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Research

Evaluation of Systemic Imidacloprid and Herbicide Treatments on Flatheaded Borer (Coleoptera: Buprestidae) Management in Field Nursery Production

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Abstract

The flatheaded appletree borer, *Chrysobothris femorata* (Olivier) (Coleoptera: Buprestidae), and related species are deciduous tree pests. Female beetles prefer to oviposit at tree bases, and larvae tunnel beneath the bark, which weakens or kills young or newly transplanted trees. In the first objective of this study, Discus N/G (2.94% imidacloprid + 0.7% cyfluthrin) applied at six lower-than-labeled rates (0.0, 0.98, 1.97, 3.94, 5.91, and 7.87 ml/cm of average trunk dia.) was evaluated for protection of field-grown maples. A second objective evaluated imidacloprid with and without herbicides to assess the impact of weed competition at the tree base on insecticide effectiveness. A third objective determined relative imidacloprid concentrations in leaf tissue samples with ELISA and related to insecticide rates, herbicide treatments, and the level of flatheaded borer protection. In two trials, higher rates of insecticide were more effective at protecting trees, with rates ≥3.94 ml product/cm trunk diameter performing equivalently. Weed-free trees had more borer attacks and grew faster than trees in weedy plots. Imidacloprid content in leaf tissues had a trend for higher concentrations in smaller, weedy trees in the first season, but that pattern disappeared in subsequent years. Based on fewer attacks in weedy versus weed-free trees (60–90% reduction), it was concluded that weed presence can reduce borer attack success in nurseries independent of insecticide treatment, but tree growth was reduced by weed presence. In addition, Discus applied at rates >3.94 ml/cm did not confer added borer damage protection in weedy plots.

Key words: *Acer*, maple, competition, insect suppression, neonicotinoid

Insect damage is a major source of revenue loss in tree crops. Flatheaded borers are among the top pest concerns because the damage is so severe and these borers are difficult to control (Adkins et al. 2010). *Chrysobothris* is an important genus of indigenous nursery-attacking flatheaded borers that have a wide distribution in North America (Wellso and Manley 2007). The most common pest species in ornamental trees are members of the *Chrysobothris femorata* (Olivier) (Coleoptera: Buprestidae) (FAB) species complex (Hansen et al. 2015, Oliver et al. 2019). The FAB species complex and other related species (e.g., Pacific flatheaded borer [*Chrysobothris mali* Horn]) are major pests of specialty tree crops across the United States (Burke 1929, Fenton and Maxwell 1937, Potter et al. 1988, Oliver et al. 2010, Seagraves et al. 2013). The FAB is detrimental to deciduous trees in nursery and newly planted fruit and nut orchard crops in states such as Alabama, Georgia, Kentucky, North Carolina, Ohio, South Carolina, and Tennessee (Addesso 2019). For example, in the southeastern United States, FAB routinely causes less than 40% loss in some nursery tree species (Oliver et al. 2010). Female beetles deposit eggs primarily at the base of trees, and upon hatching, the larva burrows into the tree and excavates a gallery beneath the bark. Larval tunneling can girdle young trees, which can weaken vascular structure or cause death. If the tree survives, it is more likely to be attacked again or die later from stress issues. In addition to structural trunk damage, the borer usually ruins the aesthetic quality of the tree trunk for nursery sales, even if the tree survives the initial attack.

Flatheaded borer management in nursery crops has traditionally used trunk sprays of contact insecticides like pyrethroids (e.g., bifenthrin, lambda-cyhalothrin, permethrin) or organophosphates (e.g., chlorpyrifos). However, multi-year studies evaluating the

efficacy of chlorpyrifos and bifenthrin trunk sprays indicated minimal control of *Chrysobothris* borers compared with untreated trees (Potter et al. 1988, Oliver et al 2010). For trunk sprays to work, not only must flatheaded borers be susceptible to active ingredients but also the application timing, dosage, and residual activity must overlap with the vulnerable life stages of the beetle. The timing of contact insecticide treatments is challenging because it is unknown whether active ingredients target the adult (i.e., residues need to be on trees before landing), egg (i.e., residues need to be applied before larva exits the egg), larva (i.e., residues need to be on the bark before the egg is laid), or all of these stages (Oliver et al. 2019). In Tennessee, flight activity of *Chrysobothris* spp. primarily begins in May, but flights can extend late into July (Klingeman et al. 2015). In Oklahoma, FAB adult emergence primarily occurred from mid-May through June and adults were able to lay eggs after 4–8 d, followed by another 6–8 d of incubation before larval eclosion (Fenton and Maxwell 1937, Fenton 1942). Fenton (1942) also reported considerable adult oviposition from July to early August based on eggs laid on cut branches deployed in trees. A complicating factor in using phenological data from older studies to time trunk spray treatments are the recent changes in *Chrysobothris* taxonomy (Wellso and Manley 2007, Hansen et al. 2015), which creates more uncertainty about the actual activity periods of different species. The extended flight periods of nursery-attacking *Chrysobothris* species and the uncertainty regarding the taxonomy of species involved with attacks necessitates multiple spray applications when using trunk sprays to protect trees.

In recent years, many nursery growers have begun to utilize systemic neonicotinoids for flatheaded borer management because a single application can provide long residual activity reducing the need to closely time treatments with borer phenological periods. In one study, imidacloprid was the most effective neonicotinoid for preventing flatheaded borer damage, and it also outperformed bifenthrin and chlorpyrifos contact insecticides (Oliver et al. 2010). A single application of 0.27 or 0.54 g imidacloprid/cm of trunk diameter provided up to 4-yr of flatheaded borer control, and applications applied earlier in the spring (March) were more effective than later (May), probably due to more time for active ingredient translocation (Oliver et al. 2010). Current imidacloprid product labels limit active ingredient to 0.45–0.56 kg/ha/yr, so rates labeled for flatheaded borers (0.27–0.54 g imidacloprid/cm of trunk diameter) translate into a maximum of 833–2,074 trees (1-cm dia.)/ha/yr, respectively. Larger trunk diameters would further reduce tree numbers that can be treated due to larger quantities of active ingredient needed. Field-grown nursery growers commonly grow close to 3,000–4,000 trees/ha, which is much higher than imidacloprid active ingredient acreage restrictions. Therefore, there is a need to identify systemic treatment options that can allow more trees per unit area to be treated. An added benefit of lower rates could be less cost to growers, lower active ingredient loads in soil resulting in reduced persistence and off-site movement in the environment, and reduced impacts on nontargets like pollinators (Krupke et al. 2012, Bonmatin et al. 2015, Botías et al. 2015, Mörtl et al. 2020).

To improve the utility of systemic insecticides for nursery growers attempting to maximize the numbers of treated trees, there are a couple of potential options. The first would be a lower treatment rate, assuming borer efficacy can be sustained. Many neonicotinoids have soil half-lives >1,000 d (Bonmatin et al. 2015), and residues may persist in the soil for years and continue to supply crop plants with active ingredients (Botías et al. 2015). The chemical properties of different neonicotinoids, as well as environmental factors such as soil type, moisture, and temperature can all impact how much active ingredient remains available to crop plants over time, as well as how low rates can be before borer efficacy is lost. The second option could be to improve tree root access to active ingredient residues in the soil. In herbaceous crops, uptake of imidacloprid was about 5% with a range of approximately 2–20% depending on the crop plant (Sur and Stork 2003). Imidacloprid not removed by crop plants either remains in the soil or is lost to degradation, leaching, or uptake by other plants (Sur and Stork 2003, Bonmatin et al. 2015). Because systemic insecticides are translocated by plants, other adjacent vegetation like weeds may compete with nursery tree crops for the same insecticide residues (Krupke et al. 2012, Botías et al. 2015, Mörtl et al. 2020). If so, then management of competing weed vegetation in nursery rows with herbicides may improve tree root access to insecticide residues in the soil.

To improve the utility of systemic imidacloprid for flatheaded borer management in nurseries, the objectives of this study were to determine the lowest effective rate of imidacloprid to maximize the number of trees that can be treated on a per-area basis and to determine the effect of herbicide application on imidacloprid efficacy. Based on previous work, we hypothesized that trees with weed-free areas at the base would have more available imidacloprid to uptake from the soil drench and therefore be better protected from flatheaded borer attacks. A third objective of our study was to perform an imidacloprid ELISA test on leaf tissue samples to determine whether a relationship exists between the relative amount of imidacloprid in maple trees and insecticide rate or herbicide treatments. We hypothesize that higher imidacloprid levels in leaf tissues will be an indirect indication of greater flatheaded borer protection in the tree trunk.

