Effects of Fertilizer and Low Rates of Imidacloprid on *Adelges tsugae* (Hemiptera: Adelgidae)

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ABSTRACT Healthy hemlock trees, *Tsuga canadensis* (L.) Carrière, and hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), populations should favor retention and population growth of adelgid predators such as *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) and *Sasajiscymnus tsugae* (Sasaji & McClure) (Coleoptera: Coccinellidae). Eastern hemlock trees between 15 and 38 cm diameter at breast height (dbh) were treated with 0, 10, or 25% of 1.5 g imidacloprid (Merit 75 WP) per 2.5 cm dbh and were either fertilized or not, in a 3 by 2 factorial design. After 2 yr, imidacloprid reduced the numbers of ovisacs and eggs found on trees in a dosage-dependent manner, while enhancing tree growth parameters such as new shoots or needles and the length of new shoots. Fertilized trees had greater adelgid fecundity, which was positively correlated with total foliar N in both winter generations. In February 2009 (27 mo after imidacloprid treatment), higher imidacloprid dosages to unfertilized trees resulted in reduced adelgid fecundity. Concentrations of N, P, and K were higher in the foliage of trees treated with insecticide, whereas foliar aluminum concentrations were consistently lower in trees with higher insecticide dosages. Trees treated with low rates of imidacloprid were healthier than untreated trees, but only trees treated with the 0.1× dosage had sufficient adelgids to possibly sustain predators over extended periods.

KEY WORDS hemlock woolly adelgid, eastern hemlock, predators, imidacloprid, fertilizer

The hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a serious threat to two native hemlock species: eastern hemlock, *Tsuga canadensis* (L.) Carrière and Carolina hemlock, *Tsuga caroliniana* Engelmann, of eastern North America. Native to Japan, *A. tsugae* was first reported in the eastern United States near Richmond, VA, in 1951, and it quickly expanded its range to include 17 states from New England to the northern part of Georgia in the southern Appalachians (Havill et al. 2006). It was first documented in Georgia in 2004 (Johnson 2005) where it now occurs in at least 11 northern counties and is spreading at 15 km/yr (Evans and Gregoire 2007). *A. tsugae* causes direct damage by feeding on the storage parenchyma cells containing xylem rays at the base of the needle (Shields et al. 1996). Populations of *A. tsugae* increase to very high densities within a few years reducing new shoot production and causing branch dieback. Heavy infestations can lead to tree mortality in as little as 2–3 yr in the southern states (Trotter and Shields 2009). Hemlock woolly adelgid has two parthenogenic generations per year called sistens and progrediens (McClure 1989). In addition, progrediens produce a winged, sexual generation that fails to colonize due to the absence of a suitable primary host [*Picea torano* (Koch) Koehne] in the continental United States (Montgomery et al. 2009). Adelgid populations grow rapidly on eastern and Carolina hemlock because these trees lack resistance and effective, native natural enemies are absent (McClure 1991a).

Potential management strategies for hemlock woolly adelgid include insecticides (McClure 1991a, Cowles et al. 2006), host plant resistance (Lagalante et al. 2006, Jetton et al. 2009, Montgomery et al. 2009), and natural enemies (McClure 2001, Zilahi-Balogh et al. 2003b, Cheah et al. 2004, Lamb et al. 2006). Although effective, use of insecticides to suppress adelgid infestations is limited in remote forest landscapes because of inaccessibility, expense, and potential post-treatment environmental risks to aquatic life in nearby rivers or streams (Cowles et al. 2006). Classical biological control is a more feasible and sustainable alternative to insecticides for hemlock woolly adelgid control in forests (McClure 1991a), although its effectiveness has yet to be proven across the range of hemlock.

Two predators, *Laricobius nigrinus* Fender (Derodontidae) and *Sasajiscymnus tsugae* (Sasaji & McClure) (Coccinellidae), have been widely released to manage hemlock woolly adelgid (McClure and Cheah 1999, Lamb et al. 2006, Mausel et al. 2010) and have been established in some areas (Mausel et al. 2010). *L. nigrinus* is a univoltine specialist predator

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native to the Pacific Northwest that has a life cycle synchronized with *A. tsugae* development (Zilahi-Balogh et al. 2003a,b). Pupae of *L. nigrinis* reside in soil from April to September, whereas first-instar nymphs of *A. tsugae* aestivate. Adult beetles and hemlock woolly adelgid become active late October through late June. Female *L. nigrinus* oviposition and larval eclosion are in synchrony with oviposition by *A. tsugae*; beetle larvae then prey on adelgid eggs (Zilahi-Balogh et al. 2003a,b).

In the mid-1990s, *S. tsugae* was responsible for remarkable success of adelgid control in Connecticut, New Jersey, and Virginia by reducing 47–88% of the *A. tsugae* population after release of 130,000 adult beetles (McClure and Cheah 1999). Lamb et al. (2005) indicated that *S. tsugae* primarily feeds on progrediens in late spring; however, their recovery after release has been inconsistent. *S. tsugae* are bivoltine and the overwintering adults are generally active beginning in early summer. Egg laying begins in early April and continues through June (Cheah and McClure 1998, 2000).

Both predator species are being mass reared for field release in various biocontrol facilities on the East Coast. *L. nigrinus* is difficult to rear in the laboratory due to asynchrony of adult emergence with prey development, which often results in high beetle mortality (Lamb et al. 2007). Mausel et al. (2008) established a field insectary in 1.2- to 2.4-m-tall *T. canadensis* artificially infested with adelgids before releasing *L. nigrinus* on them. This insectary program resulted in a population of *L. nigrinus* synchronized with its adelgid host and also produced significant numbers of beetles for release.

