Activity and Residues of Imidacloprid Applied to Soil and Tree Trunks to Control Hemlock Woolly Adelgid (Hemiptera: Adelgidae) in Forests

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ABSTRACT We studied imidacloprid application methods and timing to control the hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), in forests. The methods compared were 1) soil injection near the trunk; 2) soil injection dispersed throughout the area under the canopy; 3) soil drench near the base of the trunk; and trunk injection with the 4) Arborjet, 5) Wedgle, and 6) Mauget systems. The applications were made in the fall and the following spring. Adelgid populations on the hemlocks (*Tsuga* spp.) were assessed in the fall of two successive years after the treatments. Relative to the untreated control trees, all the soil applications resulted in population reductions, but none of the trunk injections resulted in reductions. Fall and spring treatment efficacy did not differ. Reductions by the soil treatments were between 50 and 100% (avg 80%) by the first fall and 83–100% (avg 98.5%) by the second fall. Analysis of imidacloprid residues using enzyme-linked immunosorbent assay found residues in sap, needles, and twigs 1 mo to 3-yr after application. A laboratory dose–response bioassay using excised, adelgid-infested hemlock branches with cut ends immersed in serial dilutions of imidacloprid determined the LC_{50} value to be 300 ppb, based on an exposure of 20 d. A high degree of suppression of the adelgid on forest trees was associated with residues in hemlock tissue >120 ppb 2 yr after soil treatment. Although precise relationships between residues and efficacy are elusive, it is clear that soil application of imidacloprid resulted in chronic residues of imidacloprid in tissues and suppression of adelgid populations for >2 yr.

KEY WORDS systemic, insecticide, *Adelges tsugae*

Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), threatens the health of hemlock (*Tsuga* spp.) forests in eastern North America. In contrast with the species of hemlocks in Asia and western North America, where the adelgid is endemic (Havill et al. 2006), the species native to eastern North America, eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *T. caroliniana* Engelmann, are intolerant of adelgid feeding. Adelgid densities more than four per 2-cm length of twig inhibit the production of new foliage and cause progressive discoloration of needles and death of branches (McClure 1991b). Hemlocks play a unique and crucial role in the ecology of forests in the eastern United States. They are a shade-tolerant and long-lived species, whose dense evergreen canopy provides a preferred habitat for many species of birds and mammals (Ward et al. 2004). They are also an important riparian species; their shade cools streams and improves habitat for fish (Evans 2002, Snyder et al. 2004).

Long-term sustainable management of *A. tsugae* in forests requires establishment of natural systems to restore the balance between hemlocks and adelgids, such as classical biological control and host plant resistance (Bentz et al. 2002, Cheah et al. 2004, Del Tredici and Kitajima 2004). Until a sustainable solution to this pest problem is realized, forest managers need options to provide impermanent protection of hemlock trees. Certain hemlock stands may be especially important to protect for their ecological value, as a genetic resource, (especially important for *T. caroliniana*), or for the hazard their death would cause (e.g., hemlocks are often associated with high public use areas in parks such as picnic areas and trails). For these stands, the benefits of maintaining the hemlock trees need to be weighed against the environmental and monetary costs of intervention with insecticides.

The hemlock woolly adelgid can be controlled in landscape trees with foliar sprays and systemic insecticides (McClure 1991a; Steward and Horner 1994; Cowles and Cheah 2002a,b; Doccola et al. 2003; Webb et al. 2003). Treating hemlock trees in forests, however, is a difficult proposition. Among the constraints

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Table 1. Name and location of forests in Connecticut used as study sites

Site	Town	Location	dbh (cm, mean ± SD)[a]
Mashantucket Pequot	N. Stonington	41.466° N 71.948° W	28.4 ± 12.4
Nathan Hale State Forest	Coventry	41.767° N 72.354° W	35.3 ± 14.2
Sequassen Boy Scout Camp	New Hartford	41.871° N 73.050° W	33.5 ± 7.9
Shenipsit State Forest (A)	Somers	41.963° N 72.403° W	28.2 ± 5.6
Shenipsit State Forest (B)	Somers	41.967° N 72.398° W	31.2 ± 3.8
Tunxis State Forest	East Hartland	42.002° N 72.897° W	36.6 ± 14.7

[a] Diameter at breast ht (1.5 m).

are the operational difficulty of bringing application equipment into a forest and the environmental risks. For example, the most ecologically valued trees may be adjacent to streams, and all insecticides effective against adelgids are toxic to aquatic organisms. Furthermore, any insecticide used for control of adelgids should be chosen to minimize the potential impact on its natural enemies.

Previous work has shown imidacloprid to have excellent activity for controlling hemlock woolly adelgid in landscapes (Steward and Horner 1994, Cowles and Cheah 2002b, Doccola et al. 2003, Webb et al. 2003). When applied as a systemic, either into the soil for absorption by the roots or into the trunk, imidacloprid can be translocated throughout the tree (Tattar et al. 1998). Thus, it theoretically could reach the entire population of adelgids on individual trees. Because mature hemlocks in forests typically are taller than 30 m (Godman and Lancaster 1990), reaching the crown with pesticide sprays is impractical. Placing a systemic insecticide inside the tissues of the trees should minimize the exposure of nontarget organisms, including wildlife and adelgid predators.

