects				
Day	8	40.97*	8	38.64*
Species × Day	8	1.92	N/A	N/A
Whorl(Tree) × Day	69	0.66	69	1.13
Whorl(Tree) × Species × Day	63	0.70	N/A	N/A
Branch Position(Whorl)	4	35.55*	4	33.47*
Species × Branch Position(Whorl)	4	0.64	N/A	N/A
Branch Position(Whorl) × Day	24	0.75	24	0.85
Species × Branch Position(Whorl) × Day	24	0.36	N/A	N/A

were examined using a CS covariance structure. Analysis of variance (ANOVA) was used to determine which main effects and interactions were significant ($P < 0.05$) for whorl, branch position, tree species and day. When main effects were significant (day and branch position only), least-squares mean procedures were used for mean separation.[29] Species fixed-effect interactions were not evaluated for trees injected at 90° in relation to the first whorl of branches because this treatment was applied only to *F. pennsylvanica* trees.

3 RESULTS

3.1 Spatial and temporal variation in imidacloprid equivalent concentration in leaves

Imidacloprid equivalent concentration in leaves varied ($P < 0.05$) with time and orientation to the injection point (Table 1). Species and branch height did not affect imidacloprid equivalent concentration ($P > 0.05$) (Table 1); therefore, data were pooled between species to simplify the presentation. Imidacloprid equivalent concentration increased rapidly following trunk injection until 21 DAT (Fig. 3). For a given whorl, imidacloprid equivalent concentration was consistently higher ($P < 0.05$) in leaves from branches in the plane of injection (0°) than in leaves from branches opposite the plane of injection (180°) throughout the 2006 growing season (Fig. 3). However, the effect of branch orientation decreased with height, suggesting that flow became more integrated as trees became taller.

Imidacloprid equivalent concentration of leaves collected in 2007 was 20-fold lower than that of leaves sampled in 2006 (Fig. 3). In 2007, branch orientation did not affect ($P > 0.05$) imidacloprid equivalent concentration in leaves (Fig. 3). Leaves from L90° branches had lower imidacloprid equivalent concentration ($P < 0.05$) than leaves on R90° branches throughout the 2006 growing season, but in 2007 only leaves from whorl 3 were different. Imidacloprid equivalent concentration in leaves collected from the terminal leader increased ($P < 0.05$) until 60 DAT in 2006 (Fig. 3).

For trees injected at 90° to the injection point, the concentration of imidacloprid equivalents in leaves followed similar patterns as for trees injected at 0° to the first whorl of branches in 2006. Imidacloprid equivalents increased in leaves rapidly after injection (Fig. 3). The orientation of branches relative to the injection point again affected imidacloprid accumulation. Branches at R90° and 0° to the injection point accumulated higher levels of imidacloprid in leaves than opposite branches (L90° and 180°). In 2007, however, the difference in leaf imidacloprid equivalent concentrations was less pronounced. In general, branch height did not affect ($P > 0.05$) imidacloprid equivalent concentration in leaves from 0° or 180° branches (Fig. 3).

3.2 Variation in imidacloprid equivalent concentration among tree tissues

Sampling of trees injected directly below a branch in the first whorl (0°) or between the branches (90°) resulted in accumulation of imidacloprid and metabolites in stem tissues (Fig. 4). Imidacloprid equivalents were not different from zero in fine roots or trunk cores collected at 60 DAT (Fig. 4). Stems had lower ($P < 0.05$) imidacloprid equivalent concentration than leaves from corresponding branches and whorls (Fig. 4). Stems from 0° and R90° branches had higher ($P < 0.05$) imidacloprid equivalent concentration than stems from 180° and L90° branches respectively. Overall, imidacloprid equivalent concentration in stems from 0° and R90° branches were not different ($P > 0.05$), and stems from 180° and L90° branches were not different ($P > 0.05$) (Fig. 4). Negative values for imidacloprid concentration occurred for some core tissues after subtracting background activity; however, these means were not different from zero ($P < 0.05$).