Materials and Methods

2010 Trial

A Warren County, TN, commercial nursery (35.6375429, –85.8387379) transplanted bare root dormant liners of various maples into blocks of one uniform cultivar type in April 2010 using standard practices for a 3-yr planting cycle (~1.5 m between trees and 1.8 m between rows; ANSI 2014). The choice of cultivars used in these experiments was based on the selections planted by the nursery. Trees were assigned to 1 of 12 treatments in a factorial design using maple cultivar, insecticide, and herbicide as the factors. Maple cultivars included 'Franksred' (Red Sunset; 17 replicates), 'New World' (8 replicates), 'October Glory' (15 replicates), and 'Brandywine' (16 replicates) red maples (*Acer rubrum* L.) (Sapindales: Sapindaceae); 'Jeffersred' (Autumn Blaze; 16 replicates) hybrid maple (*Acer* × *freemanii* A. E. Murray); and 'Legacy' (12 replicates) sugar maple (*Acer saccharinum* L.), for a total of 1,008 trees. On 17 May 2010, initial tree height (cm; soil line to the highest branch tip) and trunk diameter (mm; 15 cm above the soil surface) were measured. Insecticide application rates were based on the initial average trunk diameter (±SE) with cultivars grouped into two average sized cohorts consisting of 'Legacy' and 'New World' (24.6 ± 0.2 mm [range 17.2–31.1 mm]) and 'Jeffersred', 'Brandywine', 'October Glory', and 'Franksred' (17.9 ± 0.1 [range 12.6–24.2]) (Table 1). Trees were measured at test termination on 17 October 2011 to determine the change in trunk diameter and height growth.

On 18 May 2010 (~4 wk posttransplant), insecticide treatments applied to individual trees included Discus N/G (0.0314-g imidacloprid and 0.0074-g cyfluthrin/ml product; OHP, Bluffton, SC) at rates of 0, 0.98, 1.97, 3.94, 5.91, or 7.87 ml product/cm trunk diameter (hereafter reported as 0, 1, 2, 4, 6, or 8 ml). Insecticide treatments were applied as

Table 1. Application timings, products, rates, and manufacturers for pre- and postemergent herbicides in 2013 trial

Year	Month	Product name	Pre- or postemergent	AI and percentage	Rate (kg AI/ha)	Manufacturer
2013	May	Roundup	Post	Glyphosate, 41	2.46	Bayer
		Gallery 75 DF	Pre	Isoxaben, 75	0.80	Corteva
		Barricade 65WG	Pre	Prodiamine, 65	1.68	Syngenta
	July	Roundup	Post	Glyphosate, 41	2.46	
		Pennant	Pre	S-metolachlor, 83.7	2.13	Syngenta
	Aug.	Roundup	Post	Glyphosate, 41	2.46	
		Pennant	Pre	S-metolachlor, 83.7	2.13	
2014	May	Roundup	Post	Glyphosate, 41	2.46	
		Gallery 75 DF	Pre	Isoxaben, 75	0.80	
		Barricade 65 WG	Pre	Prodiamine, 65	1.68	
	Sept.	Envoy Plus	Post	Clethodim, 12.6	0.14	Valent, Walnut Creek, CA
	Nov.	Roundup	Post	Glyphosate, 41	2.46	
		Marengo	Pre	Indaziflam, 7.4	0.056	Bayer
2015	May	Envoy Plus	Post	Clethodim, 12.6	0.14	
		Sureguard	Pre	Flumioxazin, 41.4	0.36	Valent USA
	June	Envoy Plus	Post	Clethodim, 12.6	0.14	
		Sureguard	Pre	Flumioxazin, 41.4	0.36	
2016	Mar.	Sureguard	Pre	Flumioxazin, 41.4	0.36	
		Roundup	Post	Glyphosate, 41	2.46	
	June	Envoy Plus	Post	Clethodim, 12.6	0.14	
	July	Gly Star	Post	Glyphosate, 41	2.46	Albaugh, Ankeny, IA

AI, Active ingredient.

a basal soil drench in a 60-ml volume of solution. A small circular ring was made approximately 5–8 cm from the tree base with the corner of a hoe to receive the insecticide solution and prevent runoff. The Discus application rates were based on the imidacloprid component because the nonsystemic cyfluthrin component is not intended for borer management when applied as a soil drench.

On 26 May 2010, trees randomly assigned to receive herbicide treatments (i.e., half of the trees in the experiment) had post- and pre-emergent herbicides applied to the ground in a 45 × 45-cm area around each tree base, whereas nonherbicide-treated trees (i.e., the remaining half of trees in the experiment) received no herbicide treatment. Herbicide-treated trees received a tank mix of Roundup Pro (Bayer, Research Triangle Park, NC) at 2.5-kg glyphosate/ha, Barricade 65WG (Syngenta, Greensboro, NC) at 1.7-kg prodiamine/ha and Gallery 75 DF (Corteva, Indianapolis, IN) at 0.8-kg isoxaben/ha. Herbicides were banded using an 8003 flat fan nozzle in a solution of 262 liter/ha and spray pressure of 21,093 kg/m^2. Herbicide-treated plots were maintained weed-free during the experiment with Finale (Bayer) at the spot treatment rate of 0.12-kg glufosinate-ammonium/liter (15.6-ml product/liter). Weed population was naturally occurring in all nursery blocks and included primarily Johnsongrass (*Sorghum halepense* [L.] Pers.), horseweed or marestail (*Erigeron canadensis* L.), Pennsylvania smartweed (*Polygonum pensylvanicum* L.), yellow nutsedge (*Cyperus esculentus* L.), and Bermuda grass (*Cynodon dactylon* [L.] Pers.) (Fig. 1). Weedy plots were left untreated other than routine mowing within 1 m of either side of the planted rows (Fig. 1A and C). By mid-July, weed vegetation was >30 cm tall in all weedy plots. Trees received controlled-release (4–6 mo) granular fertilizer (20-6-12; N-P-K; Florikan, Sarasota, FL) applied on 14 June 2010 at 37 g per tree to all selections except 'Legacy' sugar maple, which received 55 g per tree. Fertilizer was spread evenly in a 91-cm diameter circle around the base of the tree. No supplemental irrigation was applied in this experiment.

In October 2011, the numbers of trees attacked by flatheaded borers were quantified by examining the trunk from the soil line to ~90 cm for visible damage that included sunken, discolored or cracked bark, bark sloughing, basal epicormic shoots, fungi growing through the bark, frass, or d-shaped exit holes left by emerging adults. When the previously described larval damage symptoms were present, a knife was used to probe or remove the bark and confirm the presence of the cake-like frass unique to flatheaded borers (Brooks 1919). In instances where damage was present and frass was unapparent, more bark was removed to expose the flatheaded borer serpentine galleries (and in some cases the larva providing positive confirmation of the damage source). The damage cataloged in this study was assumed to be *Chrysobothris* spp. based on the size of the galleries and species in this genus being the only flatheaded borers we have reared from trunks of maple nursery stock in Tennessee (Oliver, unpublished data). However, the flatheaded borer species responsible for attacks was not determined in this study, and the primary study focus was to determine the number of trees with or without flatheaded borer injury.