Tree health deteriorates as hemlock woolly adelgid outbreaks progress in forests (McClure 1991b, Miller-Pierce et al. 2010). Even low density infestations of less than four adelgids per 20 mm^2 of branch can cause negative physiological changes affecting new shoot growth the following year (McClure 1991b). This reduction in new growth and overall tree health results in a subsequent decline in adelgid populations. *L. nigrinus* larvae consume an average of 265 adelgid eggs at 18°C before pupating and they prefer adelgid eggs over other stages (Zilahi-Balogh et al. 2003b). Both *S. tsugae* and *L. nigrinus* often prefer high quality adelgid eggs in lab-rearing facilities (Palmer and Sheppard 2002, Lamb et al. 2006). Therefore, declining hemlock health and *A. tsugae* densities could influence predator oviposition and survival in the forest (Lamb et al. 2006), because unhealthy trees may not sustain the quality and quantity of adelgids necessary for predator growth (Mausel et al. 2010).

The objective of our study was to manipulate tree health and adelgid populations by using imidacloprid insecticide and fertilizer to provide an adequate, uninterrupted supply of high-quality adelgids to support long-term predator population growth. We expected that fertilization would increase adelgid fecundity, whereas low levels of insecticide would prevent adelgids from killing the trees but still provide sufficient populations to maintain predators. McClure (1991c) showed that adelgid fecundity was twice as high on fertilized hemlocks and suggested that fertilization may improve hemlock health when adelgid populations are controlled.

Materials and Methods

Study Site and Experimental Design. The study was initiated in November 2006 in White Co., ≈30 km north of Helen, GA, in the Chattahoochee National Forest. Eastern hemlock trees in this area were naturally infested by adelgids beginning in 2004 (Johnson 2005). We selected 60 eastern hemlock trees between 15 and 38 cm (6–15-inch) diameter at breast height (dbh), 7.3–24.6 m tall (mean, 15.6 m), 25–70 yr old, and accessible from the road for sample collection by using a hydraulic lift. The experiment was arranged in a 3 by 2 factorial design consisting of 10 replications in five blocks (two replications per block). Trees were grouped based on proximity to one another and blocks represented changes in elevation. Trees were treated with either 0, 10, or 25% of 1.5 g of imidacloprid insecticide (Merit 75 WP, Bayer Environmental Science, Research Triangle Park, NC) per 2.5 cm of tree dbh (henceforth referred to as the untreated check and 0.1× and 0.25× dosages) and one of two levels of fertilization: fertilized or not fertilized. Insecticide was applied in a circle around the tree ≈30 cm from the tree root collar and 5 cm in depth using a Kioritz soil injector (Kioritz Corp., Tokyo, Japan) on 14 November 2006. We made one injection point for each 2.5 cm of tree diameter by pressing the Kioritz dispensing knob six times to deliver from 29.5 to 30 mL/2.5 cm dbh of insecticide solution into the soil. On 9 and 19 April 2007, half of the trees received their initial fertilizer treatment. Fertilizer rates varied based on tree size so that trees <19, 19–35, and >36 cm dbh received 455, 910, and 1360 g N, respectively. The initial fertilizer application was made using a combination of fertilizer spikes (12–6–12, N–P–K, Miracle-Gro, Marysville, OH) at a rate of one spike per 1.22 m of dripline diameter and an additional broadcast application with polymer-coated urea fertilizer (29–2–5, N–P–K, Sta-Green Broadcast, St. Louis, MO). In 2008, 910, 1,810, and 2,720 g of N (polymer-coated urea fertilizer, 29–0–5, N–P–K, Sta-Green Broadcast) in total was broadcast in two applications beneath trees in the respective diameter classes used in 2007. One half of the fertilizer was applied on 4 March and the remainder on 11 June.

Sample Collection and Evaluation. We sampled hemlock terminals from the treated trees on 14 June 2007, 19 February and 26 June 2008, and 23 February and 8 June 2009 by using a hydraulic lift truck to access all parts of the canopy. On 14 June 2007, four 30-cm-long hemlock branch terminals were sampled from each of 30 trees, representing one treatment from each block. Two branch terminals were cut from the lower, and two from the upper tree crown so that one sample at each crown location (lower and upper) was taken from the road side and the other on the opposite or forest-side of the crown. In February and June 2008

and 2009, we sampled four 30-cm-long terminal branches from each of the 60 trees. Samples were placed in polyethylene bags, labeled, and transported to the laboratory where they were stored at −5°C.

The number of ovisacs, eggs, and nymphs (crawlers and settled first instars) were counted on each 30-cm branch, and the number of new branch shoots, the length of new shoots and the number of needles on new growth were measured as an estimate of tree health.

On 10 September 2009, we used the crown measurement and sampling procedures from the U.S. Forest Service FIA Core Field Guide (USDA 2001) to assess the overall health of our study trees. Those measurements included live crown ratio, crown density, foliage transparency, and live branches and were measured in percentages (0–100%). In addition, the percentage ranges (<10, 11–50, 51–75, and >75%) were categorized on a 1–4 scale system for crown dieback and new growth. Hemlock health was assessed on an individual tree basis for all 60 trees.

Insecticide Residue Analysis. Foliar imidacloprid residues were measured on 30 trees (one for each treatment per block) to examine how insecticide residues correlated with adelgid population trends within treatments. Analyses were limited to 30 trees to minimize costs. Two to three 10-cm-long terminals were taken from upper, middle, and lower canopy locations of 30 trees (six treatments, five replications) for analysis of imidacloprid residues in the foliage during each sampling date (February and June) in 2008 and 2009. We combined samples from each tree into a single sample for analysis, except in June 2008 when two samples of 10-cm-long terminals were collected from both the lower and upper half of the crowns to examine the distribution of imidacloprid in the canopy. Samples were immediately placed in polyethylene bags and stored in a field cooler until they were brought to the laboratory and stored at −60°C. We shipped samples for imidacloprid residue analyses overnight in insulated boxes containing dry ice to maintain the temperature below 0°C to the University of California–Riverside. We measured concentrations of imidacloprid within hemlock samples by using a competitive enzyme-linked immunosorbent assay (ELISA) technique (Byrne et al. 2005). For each sample, the needles were removed from all 10-cm branch terminals. One gram of needles was then weighed and transferred to a 50-ml centrifuge tube containing 5 ml of methanol. The needles were crushed using a Teflon pestle; the tubes were capped and then shaken vigorously for 12 h at 25°C. An aliquot (10 μl) of each extract was dried completely in a TurboVap LV evaporator (Caliper Life Sciences, Hopkinton, MA) and then reconstituted in a 0.05% aqueous solution of Triton X-100 before analysis by ELISA. The imidacloprid results are expressed as ppb (nanograms per gram) of imidacloprid per gram of hemlock tissue (wet weight). The limit of detection for this assay was 50 ppb hemlock tissue (wet weight) for all sample dates except the June 2008 samples in which the detection limit was 25 ppb of hemlock tissue. Concentrations below the 25 or 50 ppb detection limit were recorded as zero.