3.3 Imidacloprid equivalent concentrations in trunk tissue and leachate

Stained wood samples taken 0.10 m above the graft union had approximately 75 times higher ($P < 0.05$) imidacloprid equivalent concentration than all other wood samples taken from tree boles (Fig. 5). Imidacloprid equivalent concentrations were the same ($P > 0.05$) for unstained trunk samples taken from 0.10, 1.0 and 2.0 m above the graft union for 0° injected trees (Fig. 5). No imidacloprid equivalents were detected in any of the pour-through leachate tests on any date.

4 DISCUSSION

4.1 Spatial and temporal variation in imidacloprid equivalent concentration in leaves

Translocation of imidacloprid has been evaluated in hemlock,[30] avocado[31] and citrus trees,[32] but specific translocation pathways for imidacloprid in *Fraxinus* trees have not been assessed. Translocation of trunk-injected insecticide can be affected by a variety of factors, including xylem anatomy, temperature, tree health, injection timing and time after injection.[33]

The present authors found that imidacloprid equivalent concentration in *Fraxinus* leaves depended on orientation of

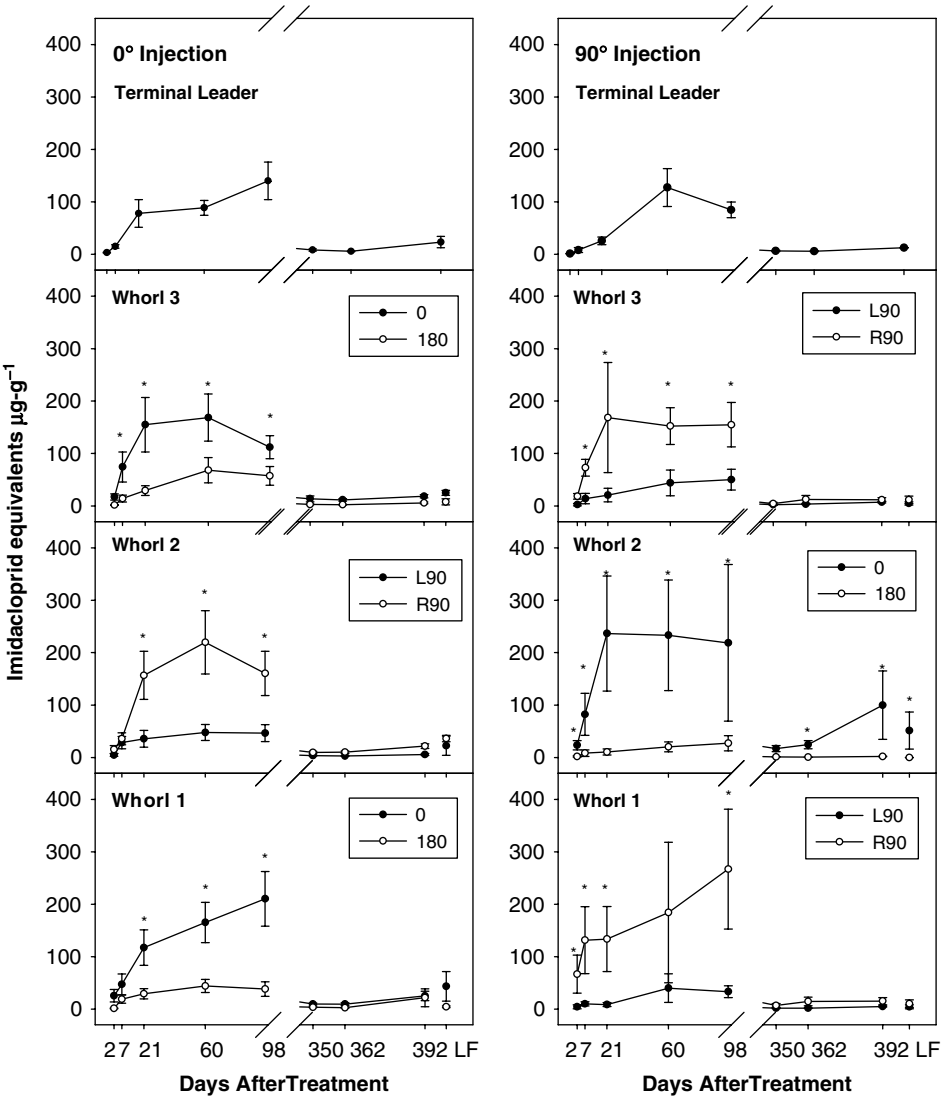

Figure 3. Mean ^{14}C-imidacloprid equivalent concentration (± SE) in whorl and terminal leader leaves from 0° or 90° (in relation to the first whorl of branches) trunk-injected *Fraxinus* trees. Data for 0° injections were pooled for *F. americana* and *F. pennsylvanica* trees. Data for 90° injections contained only *F. pennsylvanica* trees; no *F. americana* trees were injected at 90° to the injection point. Branches were labeled 0°, 180°, L90° or R90° in relation to the injection point. An asterisk (*) denotes significant differences between branches. LF = leaves collected just prior to natural abscission.