2013 Trial

Dormant bare root liners of 'Franksred' (1,092) were transplanted on 9 April 2013 in three nursery blocks at the same nursery as the 2010 trial. The field sites had recently been turned and disked several times to prepare the ground for transplanting, and trees were transplanted at the same spacing as the 2010 trial. Nursery block 1 had replicates 1–47, block 2 had replicates 48–63, and block 3 had replicates 64–91. As in the 2010 experiment, trees were assigned to 1 of 12 treatments in a factorial design that included nursery block, insecticide, and herbicide as factors. The insecticide treatments were the same as previously described. As in 2010 trial, insecticides were applied in a 60-ml solution to a small furrow near the tree base. Initial tree height and trunk diameter were measured on 25 April 2013 as previously described following transplant to calculate average trunk diameter for insecticide rates. All Discus application rates were based on the initial average trunk diameter (±SE; i.e., 15.41 ± 0.062 mm [range 10.4–22.2 mm]). Trees were measured subsequently on 20 February 2014, 20 February 2015, 7 March

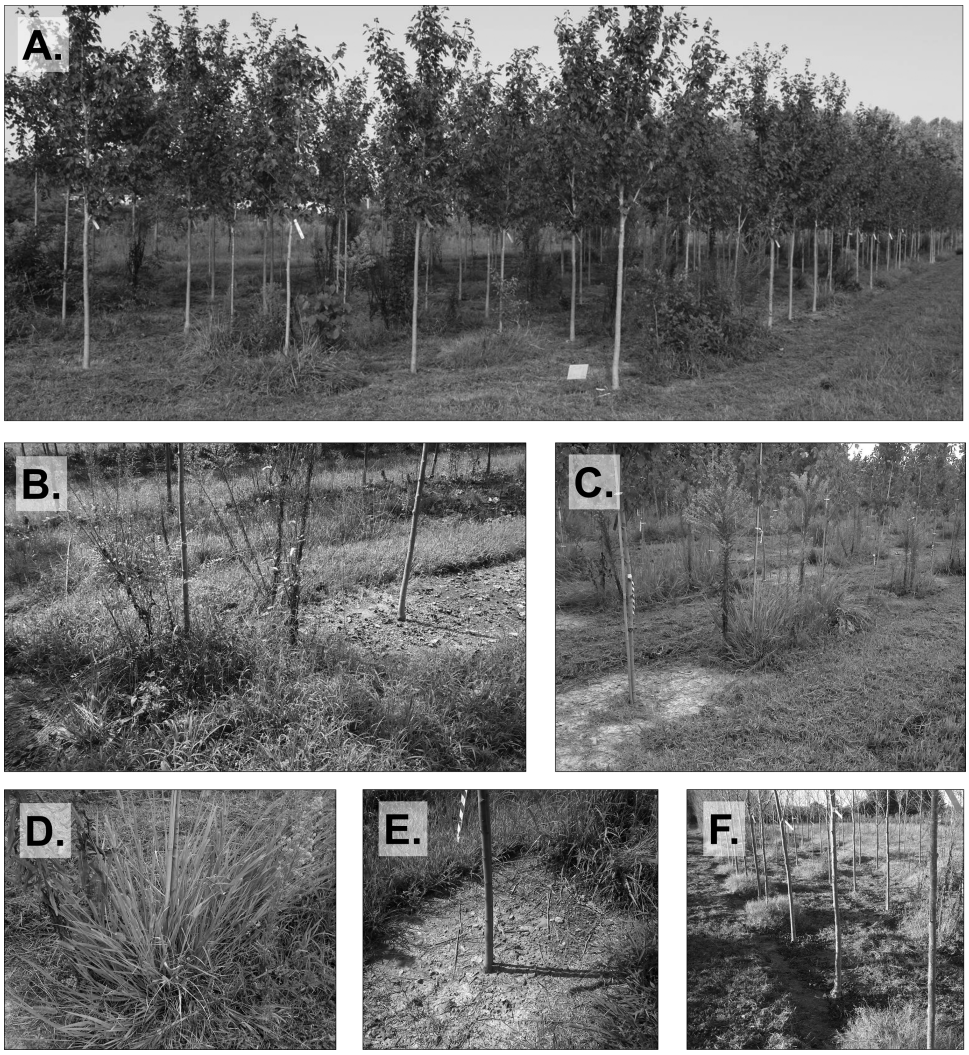

Fig. 1. Images of weedy (no herbicide) and nonweedy (herbicide-treated) maple field plots, including (A) broad view of nursery block during late August, (B) and (C) closer views of herbicide and non-herbicide plots in late August with grass and marestail weeds visible in background, (D) close-up view of weedy plot predominated by grass with some marestail during late August, (E) close-up view of a herbicide clean plot during late August, and (F) mid-November view after weed dormancy.

2016, and 28 November 2016 to determine annual trunk diameter and height growth. Insecticide treatments were applied as basal soil drenches at 3-wk posttransplanting on 30 April 2013.

Weed seeds were sown (9 May 2013) along both sides of the planted tree rows in a 30-cm wide band to ensure uniform weed pressure. Common ragweed (*Ambrosia artemisiifolia* L.), lambsquarter (*Chenopodium album* L.), and a commercial mix of annual and perennial rye grass (*Festuca perennis* Lam. 'Gulf' and *Lolium* × *boucheanum* [an intermediate ryegrass cross between perennial and Italian ryegrass]) seeds were applied with a hand-held shaker. Common ragweed was mixed with sand (1:2) at a seeding rate of 250 g/1,000 m^2. Lambsquarters was mixed with sand (1:2) and sown at a seeding rate of 641 g/1,000 m^2. The ryegrass blend was mixed with sand (2:1) and sown at a seedling rate of 404 g/1,000 m^2. A 1.2-m disc was pulled behind a tractor and made two passes in each aisle to lightly toss soil on weed seed. A utility vehicle with wide tires was driven over the sown area to ensure good soil contact with the seed. Rainfall occurred within 2 d. By 2 July 2013, weeds in plots were actively growing and included the sown weeds and other naturally occurring weeds previously described in the 2010 trial (Fig. 1). Like the 2010 trial, weed vegetation was >30 cm tall by mid-July in weedy plots. In September 2013, ragweed plants had grown to approximately 1.5- to 1.8-m tall and were hand removed. No additional weed seeds were sown in the experiment after the initial planting. There was adequate uniform weed presence in all weedy plots before herbicide applications began throughout the multi-year trial.

A rotation of pre- and postemergent herbicides began in May 2013 to maintain weed-free treatments. These products include the pre-emergent products Barricade, Gallery, Pennant, Marengo, and Sureguard and the postemergent products Roundup, Factor, Envoy Plus, and Gly Star (see Table 1 for rate, application timing, and manufacturers). Trees were maintained during the experiment using standard cultural practices. Trees were fertilized annually in spring with fertilizer applied around a ~15 cm diameter radius at the base of the trunk. Trees received granular fertilizers on 16 April 2013 (69 g per tree of 19-5-9 N-P-K; 8–9 mo; Osmocote Pro, Everris, Dublin, OH), 25 April 2014 (112 g per tree of 13-13-13 agriculture grade fertilizer), 18 March 2015 (149 g per tree of 15-15-15 agriculture grade), and 10 March 2016 (224 g per tree of 15-15-15 agriculture grade fertilizer). Trees were pruned 17 June 2014, 16 March

2015, and 10 March 2016 to maintain a central leader. No supplemental irrigation was applied in this experiment.

Following initial measurements on 25 April 2013, trunk diameter and tree height were measured on 20 February 2014, 20 February 2015, 7 March 2016, and 28 November 2016 to determine annual growth among treatments. On 29 November 2016, replicates without borer damage among all the tree treatments were identified, and 10 replicates were randomly selected from these nondamaged replicates and the trees were harvested. Trunk and shoot dry weight (g) were recorded and total biomass calculated. Trees were harvested by cutting the trunk at the soil line and then a clean trunk length of 200 cm was severed from the canopy. All shoots from the canopy were chopped into pieces and placed in paper bags. Trunks and bagged shoots were stored in a plastic covered overwintering house for 30 d and then dried in a forced-air oven at 56°C for 10 d.

Flatheaded borer attacks were assessed annually on 22 April 2014, 29 April 2015, 5 May 2016, and at the termination of the experiment on 23 November 2016 using damage criteria previously described in the 2010 trial. In the 2013 trial, sunken bark, knife probing, and bark peeling also were used to delineate the sub-bark range extent of the flatheaded borer gallery damage around the trunk circumference (left and right cardinal direction edges or 360° if completely around trunk) and lower and upper gallery height on the trunk.