Tree Nutrient Analysis. Foliar nutrient content was measured to assess N uptake within fertilized and unfertilized trees and to examine correlations of nutrient contents with adelgid densities and tree growth parameters. Samples consisted of 50 g of hemlock terminals cut from branches throughout the canopy. The February 2008 sample included both needles and fine woody branch material up to 2 mm in diameter. Subsequent samples (February and June 2009) contained only needles. All 60 trees were sampled and analyzed for each sample date. Samples were oven-dried at 40°C for 48 h and then taken to the Plant and Soil Testing Laboratory (University of Georgia, Athens, GA) for analysis. Nutrients (Mn, Fe, Al, B, Cu, Zn, Na, Pb, Cd, Ni, Cr, Mo, P, K, Ca, and Mg) were analyzed using an inductively coupled plasma emission spectrograph (Isaac and Johnson 1985, AOAC 1995), and the total percent N was quantified using the combustion method of Colombo and Giazzi (1982).

Statistical Analyses. We selected 60 trees (two trees per treatment per block) for treatment because the study area was scheduled for selective harvesting to remove hazard trees along the road. Because trees to be removed were scattered throughout our study area, we were concerned some of our treated trees would be damaged or broken off during harvesting, so we selected and treated twice as many trees as originally planned. Harvesting was delayed for a variety of reasons; so we were able to sample all 60 trees throughout the study.

We analyzed the effects of insecticide and fertilizer treatments on hemlock woolly adelgid populations and tree growth parameters as a 3 by 2 factorial experiment with interaction by using the general linear models procedure of SAS (PROC GLM, SAS Institute 2003). Data for adelgid counts and tree growth parameters from four canopy locations were averaged to provide a single value per tree. Each 30-cm-long hemlock branch used for assessing adelgid densities and tree growth parameters varied in total length because they had differing numbers and lengths of side branches. Therefore, all adelgid counts and growth parameters were standardized by dividing them by the total hemlock branch lengths within a 30-cm-long sample and expressing them as adelgids per centimeter of shoot length. These standardized independent variables, which included number of ovisacs, eggs, nymphs (crawlers and settled first instars), new shoots, total length of new shoots (in centimeters), needles on new shoots, and nymphs on new shoots were log-transformed ($\ln[x+1]$) to establish homogeneity of variance using the PROC Univariate procedure of SAS (SAS Institute 2003). The variable eggs per ovisac also was transformed using the natural log, whereas all percentage data were arcsine square-root transformed. The categorical data of tree health survey were arcsine square-root transformed after expressing them in percentages. Because imidacloprid residues in foliage from the lower and upper crown were similar ($\alpha = 0.05$), these data were averaged for

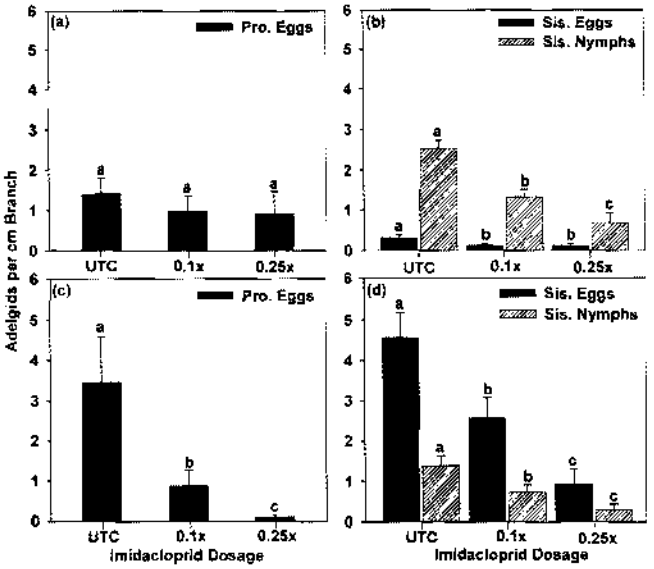

Fig. 1. Effects of imidacloprid dosage (UTC, 0.1× or 0.25× of 1.5 g imidacloprid/2.5 cm dbh) on various life stages of hemlock woolly adelgid in *T. canadensis* (means ± SE; $N = 20$) in February 2008 (a), June 2008 (b), February 2009 (c), and June 2009 (d). Insecticide was applied November 2006. Sis., sistens and Pro., progrediens. Bars of the same fill color with the same letters are not significantly different ($\alpha = 0.05$; LSD test).

each tree and that value was used for further analyses. In analyses of imidacloprid concentrations among treatments the values for the untreated checks (UTC) were excluded because they were all zeros, resulting in zero variance that can artificially reduce the estimate for pooled error. Imidacloprid residues were log transformed ($\ln[x+1]$) before analysis. Variance for foliar nutrients was homogeneous, so they were not transformed. Transformed and untransformed data for each sample date were examined using the PROC GLM procedure of SAS, and means were separated using the least significant difference (LSD) test ($\alpha = 0.05$; $N = 20$). The relationship between *A. tsugae* fecundity and concentrations of N was examined by regression analysis using PROC REG procedures of SAS (SAS Institute 2003).