branches in relation to the injection point. In 2006, imidacloprid equivalent concentration increased in leaves throughout the crown. Leaves from branches in the plane of injection (0°) had greater imidacloprid equivalent concentration than leaves on the opposite side of injection sites (180°). In addition, leaves from R90° branches had higher imidacloprid equivalent concentration than L90° branches. These results were consistent regardless of the location of the injection port. Movement of labeled compound was ordered during the first growing season. In other words, imidacloprid moved quickly into leaves and branches above the injection point in the longitudinal direction (in the plane of injection, 0°) but slowly into leaves and branches in the radial plane (opposite the plane of injection, 180°). This is consistent with research showing that *F. americana* trees have longitudinal movement six orders of magnitude higher than radial movement.[34] No deviations from the pattern of ascent occurred in any of the whorls over time. The consistent, ordered pattern of imidacloprid equivalent concentration in leaves from specific branches suggests that *Fraxinus* spp. trees have sectored xylem pathways. Sectored patterns of xylem architecture create uneven distribution of insecticide within tree crowns. Although trees in the present study were relatively small, patterns of sectoriality will likely be similar in larger trees.[15] As seen here, unbalanced injection sites could lead to uneven insecticide distribution and variable insecticide efficacy.

Most *Fraxinus* species are ring porous and are generally described as straight grained, features usually associated with sectored patterns of xylem architecture. Patterns of sap ascent in trees, however, normally deviate from purely straight and are often described as helical.[35] If translocation patterns in ash xylem were purely helical, continued spiral ascent and higher imidacloprid equivalent concentration in leaves from third-whorl 180° branches than in leaves from 0° branches would be expected. This was not the case in this study. If xylem architecture were strictly straight sectored, leaves from branches offset from the injection point (second-whorl branches from 0° injected trees, first- and third-