Leaf Tissue Imidacloprid Analysis

Leaf tissue was collected from each treatment in September 2013, 2014, and 2015 from the first 20 replicates of each nursery block and pooled into three samples (one for each block). The stems and petioles were removed and the remaining leaf tissue was dried in an oven at 40°C for 2 d. The dried samples were ground to a fine powder in a Wiley Mill (2 mm sieve, Thomas Scientific, Swedesboro, NJ). Leaf tissue was extracted following a method modified from McCullough et al. (2011). Briefly, 0.5 g of powdered leaf tissue was extracted for 3 h in 10 ml of methanol on an orbital shaker. The extract was centrifuged at 6,000 rpm for 10 min and the supernatant was diluted 20–1,000× before analysis. Imidacloprid levels were analyzed using a semi-quantitative ELISA method (QuantiPlate Kit for Imidacloprid, EnviroLogix, Portland, ME). The enclosed kit protocol was used without modification (EnviroLogix 2015). Briefly, 100-µl blank, three calibrators, and samples were added into their respective wells followed by 100 µl of imidacloprid enzyme conjugate. The samples were mixed thoroughly by moving the strip holder in a circular motion on the bench-top for 30 s. After mixing, the wells were covered with Parafilm (Pechiney Plastic Packaging, Menasha, WI) and incubated at ambient temperature for 1 h on an orbital shaker at 200 rpm. Following incubation, the Parafilm was removed and contents of the wells emptied. The wells were rinsed by flooding with cool tap water and shaken to empty. The wash step was repeated four times and dried by slapping the plate on a paper towel to remove remaining moisture. After the moisture was removed, 100 µl of substrate was added to the plates, mixed, covered in Parafilm, and incubated for another 30 min on the orbital shaker. Finally, 100 µl of Stop Solution (1.0 N hydrochloric acid) was added to each well and mixed thoroughly, which turns the well contents yellow. The plate was read within 30 min of the addition of Stop Solution at 450 nm in a UV–Vis microplate reader.

Before analysis, the average untreated control OD value was subtracted from all imidacloprid samples to adjust for matrix effects. The %B0 for each sample was calculated according to the following equation %B0 = (average OD of Calibrator or sample/average OD of Negative Control) × 100. Based on the kit protocol, the %B0 of each Calibrator fell within the following ranges: 0.2 ppb (75–86%), 1 ppb (40–57%), and 6 ppb (14–24%). If the CV for any pair of Calibrator or sample OD values exceeded 15%, the samples were re-run. The %B0 of each Calibrator was graphed against its imidacloprid concentration on a semi-log scale. The resulting equation was used to estimate unknown sample quantities. If the sample %B0 values fell above the range of the calibrators, the samples were diluted. If the samples fell below the range of the calibrators in the undiluted samples, they were reported as below the value of detection.

Statistical Analysis

In the 2010 trial, tree growth (height, trunk diameter) data were analyzed using a generalized linear model on untransformed data (Proc GLM; SAS Institute Inc., Cary, NC), whereas tree numbers with flatheaded borer damage were fitted to a binomial model with a log-link (Proc Genmod). For the 2010 trial model, data were analyzed using a factorial design with the equation *DependentVariable = Cultivar Insecticide Herbicide Insecticide*Herbicide*.

For the multi-year 2013 trial, tree growth (height, trunk diameter) data were analyzed with a repeated-measures analysis (Proc Mixed) using a factorial design with the equation *DependentVariable = NurseryBlock Insecticide Herbicide Year Insecticide*Herbicide* with tree replicate repeated over year: *Repeated Year/Sub = Rep Type = CS*. Tree numbers with flatheaded borer damage were analyzed using a logistic regression fitted to binomial distribution on the final number of attacked trees because yearly tree attack numbers were too small for sufficient analysis: *Attacks = NurseryBlock Insecticide Herbicide Insecticide*Herbicide*. Tree biomass (canopy, trunk, and total), imidacloprid quantification and cardinal direction range of borer damage were analyzed using a generalized linear model on untransformed data (Proc GLM; *Biomass = Insecticide Herbicide Insecticide*Herbicide*). Height range of borer damage was analyzed using a Generalized Linear Interactive Model (GLIM; Proc Genmod; *CardinalDirection = Herbicide*) fitted to a binomial distribution with a logit link to model presence–absence data. Pair-wise comparisons of significant variables were made using the Tukey–Kramer adjustment. Imidacloprid analysis was conducted with samples pooled across *NurseryBlock* with a repeated-measures analysis (Proc Mixed) using a factorial design with the equation *Parts Per Million = Insecticide Herbicide Year Insecticide*Herbicide* repeated over year: *Repeated Year/Sub = NurseryBlock Type = CS*.

Results

2010 Trial

For tree height, there were significant differences in growth by cultivar (F = 339.1; df = 5, 882; P < 0.0001; Table 2) and herbicide (F = 192.75; df = 1, 882; P < 0.0001) factors, but not insecticide rate (F = 1.39; df = 5, 882; P = 0.24). There was no interaction of insecticide rate and herbicide detected (F = 1.02; df = 5, 882; P = 0.41). 'Jeffersred' hybrid maple had more height growth, followed by 'Brandywine' and 'Franksred' red maples, 'Legacy' sugar maple, and 'October Glory' and 'New World' red maples. Similarly, trunk diameter growth was affected by cultivar (F = 582.70; df = 5, 880; P < 0.0001; Table 2) and herbicide (F = 423.45; df = 1, 880; P < 0.0001) factors, but not insecticide rate (F = 0.27; df = 5, 880; P = 0.93). There was no interaction of insecticide rate and herbicide detected (F = 0.86; df = 5, 880; P = 0.51). In descending order, trees with the greatest increase in trunk diameter were 'Jeffersred',

Table 2. Herbicide and maple cultivar effect on average (±SE) tree diameter and height growth after 1 yr in the 2010 trial

			Average ± SE [range] initial size		Average ± SE growth[b]	
Herbicide (Y/N)[a]	Cultivar	Maple species	Trunk diameter (mm)	Height (cm)	Trunk diameter (mm)	Height (cm)
Y	'Jeffersred'	Freeman	19.1 ± 0.2 [16.4–24.2]	197.4 ± 1.0 [177–228]	24.7 ± 0.4a	149.7 ± 3.0a
	'Brandywine'	Red	17.5 ± 0.2 [14.2–20.9]	180.4 ± 1.1 [153–202]	19.2 ± 0.4b	93.3 ± 3.9b
	'Franksred'	Red	16.6 ± 0.2 [12.6–22.4]	192.3 ± 1.3 [163–222]	9.7 ± 0.2d	76.2 ± 3.8c
	'Oct. Glory'	Red	18.4 ± 0.2 [13.6–22.7]	227.1 ± 1.6 [189–261]	14.8 ± 0.4c	28.4 ± 3.3e
	'New World'	Red	24.8 ± 0.2 [21.4–28.0]	324.5 ± 2.9 [285–363]	6.9 ± 0.3f	2.0 ± 1.5f
	'Legacy'	Sugar	24.9 ± 0.3 [18.9–31.0]	226.1 ± 1.8 [188–259]	8.1 ± 0.3e	37.0 ± 2.1d
N	'Jeffersred'	Freeman	19.0 ± 0.2 [15.7–22.3]	196.2 ± 1.0 [164–220]	18.0 ± 0.4a	114.2 ± 3.9a
	'Brandywine'	Red	17.5 ± 0.2 [13.3–21.3]	182.0 ± 1.1 [150–204]	13.3 ± 0.5b	39.2 ± 4.7b
	'Franksred'	Red	16.9 ± 0.2 [13.4–21.1]	190.9 ± 1.4 [156–248]	6.7 ± 0.2d	46.6 ± 3.4b
	'Oct. Glory'	Red	18.4 ± 0.2 [13.8–22.1]	227.0 ± 1.6 [193–256]	10.7 ± 0.4c	9.7 ± 2.8d
	'New World'	Red	24.9 ± 0.2 [20.3–28.9]	330.3 ± 2.7 [272–363]	3.5 ± 0.2f	−1.9 ± 0.7e*
	'Legacy'	Sugar	23.9 ± 0.4 [17.2–31.1]	225.2 ± 1.7 [195–256]	5.5 ± 0.3e	21.3 ± 1.6c

[a]Herbicide-treated trees (Y) received a tank mix of Roundup Pro (Bayer) at 2.5-kg glyphosate/ha, Barricade 65WG (Syngenta) at 1.7-kg prodiamine/ha, and Gallery 75 DF (Corteva) at 0.8-kg isoxaben/ha. Herbicide-treated plots were maintained weed-free during the experiment with Finale (Bayer) at the spot treatment rate of 15.6 ml product/liter. Weed population was naturally occurring in all nursery blocks and weedy plots received no herbicide treatment (N).