Results

Effects of Insecticide and Fertilizer. Because there was a constant relationship of eggs per ovisac for each generation of adelgids ($P > 0.09$), only the number of eggs and nymphs are presented in Fig. 1. Imidacloprid applied in November 2006 did not affect *A. tsugae* progrediens ovisac or egg densities in June 2007, regardless of application rate (Table 1). Likewise, sistens *A. tsugae* densities in February 2008 were unaffected by imidacloprid. Imidacloprid residues in needles

Table 1. Analysis of variance of insecticide effects on *A. tsugae* numbers per centimeter of branch, tree growth parameters per centimeter of branch, and imidacloprid residues in hemlock tissues from 2007 to 2009 of trees treated with low rates of imidacloprid insecticide in November 2006

Variable	2007 June			2008 Feb.			2008 June			2009 Feb.			2009 June		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Adelgid counts															
Ovisacs[a]	0.3	2, 20	0.72	1.4	2, 45	0.24	7.1	2, 45	<0.01	9.6	2, 45	<0.01	18.9	2, 45	<0.01
Eggs[b]	0.4	2, 20	0.68	1.9	2, 45	0.15	5.0	2, 45	0.01	25.3	2, 45	<0.01	16.8	2, 45	<0.01
Eggs/ovisac	1.3	2, 20	0.29	0.2	2, 33	0.82	1.8	2, 35	0.18	2.5	2, 23	0.09	1.1	2, 32	0.34
Nymphs	1.7	2, 20	0.19	0.8	2, 45	0.46	28.1	2, 45	<0.01				12.2	2, 45	<0.01
Tree growth															
New shoots[c]	0.5	2, 20	0.57				6.9	2, 45	<0.01				22.4	2, 45	<0.01
Length of new growth	0.4	2, 20	0.63				4.4	2, 45	0.02				29.9	2, 45	<0.01
New needles	0.6	2, 20	0.54				6.9	2, 45	<0.01				28.5	2, 45	<0.01
Imidacloprid															
Residue				4.3	1, 9	0.06	1.3	1, 9	0.28	6.1	1, 9	0.03	3.4	1, 9	0.09

Analysis performed on log-transformed adelgid population growth, tree growth parameter data, and imidacloprid residue data.
[a] Ovisacs represent progrediens generation during June and sistens generation during February.
[b] Eggs and nymphs represent progrediens generation during February and sistens generation during June.
[c] Growth parameters were only measured on hemlock branches during summer.

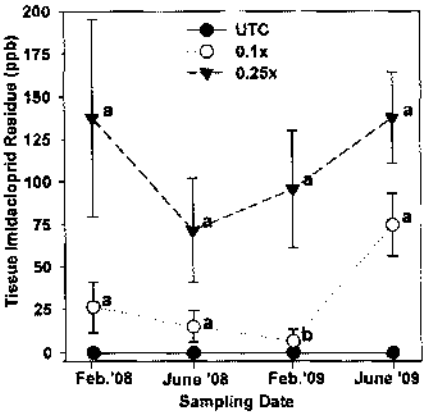

Fig. 2. Foliar imidacloprid residues in 2008–2009 detected in *T. canadensis* (means ± SE; $N = 10$) treated with different imidacloprid dosage (UTC, 0.1× or 0.25× of 1.5 g imidacloprid/2.5 cm dbh) in November 2006. For all sample dates, detection limit was 50 ppb hemlock tissue (wet weight) except in June 2008 samples when the detection limit was 25 ppb. Concentrations below the 25 or 50 ppb detection limit were recorded as zero. Symbols within the same sample date with the same letters are not significantly different ($\alpha = 0.05$; LSD test).

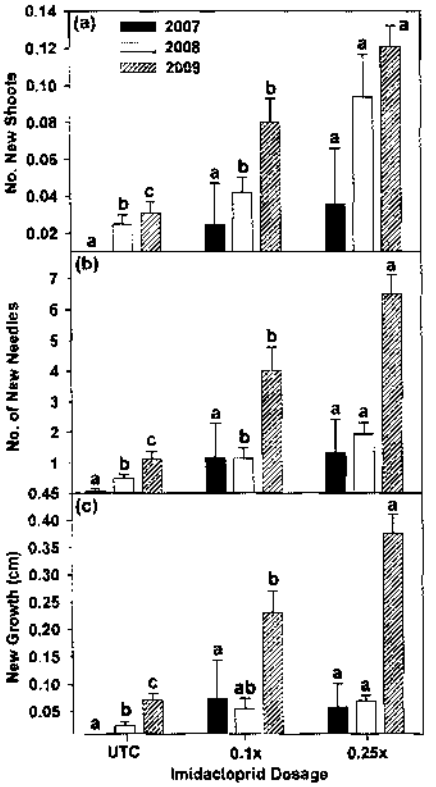

Fig. 3. Mean ± SE growth response of *T. canadensis* trees treated with different imidacloprid dosage (UTC, 0.1× or 0.25× of 1.5 g imidacloprid/2.5 cm dbh) expressed as new shoots (a), needles on new shoots (b), and length of new shoots (c) (in centimeters) per centimeter of total branch length ($N = 20$). Insecticide was applied November 2006. Bars with the same fill and the same letter are not significantly different ($\alpha = 0.05$; LSD test).

were not significantly different between the 0.25× dosage trees and the 0.1× dose except for the February 2009 samples when 25× treated trees had significantly higher residues than the 10× treated trees. (Figs. 1a and 2; Table 1). Both treatments had detectable levels of imidacloprid throughout the study, whereas untreated trees had none and for all sample dates the 25× treated trees had higher levels of imidacloprid than the 10× treatment. However, by June 2008 progrediens ovisacs and their sistens egg densities were higher on untreated check trees than on trees treated at 0.1× or 0.25× dosages (Fig. 1b; Table 1). In addition, sistens nymphs were denser on untreated trees than on 0.1× dosage trees, which had higher densities of nymphs than 0.25× dosage trees. Insecticide residues in foliage were not significantly higher in trees that received the 0.25× dose of imidacloprid than in trees that received the 0.1× dose, although both had detectable levels of imidacloprid but untreated trees did not (Fig. 2).