Among the many unanswered questions regarding application of imidacloprid for control of homopteran insect pests in trees, we address three: 1) What is the most efficient spatial placement in the soil for a systemic insecticide? 2) Is the several years of control after a single soil application previously observed (Cowles and Cheah 2002b) because of continued presence of imidacloprid within the tree or to very slow recolonization? 3) Does trunk injection of imidacloprid result in adequate mobilization of imidacloprid and control of the adelgid?

Analysis of imidacloprid residues in sap and tissues would help answer questions 2 and 3. However, the standard techniques used to quantify most other insecticides, gas–liquid chromatography and high-performance liquid chromatography, are difficult and costly for imidacloprid because of its moderate polarity and its biological activity at concentrations on the order of parts per billion (Placke 1994, Nauen et al. 1998, Nauen et al. 1999, Fernández-Alba et al. 2000). Commercially available enzyme-linked immunosorption assay (ELISA) kits for imidacloprid are an effective, rapid, and relatively inexpensive method to analyze residues from plant sap and have been suggested for analysis of plant tissue extracts (Li and Li 2000, Lee et al. 2001, Watanabe et al. 2004, Byrne et al. 2005).

Recently, several manufacturers have developed specialized equipment and proprietary formulations for microinjection of imidacloprid into tree trunks. Thus, we designed an experiment to examine application methods and timing by using various formulations of imidacloprid to control *A. tsugae* in forests. Posttreatment assessment of adelgid populations was coupled with analysis of imidacloprid residues within hemlock sap and tissues. A bioassay of adelgid mortality on excised hemlock branches immersed in imidacloprid solutions in the laboratory was used to determine lethal concentrations after short-term exposure.

Materials and Methods

Experimental Design for Forest Experiment. The application methods compared were 1) soil injection placed near the base of the trunk; 2) soil injection dispersed in the area beneath the tree canopy; 3) soil drench near the base of the trunk; and trunk injection with the 4) Arborjet, 5) Wedgle, and 6) Mauget systems. These treatments, along with an untreated control, were applied in the spring and fall at six sites (=blocks) in a 7 by 2 factorial randomized complete block design.

Selection of the six sites (Table 1) for the study was based on the presence of moderate populations of *A. tsugae* during late summer 2002, the availability of branches that could be reached with a pole pruner, and >50-m separation between 14 suitable trees in each site. We avoided high populations of the adelgid. Adelgid populations are known to be self-regulating and high populations crash through a feedback mechanism caused by reduced suitability and limited new growth of heavily infested terminal shoots (McClure 1991b). Each tree chosen for this study was marked with a permanent aluminum tag and its coordinates recorded by a global positioning system to help locate the tree at later dates.

Insecticide Application. Insecticides were applied between 1 and 29 October 2002 and between 28 May and 6 June 2003.

Two soil treatments used the Kioritz applicator (Kioritz Corp., Tokyo, Japan) for subsurface soil injection of Merit 75 WP insecticide (Bayer, Kansas City, MO) diluted to provide 1 g of active ingredient (AI) in 60 ml of water per 2.5-cm diameter at breast height (dbh). This was mid-range for the labeled treatment dosages of 0.7–1.4 g (AI)/2.5 cm dbh. One method, designated as "near trunk," placed the dosage within 45 cm of the base of the trunk of the tree with individual injections of 30 ml or less, spaced evenly around the trunk. The other method, designated as "under canopy" distributed the injections in a grid pattern beneath the tree's canopy (from the trunk to the dripline). The footplate of the Kioritz applicator was adjusted to place the injection orifices 6 cm below the soil surface. Hemlock has many fine roots near the soil surface; so shallow subsurface placement gives maximum opportunity for the trees' roots to intercept the insecticide.

The third soil application method used a formulation developed for homeowners, Bayer Advanced Garden Tree and Shrub Insect Control (Bayer Advanced LLC, Birmingham, AL). The treatment dosage was reduced 37% from the label instructions to provide the same 1 g (AI)/2.5 cm dbh dosage (68 ml of product) used in the soil injection treatments. The amount of product needed for a tree was diluted in 3.8 liters of water and drenched around the trunk of the tree outward to a distance of 45 cm.

Three treatments were microinjections into the tree trunk, each using a formulation of imidacloprid, equipment, and protocols developed by a manufacturer

The Mauget System (J. J. Mauget Co., Arcadia, CA), consisted of 3 ml of Imicide, a 10% formulation of imidacloprid in a capsule with a feeder tube. One capsule was used per 15-cm circumference (=0.15 [AI]/2.5-cm dbh). Holes (4.4 mm in diameter) were drilled 1 cm into the xylem at the root flares of the tree, 15 cm above the soil surface. Feeder tubes were inserted into the tree, pressurized capsules placed onto the feeder tubes, and the internal seal broken to allow the insecticide solution to flow into the tree. The Mauget System allows visual monitoring of uptake of the formulated product into the tree. If a capsule did not empty within 3 h, it was removed. Any remaining material was lost onto the bark of the tree; hence, it was not possible to measure partial uptake.