[b]Within each herbicide treatment, cultivar values with different lowercase letters are statistically different by Tukey's pair-wise comparison ($\alpha = 0.05$). Trees in herbicide treatments grew significantly more in trunk diameter ($F = 423.45$; df = 1, 880; $P < 0.0001$) and height growth ($F = 192.75$; df = 1, 882; $P < 0.0001$) than trees in weedy treatments for all cultivars (mean separations not shown). Insecticide treatments were not significant and were subsequently pooled.

*Negative growth value resulted from tip moth and potato leafhopper damage.

'Brandywine', 'October Glory', 'Franksred' and 'New World' red maples, and 'Legacy' sugar maple.

There were significant differences in the number of trees with flatheaded borer damage by tree cultivar, insecticide rate, and herbicide factors. Tree cultivar did impact the numbers of trees with borer damage ($\chi^2_{(5)} = 74.04$, $P < 0.0001$), with 'October Glory' red maple having the most attacks and 'Jeffersred' having the fewest. The other maple cultivars were between the two extremes. Flatheaded borer attacks on trees decreased with increasing rates of imidacloprid ($\chi^2_{(5)} = 96.81$, $P < 0.0001$; Fig. 2A). Also, there were more attacks on trees with weed-free ground at the base (herbicide-treated) than trees with weeds growing at the base at all imidacloprid rates ($\chi^2_{(1)} = 16.71$, $P < 0.0001$).

2013 Trial

In this trial, trees were blocked by nursery location ($F = 14.83$; df = 2, 88; $P < 0.0001$). Trunk diameter growth of 'Franksred' red maples differed over the four trial years ($F = 1{,}213.21$; df = 3, 270; $P < 0.0001$; Table 3). Trunk diameter growth was consistently greater in herbicide-treated trees ($F = 2{,}683.51$; df = 1, 90; $P < 0.0001$; Table 3). Insecticide rate did not affect trunk diameter growth ($F = 2.01$; df = 5, 450; $P = 0.08$). There was no interaction detected for herbicide and insecticide on trunk diameter growth ($F = 1.73$; df = 5, 450; $P = 0.13$). Tree height growth was significantly affected by nursery block location ($F = 67.22$; df = 2, 88; $P < 0.0001$) and herbicide ($F = 506.46$; df = 1, 90; $P < 0.0001$) factors (Table 3). Height growth increased each year, however by year 4, canopy development was increasing in width, as well as plant height, so height growth alone did not represent all tree growth (Table 3). Thus, total biomass was collected at the end of the trial to differentiate canopy development (Table 4). There was no effect detected for the insecticide rate ($F = 0.76$; df = 5, 450; $P = 0.58$) or the interaction of insecticide and herbicide ($F = 0.33$; df = 5, 441; $P = 0.90$) factors on height growth. At the conclusion of the trial, shoot weight, trunk weight, and total dry biomass were affected by the use of herbicide, but not by insecticide treatment rates (Table 4). Shoot weight ($F = 342.87$; df = 1, 113; $P < 0.0001$), trunk weight ($F = 446.59$; df = 1, 113; $P < 0.0001$), and total biomass ($F = 440.43$; df = 1, 113; $P < 0.0001$) were greater in trees with the base of trunks kept weed-free with herbicide (Table 4).

There were more flatheaded borer attacks on trees in the weed-free (herbicide-treated) than trees with weeds growing at the base ($\chi^2_{(1)} = 50.45$, $P < 0.0001$). Flatheaded borer attacks on trees decreased with increasing rates of imidacloprid ($\chi^2_{(5)} = 41.04$, $P < 0.0001$) in both herbicide and weedy treatments (Fig. 2B). In the herbicide treatments (clean plots), larval tunneling was greatest within the first 20 cm and decreased with height (Table 5). No height pattern was observed in the nonherbicide-treated (weedy plot) trees. Larval damage often encircled the trunk of the trees but was more concentrated in the southwest quadrant in the herbicide-treated trees with a mean location of 201.4° compared with 153.1° in weedy trees (Table 6). Larval tunneling extended further in the weedy trees, encompassing 263.8° of the trunk circumference compared with 166.1° in herbicide-treated trees. The edge of the tunneling damage extended further into the northeastern quadrant (43.8°) all the way to the southwest (262.5°) in weedy trees, whereas damage in herbicide-treated trees ranged from the southeast to southwestern quadrants (154.6°–248.1°). New trees were damaged every year of the experiment in the herbicide treatment with no imidacloprid (year 1 = 10, year 2 = 19, year 3 = 13, year 4 = 4; Supp Table 1 [online only]). With most of the rates (2, 4, 6, and 8-ml Discus/cm dia.) in the herbicide-treated trees, protection began to diminish in the third year. Attacks on the weedy trees were low across all Discus rates and sporadic across years.

Leaf Tissue Imidacloprid Analysis

The amount of imidacloprid present in leaf tissue decreased over time ($F = 29.63$; df = 2, 4; $P = 0.004$; Fig. 3) and differed by initial insecticide rate applied ($F = 4.00$; df = 4, 8; $P = 0.045$). Herbicide treatment had little effect on imidacloprid levels ($F = 5.85$; df = 1, 2; $P = 0.14$) and no interaction of herbicide and insecticide rate was observed ($F = 0.79$; df = 4, 8; $P = 0.56$). At the end of the 2013 season (year 1), leaf tissue

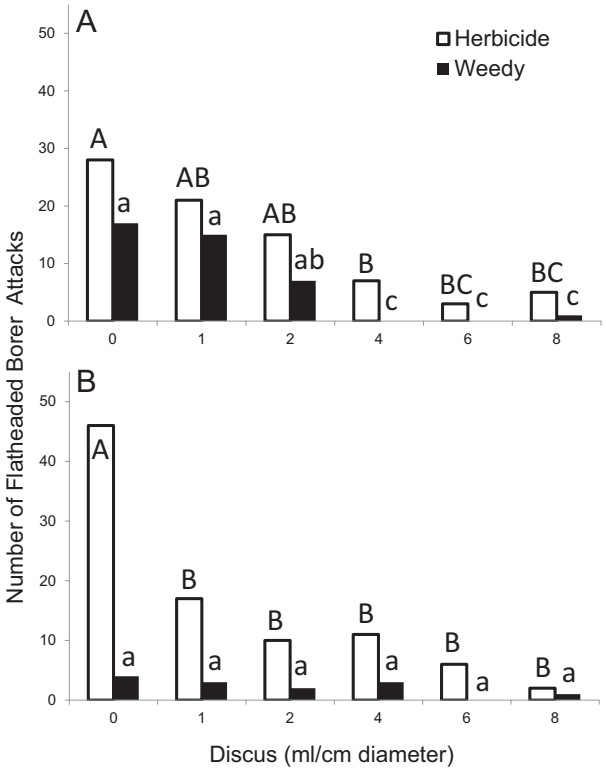

Fig. 2. Total number of flatheaded appletree borer attacks on trees with the root zone kept bare using herbicide (white bar) or weedy (black bar) at increasing rates of Discus N/G in the (A) 2010 trial and (B) 2013 trial. Herbicide treated trees had more damage than weedy trees in both the 2010 ($\chi 2_{(1)} = 16.71$, $P < 0.0001$) and 2013 ($\chi 2_{(1)} = 50.45$, $P < 0.0001$) trials. Imidacloprid treatment levels with different letters (uppercase = herbicide treated, lowercase = weedy) are statistically different by Tukey's pair-wise comparison ($\alpha = 0.05$).

imidacloprid levels increased with increasing insecticide application rates (Fig. 3). Additionally, slightly higher levels of imidacloprid were found in trees with weeds at the base (no herbicide). In 2014 (year 2), there were still differences in levels of imidacloprid among insecticide treatments. By 2015 (year 3), imidacloprid levels had dropped to below 0.04 ppm for all treatments.