The winter generation of 2009 had lower sistens ovisacs and subsequent progrediens eggs densities on trees that received imidacloprid in a significant, dosage-dependent manner (Fig. 1c; Table 1). Moreover, imidacloprid residues in trees with the 0.1× and 0.25× dosage differed significantly. The 0.1× treated trees had detectable levels of insecticide but the levels were very low. In June 2009, the sistens eggs and nymphs exhibited the same dosage-dependent trend as before (Fig. 1d), although we were unable to detect significant differences in the imidacloprid residues in the foliage (Fig. 2).

Imidacloprid did not have an immediate effect on tree growth parameters. Length of new growth, numbers of new needles, and numbers of new shoots were not significantly higher in the June 2007 sample (Table 1; Fig. 3). However, by June 2008, trees treated with the highest dose of imidacloprid produced more new shoots and more new needles per centimeter of branch relative to the 0.1× dosage and untreated trees. The 0.25× dosage trees also had a greater length of new growth than untreated check trees, whereas the 0.1× dosage trees did not (Fig. 3c). By June 2009, a dose response was evident in tree growth parameters, with the highest number of new shoots, new needles, and length of new shoots per unit branch length observed on 0.25× dosage trees followed by the 0.1× dosage and then by untreated check trees. Fertilization had no effect on tree growth parameters throughout the study at $\alpha = 0.05$; hence, means are not presented. In addition, no interaction was observed between fertilizer and imidacloprid for adelgid life stages or tree growth parameters ($P > 0.1$).

Nitrogen fertilization did increase fecundity of sistens females in 2008 and 2009 (Fig. 4; Table 2). Although the initial fertilization did not result in significantly higher foliar N levels in winter 2008, fecundity exhibited a positive linear relationship with foliar

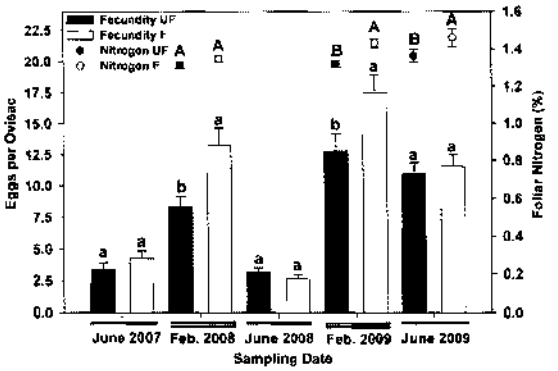

Fig. 4. Effects of fertilizer applied in spring 2007 and spring and summer 2008 on hemlock woolly adelgid fecundity and total percentage N content of *T. canadensis* foliage (means ± SE). Number of trees sampled per fertilizer treatment were $N = 15$ for the June 2007 sample and $N = 30$ for the rest of the sample dates. UF, unfertilized; F, fertilized; single line (—), progrediens; and double lines (=), sistens ovisacs. Bars or dots within the same sample date with the same letter are not significantly different ($\alpha = 0.05$; LSD test).

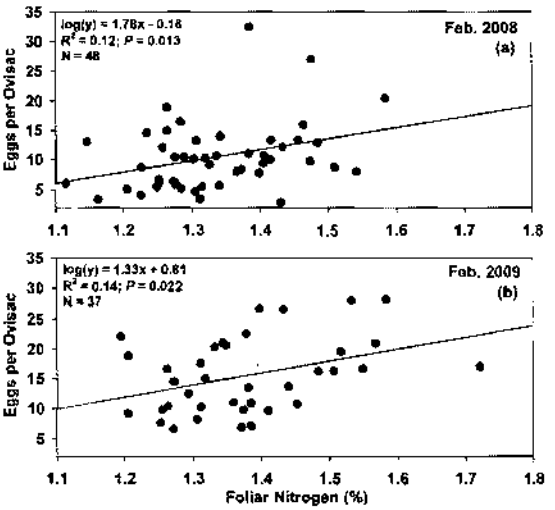

Fig. 5. Linear regression of total *A. tsugae* eggs laid per female and total foliar N content of *T. canadensis* trees on which they developed.

N content of sample trees ($F = 6.6$; df = 1, 46; $P = 0.01$) (Fig. 5a). By winter 2009, after two additional fertilizer applications in spring and summer 2008, foliar N was significantly higher in fertilized trees ($F = 18.2$; df = 1, 45; $P < 0.01$) (Fig. 4) than in unfertilized trees. Likewise, sistens fecundity had a positive linear relationship with foliar N content of individual sample trees ($F = 5.7$; df = 1, 35; $P = 0.02$) (Fig. 5b). Although foliar N content was significantly higher in fertilized trees ($F = 5.6$; df = 1, 44; $P = 0.02$) (Fig. 4), it did not contribute to higher fecundity in summer 2009 (Fig. 4). Although no significant interactions between fertilizer and imidacloprid were observed for any sample period ($P > 0.1$), in February 2009 unfertilized trees treated at the 0.1× dosage of imidacloprid had lower adelgid fecundity than fertilized trees treated at the same rate (Fig. 6).

Tree Health Survey. Because fertilization did not affect tree growth parameters, we grouped fertilized and unfertilized trees together within insecticide treatments when analyzing tree health characteristics. Live crown ratio was similar within trees that received insecticide or not. Tree crown density of 0.25× dosage trees was higher than untreated check trees but not 0.1× dosage trees (Table 3). In a related measurement, canopies of untreated check trees allowed more sunlight to pass through compared with trees that received 0.1× dosage then followed by 0.25× dosage trees. One symptom of tree decline, crown dieback, was greater on untreated check than on insecticide treated trees. The percentage of living branches was greater in the 0.1× or 0.25× dosage groups than in the untreated check group. The percentage of newly growing branches was significantly greater in the following order: 0.25× > 0.1× > untreated check trees.