The Wedgle Direct-Inject Tree Treatment System (ArborSystems, Omaha, NE) uses Pointer insecticide applied at a dosage of 1-ml of 12% formulation for every 10-cm circumference (=0.09 [AI]/2.5-cm dbh), at a height of 80 cm above the soil surface. The standard procedure is to remove a plug of bark to the depth of the cambium and replace it with a plastic plug to retain injected liquid. The device's needle is then inserted through the plastic plug to the xylem. Injected liquid causes separation of the bark from the sapwood, forming a reservoir for the insecticide. During the fall application, the injection needle plugged on several occasions. Although the Wedgle device has settings for injecting 0.5 or 1 ml, there was no method to confirm the amount of liquid dispensed into the tree. Some trees may have been injected with little or no insecticide. During the spring application, after removing the plug of bark, a 2.8-mm-diameter hole was drilled 1 cm in depth into the xylem. The plastic plug was installed as described above, and the needle was inserted through the plug into the smaller diameter hole. Application this way resulted in observable separation of the bark at the cambium layer, unlike the fall application. The amount of product injected into each tree was measured by weighing the insecticide reservoir bottle before and after application with a portable electronic centigram balance. Calibration marks on the Wedgle device did not correctly represent the volume of liquid injected into the tree, and four additional pressurizations per injection site were used to deliver the dosage.

The Arborjet VIPER system (Arborjet, Winchester, MA) was used to apply a 6-ml dose of Ima-Jet, a 5% imidacloprid formulation, for every 24-cm circumference (0.1 g [AI]/2.5-cm dbh). For this device, a 0.74-cm-diameter hole is drilled 1.5 cm in depth into the sapwood. The hole is plugged with a brass or plastic plug containing a septum. The needle of the device is inserted through the septum, and liquid is forced under pressure into the sapwood of the tree (Doccola et al. 2003). Placement of injection sites was the same as with the Mauget system, on the root flares 15 cm above the soil. The VIPER hydraulic device has a pressure gauge and a reservoir calibrated in milliliters attached to the injection needle that informs the operator the rate the product is moving into the tree as well as the volume injected.

Adelgid Population Assessment. Cold temperatures during the 2002–2003 winter resulted in mortality at study sites in nearby untreated trees of 85–95% (C.A.S.-J.C., unpublished data). Therefore, mortality was not evaluated for the overwintering, sistens generation but delayed until 7–15 July 2003 when the progrediens generation had developed. In July, shoots with adelgids were brought back to the laboratory in a cooler and evaluated under a dissecting microscope. Percentage of mortality was determined by examining and probing (to see whether there was movement of legs or mouthparts) 100 adelgids in each tree sample. These data were analyzed after arcsine square root transformation of the percentage of mortality data.

Adelgid populations were assessed in late November 2003 and in December 2004 by counting the developing nymphs in the field. During late fall and early winter, it is possible to quantify adelgid populations in the field relatively quickly using the unaided eye and some confirmation with a hand lens. In the fall, the sistens generation of adelgids breaks aestivation, and the nymphs resume development, producing characteristic woolly wax as they grow. Counting the number of woolly tufts at this time is a reliable, easy way to determine the number of live adelgids. Ten samples (branches) were cut from each tree for counts of live adelgids: five branches from the canopy reachable from the ground (<3 m) and five branches at a height of 7–10 m, by using a pole pruner. Adelgids were

counted on the terminal 25 cm of each branch, including all branchlets, up to a total of 10 adelgids. The total for the 10 samples constituted a 0–100 adelgid rating for each tree. This rating method was used because it permits rapid assessment of adelgid populations and reduces skew from the very few branches with high counts of adelgids, improving the sensitivity of population assessments relative to adelgid counts (R.S.C., unpublished data). Data were square root transformed as necessary to establish homogeneity of variance and analyzed as a randomized complete block factorial design with Statistix 8 (Analytical Software, Tallahassee, FL) as a model consisting of the six sites as blocks, the seven treatments and two application seasons as factors, and interactions between factors.

Imidacloprid Concentration in Sap and Plant Tissues. We measured imidacloprid residues with a commercially available ELISA kit (EnviroLogix 2003, Byrne et al. 2005). This kit uses a competitive assay to determine imidacloprid in samples at concentrations of 0.2–5 ppb. Each well received a 100-μl aliquot of sample, initially undiluted. If the concentration of imidacloprid was found to be >5 ppb, the remaining sample was diluted 1:10, 1:100, and 1:1000 and reanalyzed successively until the concentration was within the range of the standards. The sample and conjugate were premixed in a 96-well plastic tray; and an eight-channel pipette was used to transfer samples and reagents to the ELISA plate. Three sets of imidacloprid standards (0, 0.2, 1, and 5 ppb) were placed at the diagonal corners and the center of each 96-well plate. The plate was shielded from temperature gradients during incubation by placing it in an insulated box.