Discussion

Flatheaded borer management was achieved by the application of systemic imidacloprid. The results of this experiment demonstrate that rates of imidacloprid lower than the labeled Discus N/G flatheaded borer rates (i.e., 8.7- and 17.3-ml product/cm dia.) previously evaluated (Oliver et al. 2010) can protect trees for up to 4 yr. In both the 2010 and 2013 trials, 90% or more of trees treated with near-half the labeled rate of imidacloprid (4-ml product/cm dia. at 15-cm height) were protected from flatheaded borer damage. The use of imidacloprid rates >4 ml did not provide a statistically significant management advantage in either the 2010 or 2013 trial for either the herbicide- or nonherbicide-treated groups (Fig. 2). However, from a nursery-grower perspective, flatheaded borer damaged tree counts in the 2013 trial, especially in the herbicide treatment group, may have exceeded acceptable damage thresholds for 4-ml rate (i.e., 11 trees) compared with higher 6-ml (six trees) and 8-ml (two trees) rates. In the multi-year 2013 trial, the 4-ml rate provided 3 yr of protection.

Although this study only quantified damage attributable to flatheaded borers, it was likely all of the damage was from borers in the genus *Chrysobothris*. In over 15 yr of rearing flatheaded borer adults from nursery maple trees, *Chrysobothris* species are the only flatheaded borers we have reared from the lower main trunk (Oliver, unpublished data). Other flatheaded borer genera and species are reared from older maple trees and higher in the canopy, and *Acmaeodera* spp. are sometimes found overwintering in dead branches of maple, but *Chrysobothris* species appear to be the main maple trunk-attacking group in nurseries. *Chrysobothris* species reared from Tennessee maple nursery stock in the past and possibly involved with tree attacks in this study include *Chrysobothris adelpha* Harold, *Chrysobothris azurea* LeConte, *Chrysobothris femorata*, *Chrysobothris rugosiceps* Melsheimer, and *Chrysobothris viridiceps* Melsheimer (Oliver et al. 2019). *Chrysobothris chlorocephala* Gory also has been reared from *Acer* sp. in Georgia and may be another possible attacker of nursery maple trees (Hansen et al. 2012).

The efficacy of the lower imidacloprid rates has a twofold benefit. First, it would allow more individual trees to be treated per unit area. The effective Discus rates in this study (4- to 8-ml Discus [0.13- to 0.25-g imidacloprid]/cm trunk diameter) were 53.81–7.62% below the current lowest labeled rate, respectively. Since the Discus label limits total active ingredient per year to 560.4 g/ha, the 4–8 ml/cm study rates would potentially allow treatment of 4,311–2,242 trees (1-cm dia.) compared with just 2,061 trees at the labeled 8.7 ml/cm labeled rate. Dawadi et al. (2019) also found the half-rate of imidacloprid to be effective in protecting 99% of the treated trees for 2 yr. Second, lower effective imidacloprid rates reduce insecticide cost, as well as lessen the impact to both the environment and nontarget organisms (Bonmatin et al. 2015). Herbaceous crops only uptake ~1.6 to 20% of imidacloprid residues available in the soil (Sur and Stork 2003), but removal rates in this study are unknown since we did not measure imidacloprid soil concentrations directly. The remaining imidacloprid residues in soil are presumably lost to volatilization, microbial and chemical degradation, uptake by other plants, and lateral or vertical movement by leaching or on eroded soil particles (Bonmatin et al. 2015, Botías et al. 2015). Imidacloprid residues that are not removed by the maple trees could be a hazard to nontarget organisms like aquatic invertebrates or pollinators, since imidacloprid is commonly found outside of crop areas in surface and ground water or in other vegetation (Krupke et al. 2012, Botías et al. 2015, Mörtl et al. 2020). Since neonicotinoids can have soil half-lives >1,000 d (Bonmatin et al. 2015), the effective lower application rates in this study may help to reduce accumulation persistence and off-site movement. The soil drenches that were applied directly to the tree base in low water volumes (i.e., 60 ml) would facilitate the downward movement of imidacloprid in the soil profile via the tree roots (Radolinski et al. 2019), while minimizing off-site lateral movement and nontarget impacts. The consistent imidacloprid ELISA leaf sample concentrations observed across the imidacloprid rate levels also suggested that lateral imidacloprid movement may have been minimal among tree treatments spaced at 1.5 m apart within row and 1.8 m between rows. In this study, the test sites had Waynesboro loam, Waynesboro clay loam, Cumberland silt loam, or Huntington silt loam soils, which are characterized by gentle slopes (0–12%) and soil horizons that increase from loam-clay loam to clay with depth (USDA-NRCS 2020). The gentle topography likely minimized lateral imidacloprid movement, since storm-generated runoff can be the dominant mechanism of offsite neonicotinoid movement (Radolinski et al. 2019). The high clay content soils characterizing our test sites also can enhance retention of imidacloprid in soils (Bonmatin et al. 2015).

Table 3. Herbicide and Discus N/G effect on 'Franksred' red maple average (±SE) trunk diameter and height annual growth in the 2013 trial

		Average ± SE annual growth[b]							
		Year 1		Year 2		Year 3		Year 4	
Herbicide (Y/N)[a]	Discus rate (ml/cm dia.)	Trunk diameter (mm)	Height (cm)	Trunk diameter (mm)	Height (cm)	Trunk diameter (mm)	Height (cm)	Trunk diameter (mm)	Height (cm)
Y	0	6.3 ± 0.2a	59.4 ± 3.1a	10.0 ± 0.3a	66.1 ± 3.9a	9.6 ± 0.2a	101.7 ± 3.8a	11.7 ± 0.3a	47.5 ± 2.9a
	1	6.2 ± 0.2a	58.5 ± 3.4a	10.8 ± 0.2a	70.3 ± 3.0a	10.1 ± 0.2a	106.1 ± 3.4a	11.7 ± 0.3a	48.9 ± 2.7a
	2	6.3 ± 0.2a	62.8 ± 3.5a	10.9 ± 0.2a	75.3 ± 3.2a	10.3 ± 0.2a	102.6 ± 2.9a	11.8 ± 0.3a	44.6 ± 2.3a
	4	6.4 ± 0.2a	70.7 ± 3.7a	11.0 ± 0.2a	70.6 ± 2.7a	10.4 ± 0.2a	100.0 ± 3.2a	11.4 ± 0.3a	49.8 ± 2.4a
	6	6.2 ± 0.2a	70.4 ± 3.6a	11.3 ± 0.2a	70.9 ± 2.8a	10.3 ± 0.3a	101.8 ± 3.3a	11.6 ± 0.2a	50.1 ± 2.9a
	8	6.0 ± 0.2a	63.8 ± 3.5a	11.2 ± 0.2a	76.2 ± 3.1a	10.5 ± 0.2a	97.6 ± 3.0a	11.9 ± 0.3a	49.1 ± 2.5a
N	0	3.3 ± 0.2b	17.4 ± 1.9b	5.2 ± 0.3b	52.4 ± 2.6b	6.5 ± 0.2b	76.6 ± 4.2b	9.4 ± 0.4b	57.7 ± 3.4b
	1	3.0 ± 0.1b	17.4 ± 2.6b	4.8 ± 0.2b	52.0 ± 2.5b	6.6 ± 0.2b	71.4 ± 3.6b	9.1 ± 0.3b	58.1 ± 3.4b
	2	3.0 ± 0.1b	15.1 ± 1.6b	4.5 ± 0.2b	52.6 ± 0.2b	6.6 ± 0.3b	74.6 ± 3.6b	9.2 ± 0.3b	59.2 ± 3.1b
	4	2.9 ± 0.1b	15.0 ± 1.9b	4.9 ± 0.2b	58.4 ± 2.6b	7.2 ± 0.2b	71.9 ± 4.3b	9.6 ± 0.3b	60.8 ± 2.9b
	6	3.0 ± 0.1b	13.8 ± 1.7b	5.4 ± 0.3b	60.4 ± 2.5b	7.0 ± 0.3b	73.2 ± 4.9b	9.7 ± 0.3b	59.1 ± 5.1b
	8	2.8 ± 0.1b	15.2 ± 2.0b	5.3 ± 0.4b	55.2 ± 2.8b	6.7 ± 0.4b	82.2 ± 3.8b	9.9 ± 0.2b	54.5 ± 2.8b

[a]For list of herbicide treatments, see Table 1. Herbicide-treated plots (Y) and weedy plots not treated with herbicides (N) both had naturally occurring and additional weed seeds broadcast into plots, but herbicide plots were kept clean with the herbicides.