Plant Nutrient Analyses. Our results suggest that foliar tissue nutrient levels changed as tree health improved. In summer 2009, N, P, and K occurred at higher levels in foliage of insecticide-treated trees than in untreated trees. However, Ca and Zn levels were higher in untreated trees than in treated trees (Table 4). Likewise, Al concentrations were greater in control trees, whereas 0.1× dosage trees had higher concentrations of Al than those treated at the 0.25× dosage. B and Mn were also higher in untreated or 0.1× dosage trees than in 0.25× dosage trees. Besides differences in mineral nutrients in insecticide-treated trees, we also noted differences in P content in trees that received fertilizer or not. There was greater P content in unfertilized trees ($F = 5.7$; df = 1, 45; $P =$

Table 2. Analysis of variance of effects of fertilizer on *A. tsugae* life stages per centimeter of branch from 2007 to 2009

Adelgid counts	2007 June			2008 Feb.			2008 June			2009 Feb.			2009 June		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*	*F*	df	*P*
Ovisacs[a]	0.6	1, 20	0.44	3.7	1, 45	0.06	0.9	1, 45	0.35	2.4	1, 45	0.13	0.2	1, 45	0.61
Eggs[b]	0.0	1, 20	0.95	2.2	1, 45	0.14	1.1	1, 45	0.31	2.5	1, 45	0.12	0.0	1, 45	0.98
Eggs/ovisac	0.8	1, 20	0.39	8.3	1, 33	0.01	0.9	1, 35	0.35	6.1	1, 24	0.02	0.0	1, 32	0.80
Nymphs	0.1	1, 20	0.68	0.6	1, 45	0.44	0.0	1, 45	0.86				0.0	1, 45	0.91

Analysis performed on log-transformed adelgid population growth. Fertilizer was applied in spring 2007, and spring and summer 2008.
[a] Ovisacs represent progrediens generation during June and sistens generation during February.
[b] Eggs represent progrediens generation during February and sistens generation during June.

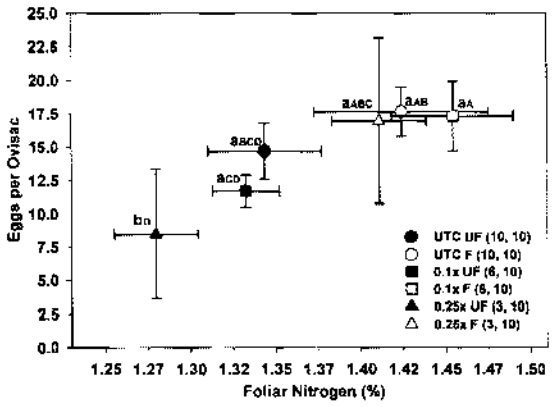

Fig. 6. Means ± SE of *A. tsugae* eggs per female versus foliar nitrogen content of *T. canadensis* trees in winter 2009 treated with different dosages of imidacloprid (UTC, 0.1× or 0.25× of 1.5 g imidacloprid/2.5 cm dbh) in November 2006. Imidacloprid treatments are indicated by different shaped symbols and fertilizer treatment by symbol color. UF, unfertilized; F, fertilized. The legend also indicates in parentheses the number of trees included per treatment for eggs per ovisac followed by the number of trees analyzed for foliar nitrogen. Lowercase letters indicate comparisons of eggs per ovisac among treatments, whereas uppercase letters indicate comparisons of foliar nitrogen content among treatments. Symbols with similar-case letters (upper or lower) are not significantly different (α = 0.05; LSD test).

0.02; mean ± SE, 0.16 ± 0.01) than fertilized trees (mean ± SE, 0.14 ± 0.01) for the same sample date.

Discussion

Because *L. nigrinus* and *S. tsugae* require healthy adelgids that, in turn, require healthy hemlocks, we sought to determine the extent to which applications of insecticide and fertilizer are likely to promote predator establishment. Cowles (2009) showed that soil injection of a 0.1× dosage of imidacloprid caused 50% suppression of adelgid populations on eastern hemlocks of the size range used in this study, and he provided a predictive model to reduce adelgid populations 90%, based on tree dbh. Empirically, adelgid densities tended to increase with increasing tree dbh (19–60 cm dbh), presumably due to increased dilution of imidacloprid in larger trees (Cowles 2009). Although we did not block the trees based on diameter, our 0.1× dosage effectively reduced sistens 75% and progrediens 40% 2 yr posttreatment and we found no influence of tree diameter on adelgid populations when tree dbh was modeled as a covariate. Our trees were all <38 cm, so the diameter or tree size effect was less likely to be a significant factor.

After 14 mo posttreatment (summer 2008), progrediens adelgids were less dense on trees treated with both rates of insecticide compared with untreated trees. However, trees that received the 0.1× dosage had a moderate level of sistens nymphs, which was greater than trees treated at the 0.25× dosage. In both winter and summer 2009, abundance of sistens and progrediens life stages exhibited a dose response where adelgids were lowest on 0.25× dosage trees and highest on untreated check trees. This suggests that a low rate of imidacloprid, 0.1× dosage or less, could allow trees to maintain a moderate density of *A. tsugae*. In addition, a tremendous improvement in tree health was found on the insecticide-treated trees proportional to the dose that they received. One year after application in summer 2008, trees treated with a 0.25× dose of imidacloprid produced more new shoots than the 0.1× dosage or untreated check trees. Unlike the previous year, new growth was denser on 0.25× dosage trees followed by 0.1× dosage trees, which had denser new growth than untreated check trees in the summer 2009. It seems that trees treated with a 0.1× dosage not only maintained a moderate density of *A. tsugae* but also improved tree health, suggesting that they could sustain predators for a longer time. In comparison, trees treated with a 0.25× dosage of insecticide had a very low population of *A. tsugae;* thus, at least for a few years, they are unlikely to be suitable for sustaining populations of predators.