To read the ELISA plates, two methods were used. One method measured absorbance at 450 nm with a 96-well plate reader (Titertek Multiskan model 310C, Eflab Oy, Helsinki, Finland). The second method created an image of the plate on a trans-illuminated flatbed scanner (Epson Perfection 1650, fitted with Film Adapter model EU-33, Seiko Epson, Nagano, Japan), and the intensity of the blue color in each well (inversely proportional to the observable yellow color) determined from the digital image with SigmaScan Image software (SPSS, Inc., Chicago, IL). Standard curves were graphed using SigmaPlot software (SPSS, Inc.) to provide a linear regression with log of the concentration versus the optical density measurements from the standards. The regression parameters of slope and intercept were then used to calculate the concentration of unknowns. Measurements of color intensity generated with digital images of ELISA plates required log transformation on both axes (imidacloprid concentration versus color intensity) to generate a linear standard curve from which concentrations of unknowns could be calculated.

Residues in sap were analyzed from single branches of 45-cm length cut from all study trees 2 to 3 m from the ground on 7–15 July and 20–27 August 2003. Sap was extracted at the field site by using a custom-built 25- by 7.5-cm-diameter hyperbaric chamber (Gregory 1966). The cut end (<5 mm in diameter) of each branch was inserted through a rubber gasket, and the entire branch was carefully folded, without breaking, to fit within the chamber. The chamber was gradually pressurized with nitrogen up to 1,400 kPa. Sap (200–700 μl) was collected with a micropipette, placed in a 1.5-ml microcentrifuge tube in an ice chest, taken to the laboratory, and kept at −20°C until analyzed. Sap samples required no additional cleanup before analysis.

Residues were analyzed in needles and twigs from the same branches used for sap analysis in August 2003 and from branches cut 13–26 November 2003 and 8–20 April 2005. The 2003 samples were dried overnight at 35°C, the needles were separated from twigs, and these parts were pulverized separately using an inexpensive grinder (Mr. Coffee, model IDS55, Cleveland, OH). In 2005, samples consisted of eight branches from each tree. The branches from each tree were dissected immediately into 2003 and 2004 growth age classes. The twigs and needles were not separated, but each age class was dried and pulverized separately. For both years, a sample (1.000 g) of pulverized tissue was added to 10.00 ml of histological grade acetone in a 60-ml environmental sample vial and shaken horizontally overnight (2 cycles/s). After allowing particulate matter to settle (≈1 h), a 1.000-ml aliquot was converted to an aqueous suspension by allowing the acetone to evaporate and vortexing the residue in 1.000 ml of distilled water. The sample was then frozen until analysis. To facilitate comparison with sap concentrations, the assay values for tissues were converted to ppb of their fresh weight equivalent. Because of the 60% moisture content of fresh versus oven-dried samples, there were 2.5 g of fresh tissue equivalent extracted in 10 ml of solvent, or a four-fold dilution, requiring a multiplicative correction factor of 4. The concentrations reported throughout this article have been converted to the estimated fresh tissue concentration. Imidacloprid concentration data required square root or log transformation to establish homogeneity of variance.

Laboratory Dose Response. The short-term response of hemlock woolly adelgid to imidacloprid was determined with a dose–response test conducted in the laboratory. Imidacloprid solutions of 0.01, 0.1, 1, 10, and 100 ppm were prepared by diluting Bayer Advanced Garden Tree & Shrub Insect Control in distilled water. Hemlock branches cut from heavily infested hemlocks from Breininger Gap, Union Co., Pennsylvania, on 10 January 2005 were shipped overnight to Windsor, CT. Upon receipt, the branch ends were recut and placed in water. One day later, branches were cut at 25-cm length from the branch terminus, and the cut ends were placed in 30–40 ml of the imidacloprid solutions or distilled water (untreated check) held in 60-ml glass vials. Each concentration was replicated with eight branches. Each vial was replenished with the same concentration of imidacloprid, so that branches always had liquid. To prevent desiccation, the branches were partially covered with a transparent plastic bag, allowing gas exchange around the base of the vials. The experiment was

conducted with diffuse natural lighting at temperatures of 12.9 ± 2.6°C (mean ± SD, range 8.2–19.4°C).

After 19–21 d, the adelgid mortality was determined by observing them with a dissecting microscope under 20–40× magnification while probing with an insect pin to detect movement of legs or mouthparts. A sample of 30 adelgids was assessed from each branch. Data were corrected for mortality in the untreated control by using the method of Abbott (1925), and the data were analyzed graphically with SigmaPlot by using a log-concentration versus probability mortality and linear regression.

Results and Discussion

Adelgid Populations on Treated Trees. Site variability and natural mortality affected adelgid survival and obscured insecticide treatment effects in the July 2003 assessment. Site effects on mortality were highly significant ($F = 6.26$; df = 5, 65; $P = 0.0001$), with the greatest mortality at the Mashantucket Pequot site, where the tree vigor was poor, and in the northwestern hills (Camp Sequassen and Tunxis State Forest). Mortality averaged over all treatments for these sites was 90, 82, and 75%, respectively. Mortality at the remaining sites (Shenipsit and Nathan Hale State Forests) was 65 and 56%, respectively. Adelgid mortality ranged from an average of 64% for the Wedgle-treated trees to 80% for the Kioritz, near trunk imidacloprid placement. Adelgids in the untreated check trees experienced 67% mortality. Although method of application and timing effects were not significant overall, among soil injection treatments the fall application caused more mortality than the spring application (85 versus 72% mortality, respectively; $P < 0.02$), and all soil applications caused significantly greater mortality (79%) than the untreated check (contrast $F = 4.33$; df = 1, 65; $P < 0.04$).