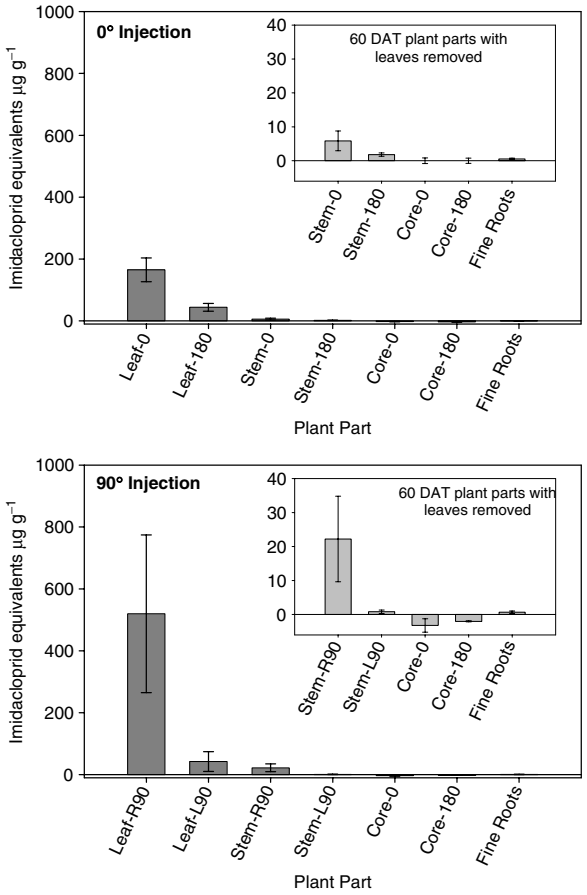

Figure 4. Mean ^{14}C-imidacloprid equivalent concentration (± SE) in first-whorl leaves and stems (0° or 180°, R90° or L90° in relation to the injection point), trunk cores (0° or 180° in relation to the injection point) and roots (0° or 90° in relation to the first whorl of branches) of trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees. Inset graphs have been reconstructed without leaf samples. All samples were collected 60 days after treatment.

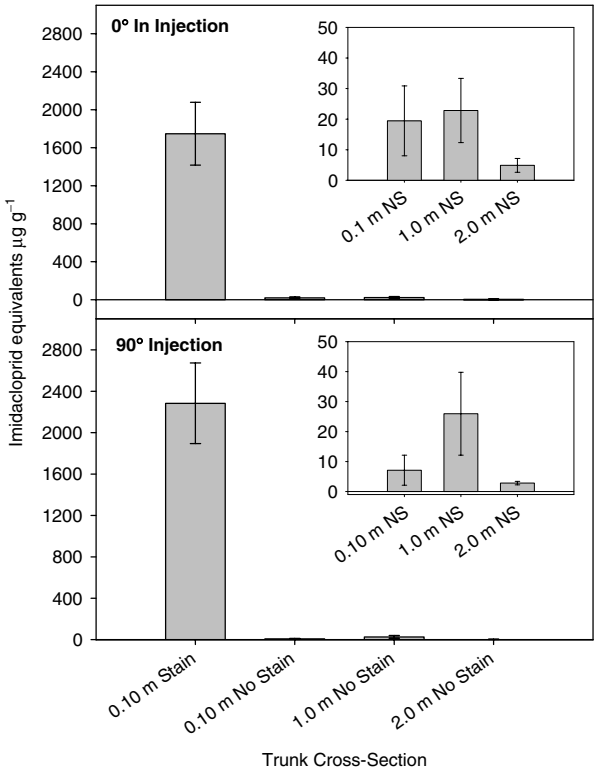

Figure 5. Mean ^{14}C-imidacloprid equivalent concentration (± SE) in stained and unstained trunk tissue taken at varying heights above the center of the injection scar from 0° or 90° (in relation to the first whorl of branches) trunk-injected *Fraxinus americana* and *F. pennsylvanica* trees harvested in October 2007. Inset graphs have been reconstructed without stained tissue samples.

whorl branches from 90° injected trees) would have intermediate imidacloprid equivalent concentrations compared with leaves from branches in the plane of injection (first whorl and three branches from 0° injected trees and second-whorl branches from 90° injected trees), but this did not occur either. Instead it was established that ash xylem architecture has a zigzag pattern of ascent. Kiten *et al.*[36] proposed that patterns of sap ascent in *F. lanuginosa* are influenced by vessel-to-vessel contact, and, even though ash is considered to be a straight-grained wood, tangential drift in vessel position does occur. Burggraaff[37] noted a zigzag pattern in the xylem architecture of *F. excelsior* and described its vessels as slanting toward the right. In addition, he states that vessels are in contact with one another in both the tangential and radial directions, but that no perforations exist that allow open contact between vessels. The present results indicate that, while xylem architecture of *F. americana* and *F. pennsylvanica* are highly sectored, they do not follow a helical or straight sectored pattern; instead they exhibit a zig-zag pattern of ascent similar to that previously described for *F. excelsior*.[37]