Imidacloprid residues in hemlock tissue were consistently higher in trees receiving the 0.25× dosage than in those trees that received the 0.1× dosage for all sample dates. Indirect effects of insecticide on *L. nigrinus* and *S. tsugae* were not tested in this study. However, Eisenback (2008) studied survival rate, feeding preference, and level of toxicity of adelgid-

Table 3. Mean ± SE tree crown health characteristics of hemlocks on 10 September 2009 for trees treated with different rates of imidacloprid insecticide (*N* = 20) in November 2006

Criteria	Imidacloprid dosage			*F*	df	*P*
	UTC[a]	0.1×	0.25×			
Live crown ratio	65.7 ± 3.5a	67.0 ± 3.0a	73.2 ± 2.9a	1.5	2, 45	0.21
Crown density	52.0 ± 3.7b	63.2 ± 4.4b	73.2 ± 4.3a	7.0	2, 45	<0.01
Foliage transparency	66.7 ± 4.5a	50.9 ± 5.4b	33.7 ± 5.0c	13.6	2, 45	<0.01
Crown dieback	2.0 ± 0.1a	1.2 ± 0.1b	1.3 ± 0.1b	12.2	2, 44	<0.01
Live branches	49.5 ± 5.5b	68.0 ± 5.4a	72.1 ± 4.9a	10.7	2, 45	<0.01
New shoots	1.8 ± 0.1c	2.8 ± 0.2b	3.5 ± 0.1a	22.2	2, 45	<0.01

Means in a row followed by different letters are significantly different ($P < 0.05$) and were separated using LSD test. Analyses of variance were performed on arcsine square root transformed data. The categorical data, crown dieback, and new shoots were expressed as percentages to enable arcsine square-root transformation. All values in the table are percentages, with the exception of crown dieback and new shoots, which used a 1–4 rating.

[a] Untreated check, 0.1× = 10% and 0.25× = 25% of recommended rate of 1.5 g imidacloprid/2.5 cm of trunk diameter.

Table 4. Mean ± SE foliar nutrient content in June 2009 of hemlock trees treated with three rates of imidacloprid ($N = 20$) in November 2006

Foliar nutrient	Imidacloprid dosage			F	df	P
	UTC[a]	0.1×	0.25×			
N	1.32 ± 0.04b	1.46 ± 0.06a	1.45 ± 0.04a	4.5	2, 45	0.02*
Ca	0.65 ± 0.03a	0.47 ± 0.03b	0.41 ± 0.03b	20.2	2, 45	<0.01*
K	0.43 ± 0.03b	0.59 ± 0.03a	0.67 ± 0.03a	5.2	2, 45	0.01*
P	0.13 ± 0.01b	0.17 ± 0.01a	0.17 ± 0.01a	5.9	2, 45	0.01*
Mg	0.13 ± 0.01a	0.12 ± 0.01a	0.11 ± 0.00a	1.9	2, 45	0.15
S	0.16 ± 0.01b	0.18 ± 0.00a	0.15 ± 0.00b	6.5	2, 45	<0.01*
Al	584.3 ± 24.8a	506.4 ± 22.5b	406.3 ± 13.9c	20.1	2, 45	<0.01*
B	39.4 ± 2.1a	34.8 ± 2.2a	29.2 ± 1.8b	7.6	2, 45	<0.01*
Cu	1.8 ± 0.2a	2.6 ± 0.3a	2.4 ± 0.2a	2.5	2, 45	0.09
Fe	79.4 ± 15.7a	70.5 ± 8.6a	53.9 ± 3.8a	1.3	2, 45	0.26
Mn	1065.6 ± 66.1a	796.6 ± 92.8b	676.6 ± 58.5b	7.8	2, 45	<0.01*
Zn	24.6 ± 1.5a	20.8 ± 1.1b	19.6 ± 1.3b	4.0	2, 45	0.02*

Means of Ca, K, Mg, N, P, and S shown above are expressed in total percentage concentration (of dry weight), whereas Al, B, Cu, Fe, Mn, and Zn are in ppm in the hemlock foliage. Means in a row followed by different letters are significantly different (*$P < 0.05$) and were separated using LSD test. Analyses of variance were performed on untransformed data.

[a] Untreated check, 0.1× = 10% and 0.25× = 25% of recommended rate of 1.5 g imidacloprid/2.5 cm of trunk diameter.

infested branches treated at the 0.25× dosage (of 1.4 g) of imidacloprid on predators. They did not find any evidence of indirect toxicity on either predator species and no traces of insecticide residues were detected in beetle cadavers. Based on their study, it is unlikely that the low rates of imidacloprid in our study would have direct or indirect effects on survival and development of predators.

Our results show that fertilized trees had greater adelgid fecundity in the winter generations. Nitrogen content was higher in fertilized versus unfertilized trees in 2009 samples. Several studies indicate that a high level of N in host plants after application of mineral N enhances the fecundity of piercing-sucking insects (Petitt et al. 1994, Kytö et al. 1996, van Emden 1996, Nevo and Coll 2001). In addition, McClure (1991c) showed an immediate increase in *A. tsugae* fecundity when they developed on young *T. canadensis* trees after a spring application of fertilizer. *A. tsugae* eggs are vital for *L. nigrinus* larval development (Zilahi-Balogh et al. 2003b). In the current study, adelgid females that developed on fertilized trees produced 14–17 eggs per female compared with seven to 13 eggs per female on unfertilized trees during winter generations. This higher adelgid fecundity would probably enhance predator survival and establishment. Our data also showed a reduction of adelgid fecundity in unfertilized trees having higher imidacloprid dosage. We know trees that received a higher dose of imidacloprid had enhanced shoot growth. Perhaps, this diluted the available N in the foliage and in turn limited fecundity, which is consistent with previous work (Finzi 2009; Miller-Pierce et al. 2010). This result clearly demonstrated that using low dosages of insecticide and fertilizer can maintain healthy trees and sufficiently moderate *A. tsugae* populations to provide predators with ample food as their populations grow.