The adelgid populations in November 2003, 13 and 9 mo after the fall and spring applications of imidacloprid, respectively, were dramatically reduced on the hemlocks treated with soil applications of imidacloprid, but those on hemlocks receiving trunk injections were no different than the populations on untreated hemlocks (Fig. 1A). Application timing (fall 2002 or spring 2003) and the timing × application method interaction were not significant ($F = 3.06$; df = 1, 65; $P = 0.08$ and $F = 0.13$; df = 6, 65; $P = 0.99$, respectively). The rating of adelgid populations on hemlocks receiving soil treatments ranged from 0 to 8, but the soil treatments were not significantly different from one another. Although the rating of adelgids on the hemlocks treated by trunk injection of imidacloprid were not significantly different than the untreated trees, there were some differences within this group; trees treated with the Wedgle had significantly

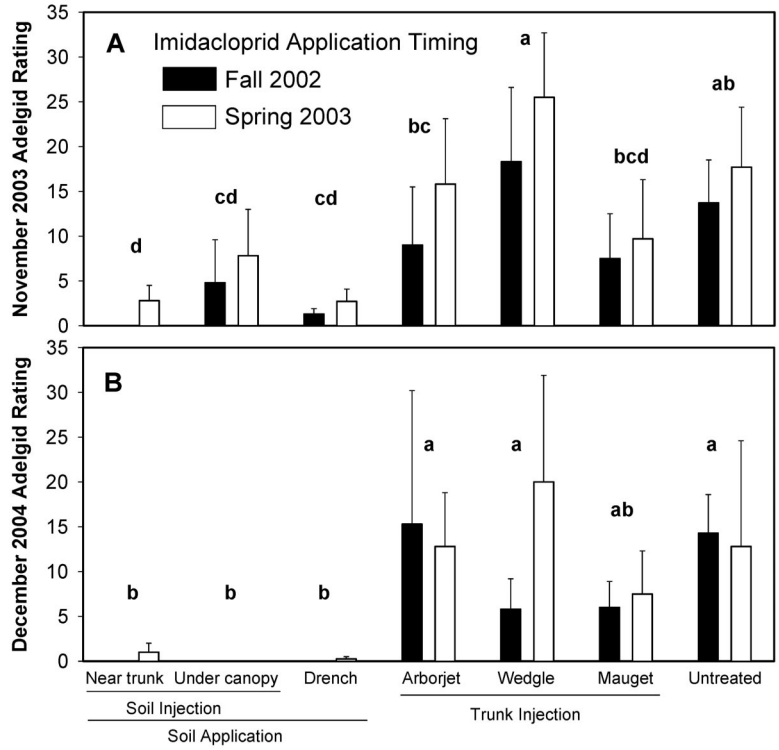

Fig. 1. Hemlock woolly adelgid population ratings: (A) November 2003. (B) December 2004. Up to 10 adelgids were counted from each of 10 30-cm shoots taken from each tree. The adelgid rating is the total from all 10 shoots. Method of application was highly significant ($P < 0.001$), but timing was not significant. Method main effects followed by the same letter are not significantly different (Student–Newman–Keuls test; $P < 0.05$).

Table 2. Imidacloprid concentration (mean ± SE) in hemlock sap, needle, and twigs determined by ELISA

Treatment	Imidacloprid concn (ppb)[a]				
	July 2003 Sap	Aug. 2003 Sap	Aug. 2003 Needle	Aug. 2003 Twig	Nov. 2003 Twig
Soil injection					
Kioritz, near trunk	4.7 ± 0.9b	8.3 ± 2.7a	52 ± 16	33 ± 10	87 ± 14bc
Kioritz, drip line in	6.3 ± 1.8b	5.0 ± 1.0abc	37 ± 10	20 ± 5.6	84 ± 14bc
Homeowner drench	4.0 ± 0.7b	5.5 ± 2.7ab	31 ± 12	26 ± 6.4	89 ± 31bc
Trunk injection					
Arborjet	3.3 ± 1.1b	2.0 ± 0.5bc	32 ± 20	44 ± 32	162 ± 35a
Mauget	37 ± 23a	1.8 ± 0.5bc	220 ± 190	64 ± 30	98 ± 18b
Wedgle	2.5 ± 0.5b	1.0 ± 0.3c	6.9 ± 1.2	17 ± 7.2	40 ± 10b
Untreated check	2.0 ± 0.4b	1.5 ± 0.1bc	8.1 ± 1.2	8.0 ± 1.1	24 ± 4.4c
Significance (P value)[b]	0.04	0.008	0.43	0.34	0.0005

Means ($n = 12$) within columns followed by the same letter are not significantly different (LSD test; $P = 0.05$).
[a] Column headings refer to the date that samples were collected and the type of sample.
[b] Statistical significance (P values) are based on the factorial analysis of variance.

more adelgids than trees treated with the Arborjet or Mauget ($P < 0.05$).