[b]Values within columns with different lowercase letters are statistically significant by Tukey's pair-wise comparison (α = 0.05). Following transplant on 9 Apr. 2013, trees were initially measured on 25 Apr. 2013 and the average (± SE) was 15.4 ± 0.062 mm (range 10.4–22.2 mm) for trunk diameter and 208.34 ± 0.66 cm (range 145.0–295.0 cm) for tree height. The average trunk diameter was used to determine Discus rates. Trunk diameter and tree height growth measurements included year 1 (25 Apr. 2013 to 20 Feb. 2014), year 2 (20 Feb. 2014 to 20 Feb. 2015), year 3 (20 Feb. 2015 to 7 Mar. 2016), and year 4 (7 Mar. 2016 to 28 Nov. 2016).

Table 4. Average (±SE) dry weight of 'Franksred' red maple trees at termination (Nov. 2016) in the 2013 trial

		Average ± SE dry weight (kg)[b]		
Herbicide (Y/N)[a]	Discus rate (ml/cm dia.)	Shoots	Trunks[c]	Total biomass
Y	0	1.80 ± 0.15a	1.76 ± 0.10a	3.56 ± 0.11a
	1	1.69 ± 0.15a	1.83 ± 0.10a	3.51 ± 0.25a
	2	1.59 ± 0.14a	1.76 ± 0.09a	3.34 ± 0.23a
	4	1.72 ± 0.12a	1.86 ± 0.10a	3.58 ± 0.22a
	6	1.74 ± 0.08a	1.87 ± 0.07a	3.61 ± 0.14a
	8	1.66 ± 0.12a	1.73 ± 0.12a	3.39 ± 0.22a
N	0	0.65 ± 0.07b	0.88 ± 0.06b	1.53 ± 0.21b
	1	0.64 ± 0.09b	0.79 ± 0.06b	1.42 ± 0.12b
	2	0.62 ± 0.06b	0.75 ± 0.06b	1.37 ± 0.09b
	4	0.58 ± 0.08b	0.85 ± 0.07b	1.43 ± 0.14b
	6	0.47 ± 0.06b	0.74 ± 0.07b	1.22 ± 0.12b
	8	0.63 ± 0.08b	0.87 ± 0.07b	1.50 ± 0.14b

[a]For list of herbicide treatments see Table 1. Herbicide-treated plots (Y) and weedy plots not treated with herbicides (N) both had naturally-occurring and additional weed seeds broadcast into plots, but herbicide plots were kept clean with the herbicides.

[b]Values within columns with different lowercase letters are statistically significant by Tukey's pair-wise comparison (α = 0.05).

[c]Trunk weight was from a 200-cm-long bolt severed above the soil line. Shoot dry weight included all branches above the 200-cm bolt. Total biomass included both shoots and trunk weight.

The height of flatheaded borer damage observed in these studies is consistent with previous reports (Seagraves et al. 2013, LeBude and Adkins 2014, Dawadi et al. 2019) with nearly 50% of damage in weed-free trees observed below 20 cm, 27% between 20 and 40 cm, and 10% from 40 to 60 cm. Damage was mostly oriented in a southerly direction; however, larval damage in trees sheltered by weeds tended to extend farther into the western and northeastern quadrants of the trunk circumference (Table 6). The greater extent of larval damage on trunks in weedy trees sites is likely due to smaller differences in temperature across the surface of shaded trunks than trees exposed to direct sunlight in the weed-free plots (Fig. 1). Dawadi et al. (2019) observed as much as a 4°C greater average trunk temperature on the sunny side of weed-free trunks compared with trunks shaded by cover crops.

The impact of weeds on flatheaded borer management was unexpected. The authors initially hypothesized that the use of herbicides to suppress weeds would increase uptake of imidacloprid by the trees via less vegetative competition for the systemic active ingredient, thereby improving tree protection against flatheaded borers. Consistently, the opposite pattern was observed, with weed-free trees being attacked by flatheaded borers more often (Fig. 2). Trees in weedy plots grew slower and were smaller than those in weed-free plots, and cultivars also varied in growth rates as expected (Tables 2–4). The greater tree mass volume in weed-free plots with faster growing trees could have diluted imidacloprid concentrations. Alternatively, applications of imidacloprid in weed-free plots could have had greater exposure to erosion weathering or chemical degrading sunlight, though this seems less likely given treatments were applied to the soil. Additionally, the higher trunk temperatures of weed-free trees (4°C) could have affected systemic chemical translocation (e.g., higher transpiration rates) or possibly the rate of active ingredient degradation by physiological processes inside tree tissues. Nevertheless, the differences in foliar concentration between herbicide (weed-free) and nonherbicide (weedy) treatment concentrations disappeared in years two and three.

The imidacloprid analysis technique used here is semiquantitative, so it is not as sensitive as a direct measure of imidacloprid by Liquid

Chromatography-Mass Spectrometry. However, it can be useful and more cost effective for this type of analysis where relative values of insecticide among treatments are more important than precise quantification. Since imidacloprid is translocated in the vascular system to the leaves, the relative values of imidacloprid in the leaf tissues also are likely to be directly related to the relative quantities of imidacloprid in the trunk tissues where flatheaded borer larvae are feeding. The ELISA kit method has been used previously to quantify imidacloprid concentrations in eastern hemlock (*Tsuga canadensis* [L.] Carrière) and ash (*Fraxinus* spp.) tissue (Eisenback et al. 2009, McCullough et al. 2011) with the caveat that the kits are more reliable when leaf tissue concentrations are higher. Eisenback et al. (2009) observed that matrix effects were more pronounced at concentrations in the lower working range of the kit, with recovery of 5 µg/liter imidacloprid being more accurate than recovery of 0.2 µg/liter. It is, therefore, possible that a more rigorous chemical analysis would have continued to show an effect of herbicide on imidacloprid concentrations in subsequent years. Increased scrutiny of imidacloprid metabolite toxicity toward flatheaded borers also might be warranted. The extended toxicity of imidacloprid toward hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), has been attributed to the olefin metabolite of imidacloprid present in the phloem of hemlock trees (Coots et al. 2013). Imidacloprid metabolites may also play a role in flatheaded borer toxicity over time but were not evaluated with the ELISA procedure in this study. Alternatively, the multi-year toxicity against flatheaded borers could be a result of time-cumulative toxicity, where long larval developmental periods of 1–2 yr and extended feeding on lower doses of imidacloprid may have the same effect as a short-interval exposure to a higher dose (Sánchez-Bayo and Tennekes 2020).

Table 5. Height range frequency of flatheaded borer damage on 'Franksred' red maple trees in the 2013 trial.

	Frequency of damage[a]			
Height range (cm)	No herbicide	Herbicide	$\chi^2_{(1)}$	*P*-value
0–20	0.17a	0.48a	3.99	0.04
21–40	0.25a	0.27b	0.02	0.89
41–60	0.33a	0.10c	5.47	0.02
61–80	0.17a	0.05c	2.34	0.13
81–100	0.08a	0.06c	0.17	0.68
101–120	0a	0.05c	0.99	0.32
$\chi^2_{(5)}$	8.29	87.66		
P-value	0.14	< 0.0001		

[a]Values within columns with different lowercase letters are statistically significant by Tukey's pair-wise comparison ($\alpha = 0.05$). For a list of herbicide treatments applied, see Table 1.

Toxicity of imidacloprid to adult flatheaded borers may be another indirect factor in the observed larval damage reductions. Leaves from *Fraxinus* trees treated with imidacloprid trunk injections or basal trunk sprays were toxic to adult emerald ash borer (*Agrilus planipennis* Fairmaire; Coleoptera: Buprestidae) in leaf feeding bioassays (McCullough et al. 2011). It is possible that adult flatheaded borers in this study also were poisoned by feeding on maple leaves with imidacloprid residues. However, unless the adults had a behavioral habit of leaf feeding and then ovipositing on the same tree, it seems unlikely that this would have been a factor in the subsequent larval infestations that were related to imidacloprid rate levels. Fenton (1942) also reported that adult *Chrysobothris* adults feed on the bark of new branch growth and not leaves on apple trees, and the low incidence of imidacloprid in phloem and bark (Mota-Sanchez et al. 2009) may limit adult exposure to at least the parent imidacloprid compound.