Results from previous studies suggest that a single injection of imidacloprid can provide highly efficacious pest control over two growing seasons,[21] but others have reported that levels of insecticide are greatly reduced during the second season after injection.[8,9,10,14,22,23] The present authors evaluated the amount of imidacloprid over two growing seasons and found that, on 11 June 2007, leaves had an imidacloprid equivalent concentration less than 12% of levels found at the end of the growing season in 2006. Mota-Sanchez *et al.*[10] determined that greater than 64 µg g^{-1} of imidacloprid equivalents in leaf tissues caused 70–100% *A. planipennis* mortality. They found that the number of affected *A. planipennis* adults ranged between 70 and 81% 45 days after injection, as opposed to only 17–30% the second season after injection. If the number of affected beetles is reduced this dramatically, efficacious *A. planipennis* control is highly unlikely the second season after injection. Bioassays conducted during the present study indicate that, during the second season, mortality was highly variable, and approximately 50% of beetles were affected, compared with 80% of beetles in 2006 (data not shown). In addition, imidacloprid equivalent concentrations for leaves of any given branch rarely exceeded 50 µg g^{-1} during the year after injection, further suggesting that efficacious control during the second season after injection was unlikely.

4.2 Variation in imidacloprid equivalent concentration among tree tissues

In addition to leaves, imidacloprid equivalents were also present at detectable levels in stem tissue. Patterns of imidacloprid distribution in stem sections harvested 60 DAT (2006) were similar to leaves for both 0° and 90° injected trees. Concentrations were highest in stems from 0° and R90° branches when compared with stems from 180° and L90° branches, which is consistent with zigzag ascent.

Several studies have demonstrated that imidacloprid transport in plants occurs almost exclusively via the xylem.[38–40] The present evaluation of imidacloprid in fine roots and trunk cores and the leachate tests that were performed addressed this issue. While imidacloprid was present in leaves and stems, no measurable imidacloprid equivalent concentration was present in roots or trunk cores. If any imidacloprid movement occurred in phloem, these tissues would likely have had detectable levels of imidacloprid.

To investigate imidacloprid movement into fine roots further, pour-through leachate tests were also performed. No detectable imidacloprid equivalents were present in leachate collected from pour-through tests. It is concluded that fine root turnover from trunk-injected trees does not likely contribute to imidacloprid residues in soil. The absence of detectable imidacloprid also indicates that imidacloprid movement occurs primarily in xylem.[38–40]

4.3 Imidacloprid equivalent concentrations in trunk tissue and leachate

In a previous study that used ^{14}C to examine imidacloprid movement in ash trees, Mota-Sanchez *et al*.[10] demonstrated that canopy leaves collected after abscission contained an imidacloprid equivalent concentration as high as those sampled before abscission. In other words, imidacloprid was not translocated from leaves back into the trunk prior to litterfall. Post-harvest analysis of woody tissue from trees used in that study had very high imidacloprid equivalent concentration in stained areas around the injection site. It is hypothesized that trunk tissue in this area serves as a reservoir for imidacloprid that is translocated to leaves during the subsequent growing season. To investigate this further, trees from the present study were destructively harvested at the conclusion of the 2007 growing season. Cross-sections of wood taken from the bole of trees at different heights above the injection point address this hypothesis. Stained areas of wood collected 0.10 m above the injection point contained extremely high imidacloprid equivalent concentrations when compared with all other plant parts. These results, combined with low but detectable imidacloprid levels in leaves from the 2007 growing season, support the reservoir hypothesis that imidacloprid is pooled in woody tissue around the injection point and gradually dissipates as it is translocated through the xylem over time. High imidacloprid equivalent concentration in woody tissue and zero evidence of imidacloprid equivalent concentration in roots and trunk cores also support previous evidence that imidacloprid moves primarily through the xylem.

5 CONCLUSIONS

In summary, it was found that imidacloprid equivalent concentration varied in leaves of ash trees, based on the position of branches in relation to injection points and time elapsed after injection. Xylem anatomy in ash trees causes trunk-injected imidacloprid to travel through the tree in a zigzag xylem ascent pattern, which could result in variable levels of insecticide in the tree crown. Levels of insecticide were substantially reduced 1 year after injection and were unlikely to provide adequate control of *A. planipennis*.

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