The December 2004 adelgid ratings showed the same patterns observed the previous year, although the ratings were lower overall (Fig. 1B). All soil treatments had significantly lower populations than the untreated check, with four of the six soil treatment combinations having no adelgids. Trunk injection treatments were not significantly different from the untreated check trees. However, the Mauget treatment continued to have adelgid populations that were intermediate between the untreated check and the soil applications. Time of application and its interaction with application method again were not significant.

Imidacloprid Concentration in Sap and Plant Tissues. Measurements of sap concentrations were highly variable (Table 2), possibly because of nonuniform distribution and using a single branch to collect sap (a logistical necessity because of the sap extraction method). In July, only the trunk injection by using the Mauget system was significantly different from the untreated check. Two of these trees had high concentrations of imidacloprid (180 and 230 ppb) in the sap; however, the August sap concentration from those trees had diminished to background levels. In August, the Kioritz near the trunk was the only treatment with imidacloprid residues significantly higher than the untreated check. At that date, the sap concentration for trunk-injected trees was consistently low.

The number of trees with detectable imidacloprid was assessed by comparing concentrations from individual trees with the background from the untreated checks. A threshold of the maximum measured from the untreated group, plus twice that group's standard error, was used as the critical threshold to define a positive detection. For trunk injection treatments, only one to three trees (of 12) per treatment method group had detectable imidacloprid present in the sap in either the July or August sap samples. In contrast, there were six to nine trees with positive detections from each soil-based treatment method.

Table 3. Imidacloprid concentration (mean ± SE) from 2003 and 2004 hemlock growth determined by ELISA from combined needles and twigs collected in 2005

Treatment	Imidacloprid concn (ppb)			
	Fall 2002 application		Spring 2003 application	
	2003	2004	2003	2004
Soil injection				
Kioritz, near trunk	140 ± 16	95 ± 18	220 ± 70	250 ± 120
Kioritz, under canopy	150 ± 51	120 ± 33	180 ± 46	170 ± 42
Homeowner drench	90 ± 16	100 ± 17	240 ± 91	240 ± 130
Trunk injection				
Arborjet	87 ± 20	97 ± 39	170± 72	220 ± 160
Mauget	250 ± 110	120 ± 30	68 ± 18	42 ± 13
Wedgle	20 ± 3.6	17 ± 3.7	64 ± 16	56 ± 19
Untreated check	22 ± 3.2	16 ± 3.2	24 ± 6.8	20 ± 4.2

Source[a]	df	MS	F	P
Block	5	0.2878		
Method	6	1.3751	14.72	0.0000
Timing	1	0.2564	2.74	0.1024
Method × timing	6	0.2302	2.46	0.0329
Error	65	0.0934		

[a] Analysis of variance is based on the log of the concentration averaged for the 2003 and 2004 tissues from the same tree.

The August 2003 sampling (Table 2) determined residues in sap, needles, and twigs from the same branch; thus, it was possible to examine correlation of the residues in these tissues. Needle concentration was significantly but weakly correlated with sap values ($r = 0.37$, $P = 0.006$), whereas twig concentration was not ($r = 0.18$, $P = 0.10$). The highest correlation existed between twig and needle concentrations ($r = 0.66$, $P < 0.0001$). Overall, twigs had an average of 1.4 times the concentration found in needles.

Sap could not be extracted from all the experimental trees in November, so measurements were made only on pulverized twigs. In November, the residues in all the trunk injection treatments were significantly higher than the untreated check, whereas none of the soil injection treatments were higher. The November twig residues were overall ≈3 to 4 times higher with less within-treatment variation than the August twig residues. The reduction in variation is probably because of the use of composite samples. The increase in tissue concentrations is difficult to explain. The increase may partly be due to sampling from a greater height within the tree canopy or partly due to back-calculation of tissue concentration after the 1:10 dilution required for analyzing all these and subsequent samples (see below).

In 2005, residues were again examined in the trees, 22–31 mo after treatment, and foliage was divided into 2003 and 2004 growth (Table 3). There was a strong correlation ($r = 0.90$, $P < 0.0001$) between the 2003 and 2004 residues taken from the same branches; treatment method and timing did not influence this tight correlation. The residues from the 2003 and 2004 foliage revealed effects of application timing (revealed as significant interactions) as well as application method (Table 3). All soil-based applications had a remarkably similar pattern for tissue concentrations, so we conclude that there are no differences among dispersion patterns (near trunk versus trunk to drip line) regarding efficiency of imidacloprid uptake by hemlock roots. The tissue concentrations were consistently greater for spring than for fall soil applications, with averages of 220 versus 120 ppb, respectively, averaged over soil application methods ($P < 0.02$ for timing main effect for soil applications). The lack of difference in the biological effectiveness is probably because of the excellent efficacy of all the soil applications. Treatment timing effects on imidacloprid concentration were observed for the Wedgle trunk injection, but the higher values observed with the spring applications ($P = 0.04$) could be due to having increased the application dosage and from having drilled into the xylem to prevent plugging of the injection needle. Treatment timing effects for the Arborjet and Mauget systems were not significant ($P = 0.3$ and 0.12, respectively).