Flatheaded borer damage in the weed-free trees could have several causes. Damage by flatheaded borers begins most often near a bud union or other wound site on the trunk (LeBude and Adkins 2014) and is also more common on the southwestern side of the tree (Oliver et al. 2010, Seagraves et al. 2013). Herbicide damage to some nursery crops from postemergents such as glyphosate (Altland et al. 2003) and herbicide-associated injury such as bark cracking or reduced cold hardiness (Daniel et al. 2009) could increase susceptibility to flatheaded borers. However, no signs of herbicide damage (bark cracking or leaf distortion) were observed among test trees in the 2010 or 2013 trials. The other pre-emergent herbicides used in this study are considered safe for use in woody ornamentals if applied under directed spray (Altland et al. 2003). Therefore, we do not believe that herbicides were a significant factor in flatheaded borer damage observed in this study.

A likely explanation for the reduction in borer attacks on nonherbicide tree sites is that the weed vegetation altered the microclimate preferences of females for oviposition sites (Fig. 1). Flatheaded borer attacks are often concentrated on the sunny (south-southwest) side of the trees in the southeastern United States (Seagraves et al. 2013, Dawadi et al. 2019). The shading of the tree base by weeds also may be less suitable for postoviposition larval development. Trunk temperatures are up to 4°C cooler in trees shaded by a live cover crop compared with trees grown in bare rows (Dawadi et al. 2019). Winter cover crops grown in nursery tree rows and senescing naturally through early summer also reduced flatheaded borer damage over a 2-yr period, resulting in tree survival of 77, 98, or 99% for untreated, cover cropped, or imidacloprid-treated trees, respectively (Dawadi et al. 2019). In this study, the weed barrier around the lower trunk could have been a nuisance barrier to adult borers attempting to oviposit (Fig. 1). Height and compass direction of the borer hits also were higher on the tree

Table 6. Average (±SE) compass direction of flatheaded borer damage on 'Franksred' red maple trees in the 2013 trial

	Average damage direction ± SE[b]			
Herbicide[a]	First	Final	Mean damage location ± SE	Mean extent of damage ± SE
No	43.8° ± 16.5	262.5° ± 45.2	153.1° ± 18.4	263.8° ± 36.4
Yes	154.6° ± 10.8	248.1° ± 12.7	201.4° ± 6.6	166.1° ± 11.9
F-value, df=1	11.0	0.08	5.56	6.43
P-value	0.0015	0.86	0.02	0.01

[a]For list of herbicide treatments, see Table 1.

[b]Damage was recorded in a clockwise direction from first sign of damage (= 'First') to the final sign of damage (= 'Final').

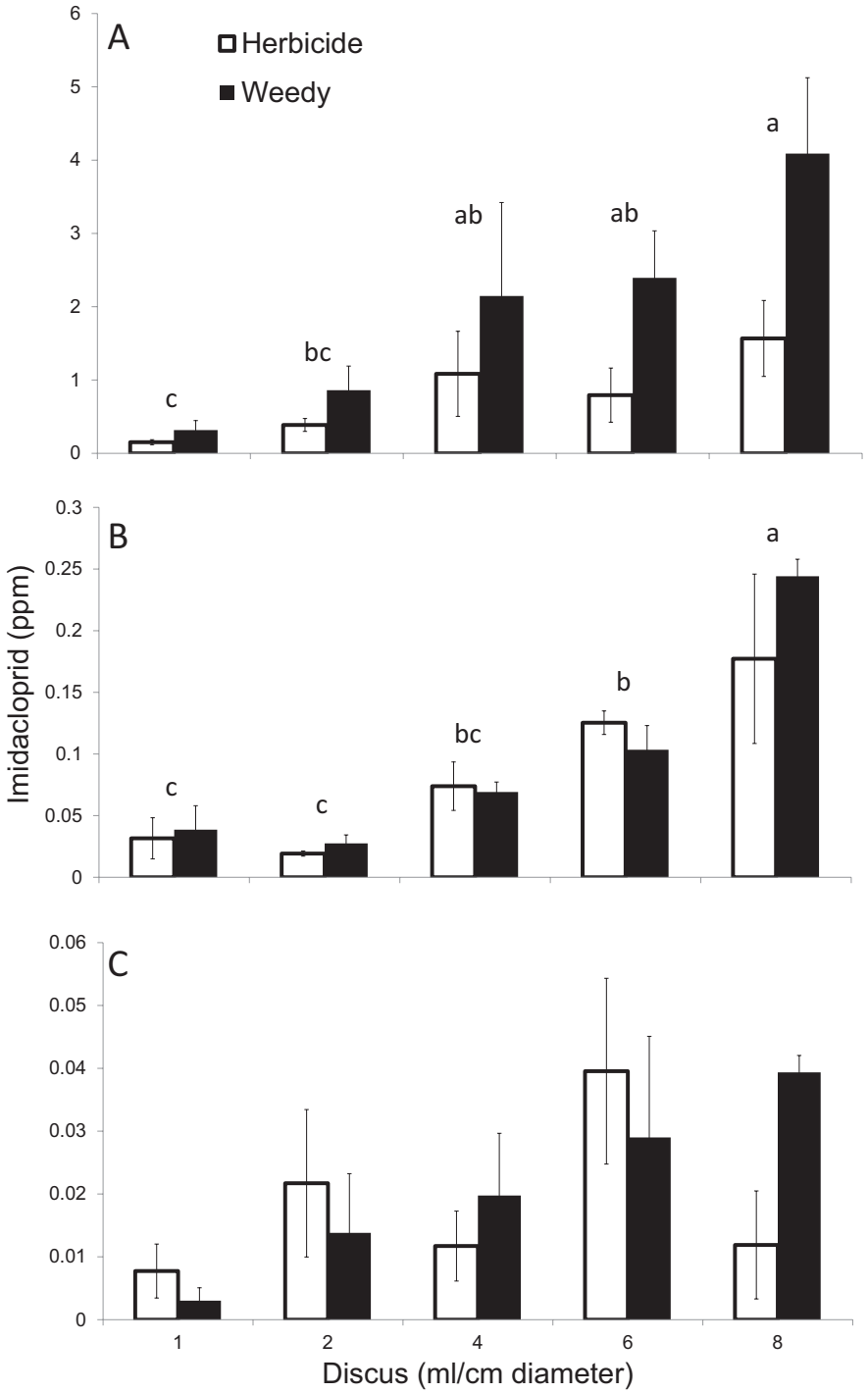

Fig. 3. Semi-quantitative ELISA analysis of imidacloprid parts per million (ppm) in maple leaf tissue in September (A) 2013, (B) 2014, and (C) 2015. Imidacloprid treatment levels with different letters are statistically different by Tukey's pair-wise comparison ($\alpha = 0.05$).

trunks in the weedy plots and were often above the height of the weeds, which may indicate modifications of female oviposition preferences. Adult *Chrysobothris* borers prefer the sunny side of the tree for mating, female movement, and oviposition choice (Brooks 1919, Fenton and Maxwell 1937). Brooks (1919) reported that flatheaded borer oviposition could be prevented on trees by shading the trunks with low-headed branches or placement of a 15-cm wide board on the sunny-side of the tree. Consequently, weedy tree sites also may disrupt mating and oviposition behavior of adult beetles and possibly postoviposition growth and survival of larvae.

Clearly, more work is needed to understand what characteristic of the weeds and cover crop are responsible for preventing flatheaded borer attacks. Additional possible causes of reduced flatheaded borer damage in weedy plots may include trunk camouflage, adult borer hindrance, and increased predation risks to adult or larval borers. Preliminary results of a new study looking at early

kill of cover crops to reduce competition with trees suggests that the dead cover crop is not as effective at reducing flatheaded borer attacks as the live cover crop (Addesso, unpublished data). A better understanding of how weeds and cover crops reduce borer attacks can aid in developing more effective barrier methods for flatheaded borer management. A previous trial using commercially available tree guards was unsuccessful in protecting newly transplanted trees from attack by flatheaded borers (Fare et al. 2018). These two additional studies suggest that a physical barrier alone (tree guard or dead cover crop) are not sufficient to protect trees from borer attacks and other factors such as allelopathic effects on larval or adult borers or chemically mediated changes in adult oviposition behavior may be involved. Experiments to tease apart the different possible factors influencing flatheaded borer damage levels are ongoing.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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