It is unclear why we observed enhanced fecundity on fertilized trees only for sistens. One explanation may be that aestivating sistens nymphs exert less stress on trees (Lagalante et al. 2006). As a result, trees could amass and store reserves in their xylem parenchyma cells. Once aestivating nymphs break diapause, they use these stored reserves and develop into healthy females. Thus, healthier sistens females that developed on fertilized trees with more reserves oviposit more eggs than those that developed on unfertilized trees. In contrast, the progrediens develop on trees that have been depleted by sistens feeding and the trees have little or no time to replenish reserves before feeding by progrediens begins. In 2008, we used both foliage and small branches in samples processed for nutrient content, whereas we analyzed only foliage in 2009 samples. The small branches in 2008 samples probably contained lower amounts of nutrients than foliage and this might have masked N content in the foliage where adelgids feed. This is consistent with Hagen-Thorn et al. (2004), where they observed greater N in Norway spruce [*Picea abies* (L.) H.Karst.] foliage than in stems.

Regardless of foliar N content, late spring samples did not show a notable effect of fertilizer on progrediens fecundity. This was consistent with a previous study where an increase in *A. tsugae* fecundity was not attained in the spring generation on older *T. canadensis* forest trees even though the fertilizer had been delivered by trunk injection (McClure 1992). Although the exact reason for this observation is unknown, there could be several possibilities. First, progrediens crawlers settle on the previous year's shoots beginning early March to late April. Montgomery et al. (2009) observed that bud break of eastern hemlock usually started from the first-week of April; thus, crawlers lack new shoots to feed upon immediately after their emergence. Second, in most trees N is mobile within the tree and usually moves from the storage parenchyma cells in older shoots to newly developing tissues in early spring, a phenomenon that seems to occur in *T. canadensis* as well (Stadler et al. 2005, Lagalante et al. 2006). This N metabolism is rapid in Pacific silver fir, *Abies amabilis* (Dougl.) Forb.,

infested with *Abies piceae* (Ratzburg), and it is quickly incorporated into new flushes of fertilized trees (Carrow and Graham 1968). High N remobilization also occurs from older leaves to new shoots of *Quercus glauca* Thunb. ex Murray (Miyazawa et al. 2004) and Norway spruce (Nommik 1966). Therefore, because the developing progrediens adelgids settle on the older tissue where N is being removed, they may not have access to sufficient N to enhance fecundity. In addition, nutrient analysis of summer 2009 samples detected more foliar N in fertilized trees than in unfertilized ones, possibly because these samples were composed primarily of new shoots and foliage. Third, mature or older trees may not be as efficient as younger ones in nutrient uptake from the soil. For example, it has been shown that fertilizer uptake decreased with tree age in young Norway spruce stands (Nommik 1966). Fourth, addition of N fertilizer is likely to affect the complex ecological relationships in the soil, especially mycorrhizae. Finzi (2009) showed that application of N alone can reduce hemlock growth even in a nutrient-limited hemlock–forest stand.

Low rates of insecticide seemed to improve foliage chemistry. Residual nutrient concentrations exhibited enhanced levels of N, P, and K in the healthy foliage of insecticide-treated trees. It is likely that high adelgid densities depleted the nutrient content of trees not treated with insecticides. Studies showed that *A. tsugae*-susceptible *T. canadensis* had greater levels of N and K, and low P relative to resistant hemlock species, making them more suitable to colonization by adelgids (Pontius et al. 2006). In contrast to N–P–K content, aluminum in the needles was consistently reduced as the insecticide dosage increased, probably as a result of greater growth of shoots and dilution of Al in the foliage rather than changes in soil availability. However, where soils have low levels of Ca, there is increased Al uptake by plants (Tisdale and Nelson 1975), which places additional stress on trees and makes them more vulnerable to insect attack (DeHayes et al. 1999, Schaberg et al. 2006). Other foliar minerals, such as Ca, Zn, Mn, and B, tended to accumulate in unhealthy trees that did not receive imidacloprid. This suggests that *A. tsugae* colonization caused an imbalance in foliar chemistry and imposed additional, indirect secondary stress to *T. canadensis*. Interestingly, we noted greater P content in unfertilized versus fertilized trees. It is possible that excessive adelgid feeding in tissues of unfertilized trees caused nutrient imbalance. For example, Miller-Pierce et al. (2010) found that hemlocks fed upon by adelgids had lower levels of foliage N% relative to trees without adelgids during the first year, but in the second year this trend disappeared.

Higher populations of adelgids and increased fecundity on fertilized trees, especially in winter, may be beneficial for survival and development of specialist predators like *L. nigrinus* and *S. tsugae*. However, the increased fecundity and corresponding intrinsic rate of growth will need to be balanced with the desire to maintain tree health. As long as trees remain healthy and the intrinsic rate of growth for predator populations exceeds that of *A. tsugae*, we anticipate that the regime of very low dose imidacloprid treatment and fertilization will optimize predator establishment and success. From a practical view, because they both should provide long-term effects, a combination of low-dose insecticide application with predator releases may optimize predator establishment and preservation of hemlocks. Prolonged tree health plus sustained, healthy adelgid populations should benefit other predators as well, allowing their populations to grow and spread to the surrounding forest. A one-time application of insecticide to preserve tree health long enough to provide predators a window of opportunity to establish should minimize the potential for adelgids to develop insecticide resistance. Future research should focus on multiple releases of predators and evaluation of their establishment on adelgid populations developing on healthy trees. Once the predator population grows in the forest, they should reduce *A. tsugae* populations and possibly eliminate the need for future insecticide treatments.

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