Remarkably, the tissue concentrations determined from November 2003 samples (Table 2) were found to be similar to those found from the 2003 and 2004 growth when these tissues were collected from trees in 2005 (Table 3). This suggests that imidacloprid (or metabolites also detected by ELISA) may be stable, once protected within the tissues of trees. The concentrations found in 2004 tissues were equivalent to those in the previous year's growth, demonstrating that imidacloprid is mobilized from within the tree and translocated to new growth. That the additional growth did not dilute the imidacloprid during this period and that the imidacloprid concentration seems to have continued to increase among the soil-based treatments, suggests that 1) the entire living portion of the tree, and perhaps the xylem, could be storing imidacloprid and from which it is remobilized; and 2) additional imidacloprid may continue to be extracted from the soil by the roots for a year or more after the application.

All soil treated trees with 2005 residue concentrations >122 ppb had no adelgids on them in fall 2004. The relationship between tissue concentrations of imidacloprid in the Arborjet- and Mauget-injected trees (Tables 2 and 3) versus the degree of control (Fig. 1) is not as clear. The concentrations of imidacloprid found in some of the trunk-injected trees was similar to those treated via soil injection, and yet the degree of suppression of adelgids was inferior. Several explanations are possible. Trunk injection may lead to less uniform distribution of imidacloprid, and the population of adelgids observed could be due to the insecticide not reaching every branch. This hypothesis is bolstered by the earlier sap residue analysis: fewer branches of trunk injected trees were found with detectable imidacloprid, even though there were a few branches with exceptionally high sap concentrations. The composite sample of branches used to establish tissue concentration of imidacloprid could have obscured uneven distribution. However, injection at the root flare on hemlocks is known to result in initial upward and downward movement of solutes in sap (Tattar and Tattar 1999). An insecticide injected at the root flare would likely be distributed evenly after distribution throughout the root xylem tissues. Another possible explanation is that imidacloprid may be converted into more active, synergistic, or mobile compounds when applied to the soil, rather than when injected directly into the xylem. Previous studies have determined that imidacloprid is readily metabolized into other compounds with these properties when applied to the soil and taken up by herbaceous plants, whereas these metabolites are not as evident when applied to the foliage (Nauen et al. 1998).

The commercial ELISA kits were found to have some limitations, but they were generally useful for approximating the concentration of imidacloprid in sap and plant tissues. Byrne et al. (2005) discovered matrix effects, apparent nonspecific binding causing a false positive response that disappears upon sufficient dilution of samples. Matrix effects also were observed in this study; however, the degree of dilution required to eliminate matrix effects was not systematically studied as was done by Byrne et al. (2005). In general, sap samples from hemlocks were only diluted when determinations were found to be in excess of 5 ppb, the upper quantification limit. However, samples prepared by extraction from tissues were always diluted

at least 1:10 before analysis, as experience quickly demonstrated this was necessary for obtaining consistent readings. The need to dilute samples before analysis correspondingly increases the minimum concentration that can be quantified from samples. The 1:10 dilution necessary for tissue samples (this study) had the effect of increasing the limit of detectable imidacloprid concentrations to ≈8 ppb. The higher limit of detection and possible incomplete elimination of matrix effects are probably responsible for the high background seen for the untreated checks, particularly for the tissue samples (Tables 2 and 3). Furthermore, the ELISA method results have to be considered as semiquantitative for imidacloprid because some of its metabolites also are detected. Although they bind less than the parent compound, the "imidacloprid" concentration determined by this method must incorporate the signal contributed by imidacloprid metabolites (EnviroLogix 2003). Because its metabolites are less readily detected than imidacloprid, the ELISA is more selective for quantifying imidacloprid than the Placke and Weber (1983) total method, which converts all the metabolites and imidacloprid into one product that can be analyzed by gas chromatography. The ELISA method provided useful semiquantitative results, was relatively inexpensive, and was adaptable for analysis of small quantities (1-g samples) of needle and twig tissues; however, the inability of this method to answer questions regarding the concentrations of parent compound versus imidacloprid metabolites leaves unexplained the disparity between suppression of adelgids with trunk versus soil applications of imidacloprid.

Laboratory Dose Response. There was an average of 28% mortality of adelgids within the untreated checks. However, after Abbott's correction for mortality (Abbott 1925) in the untreated checks, there was excellent fit to a linear model for probability (mortality) versus log (concentration) (Fig. 2). The exception was 100,000 ppb (100 ppm), which was treated as an outlier. The LC_{50} and its 95% confidence limit (CL) were graphically estimated to be 300 and 150–600 ppb, respectively.

The poor efficacy of the 100 ppm solution may have been due to incomplete solubility. This concentration seemed cloudy, indicating a suspension rather than a complete solution of imidacloprid. Microscopic particles of imidacloprid or other formulation components could have clogged the tracheids of the vascular system and reduced the total quantity of imidacloprid being moved into the branch.

The elevated LC_{50} estimate of 300 ppb found in the laboratory experiment, compared with the field-observed long-term lethal dosage of ≈120 ppb, requires explanation. The short-term study investigated the response in late instar or mature individuals, whereas the field study involved interactions with early instars, which are likely to be more sensitive to imidacloprid (Lowery and Smirle 2003). Furthermore, the long-term results in the field study involved several generations of adelgids. Consistent 90% mortality in four successive generations would translate to $\approx 1-0.1^4$, or

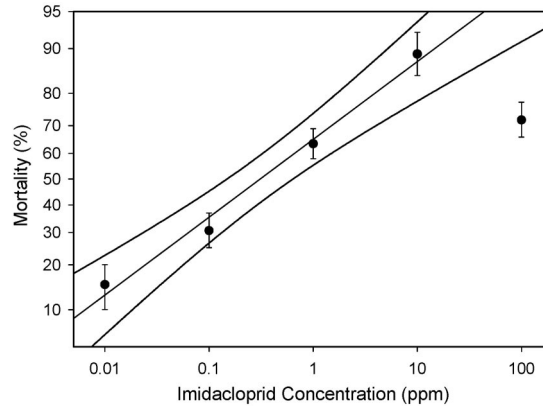

Fig. 2. Dose–response relationship with 95% CL for late instar hemlock woolly adelgids on excised foliage systemically exposed to imidacloprid solutions. The data from the 100 ppm concentration were treated as an outlier. Note that 1 ppm = 1,000 ppb. There was 28% mortality in the untreated check group. Average mortality ± SE is given for Abbott's corrected values, with 30 individuals per replicate ($n = 8$).

99.99% mortality, compared with an untreated check. Another explanation could be that uptake through the cuttings does not result in metabolites that are more active such as may occur via the root system.

Trunk injection methods were ineffective for controlling HWA, whereas soil placement of imidacloprid provided at least 2 yr of benefit. Interaction of the injected imidacloprid formulations with hemlock sap may have allowed precipitation of imidacloprid if the formulation solvent was diluted to the point at which it could no longer keep the imidacloprid in solution. Imidacloprid has water solubility at room temperature of 500 ppm, whereas the injected formulations in this study were 5, 10, and 12% active ingredient (100–240× the maximum solubility in water). Imidacloprid precipitate could have clogged the xylem tracheids, which would prevent efficient translocation of the active ingredient. This interpretation is supported by 1) the observation that low concentration formulations of imidacloprid can result in greater success in moving imidacloprid into the crown of the tree through trunk injection (Joe Doccola, Arborjet, personal communication); and 2) the decreased efficacy of the highest dosage treatment in our laboratory dose–response experiment. Efficacy of imidacloprid trunk injections might be improved if it were delivered in a manner that guarantees that it will remain in solution within the sap. This could be accomplished with a higher volume, lower concentration injection method, or improved formulation bridge solvents. Alternatives to imidacloprid could also lead to improved trunk injection results. Among insecticides with greater water solubility, the neonicotinoid insecticide dinotefuran looks especially promising. It has a water solubility of 39,800 ppm and is insecticidal to armored scales, a group against which systemic applications of imidacloprid have yielded poor results (R.S.C., unpublished data). However, the disappointing degree

of adelgid mortality relative to tissue concentrations for trunk-injected trees, the high cost of tree injection relative to soil application, and the unavoidable wounding of the trunk associated with trunk injection, suggests that soil application should be the favored method of imidacloprid application to control the adelgid on hemlocks. However, soil applications should not be made where the insecticide may migrate to aquatic environments or water supplies. Fortunately, imidacloprid binds very tightly to soil organic matter (Mullins and Christie 1995; Cox et al. 1998; R.S.C., unpublished data). Hemlock forests typically have 8 cm or more of partially decomposed needles forming a highly organic surface soil layer, in which most of the tree's absorptive roots are found. The interaction of imidacloprid with soil organic matter can be exploited by using very shallow subsurface injections of imidacloprid. Shallow placement of active ingredient maximizes the interaction of imidacloprid with organic matter as it is leached into the soil with precipitation. Once immobilized by organic matter, the gradual loss to the surrounding soil solution permits uptake by adjacent roots. An additional environmental safety factor for imidacloprid is its short half-life (1.4 d) in water when exposed to sunlight (Mullins and Christie 1995). These properties should greatly limit the potential risk for imidacloprid to enter streams and impact nontarget aquatic invertebrates.

Our research has demonstrated that soil application to forest hemlocks of imidacloprid results in a long duration of residues in tree foliage, multiple years of mobilization of the active ingredient to new growth, and reduction in adelgid populations. Insecticide treatment should be considered a stopgap measure to preserve trees that are of exceptional value until such time that biological control becomes established. Where chemical control is necessary, soil application of imidacloprid provides an efficient and long-lasting means of adelgid suppression.

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