Distribution and Abundance of *Adelges tsugae* (Hemiptera: Adelgidae) Within Hemlock Trees

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ABSTRACT We studied the distribution of hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), within hemlock trees for three summer (progrediens) and two winter (sistens) generations in northern Georgia. Eastern hemlock, *Tsuga canadensis* (L.) Carrière, trees were treated with 0, 10, or 25% of 1.5 g of imidacloprid per 2.5 cm of tree diameter at breast height and fertilized or not in a factorial design. Adelgid ovisacs per centimeter of branch were more abundant from June 2007 to June 2008 in the upper tree crown of insecticide untreated trees and when all trees were combined and that was the general trend for most comparisons. However, ovisacs were more abundant in the lower crown of insecticide treated trees in June 2008. More sistens nymphs settled on the upper crown branches than on the lower branches in summers 2007 and 2008. Higher eggs per ovisac were observed in the upper crown in February 2008 and in both the winter and summer 2009. In contrast, adelgids were more fecund in the lower crown in June 2008. On fertilized trees, eggs laid per adult were higher in the upper crown in February 2008. In summer 2008, eggs per ovisac were higher in the lower crown, but this reversed again to the upper crown by summer 2009. New growth of branches also varied among sample dates. These data demonstrate the variable distribution of adelgid and hemlock growth within trees over time and suggest that sampling only one crown area will not provide accurate estimates of adelgid densities.

KEY WORDS *Adelges tsugae*, eastern hemlock, *Tsuga canadensis*, distribution, tree height

Hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a serious threat to eastern hemlock, *Tsuga canadensis* (L.) Carrière, and Carolina hemlock, *Tsuga caroliniana* Engelmann, in the forests of eastern North America. Hemlocks are invaluable in sensitive public sites such as parks, picnic areas, and trails within their range (McClure 1991c), and they are also widely planted and valued in a variety of landscapes (Cheah and McClure 2000). Hemlock woolly adelgid was first detected in the eastern United States near Richmond, VA, in 1951 and then in Pennsylvania in the 1960s and Connecticut by 1986 (McClure 1987). Now, *A. tsugae* is distributed from north Georgia to New England and southern Canada (USDA–Forest Service 2009). Mammals, wind, and birds play active roles in hemlock woolly adelgid unintentional dispersal with the crawler stage being more amenable to movement (McClure 1990, 1991c). *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. This pest causes heavy tree mortality within as few as 2–3 yr in the southern Appalachian Mountains (Trotter and Shields 2009). *A. tsugae* has two asexual generations, sistens and progrediens, on hemlocks which are its secondary host (Annand 1928, McClure 1987). Its primary host, *Picea torano* (Koch) Koehne does not occur in the continental United States (Montgomery et al. 2009). Both the overwintering sistens and progrediens undergo parthenogenic reproduction and oviposit ≈50 and 25 eggs per female, respectively (McClure 1989).

Various sampling protocols including binomial sequential sampling (Fidgen et al. 2006) and randomized branch sampling (Evans and Gregoire 2007) have been proposed and tested to assess *A. tsugae* populations at the individual tree or forest scale (Costa 2005). Randomized branch sampling showed that sistens distribution within the tree crown depended on their population density, suggesting that sampling from the lower regions of the tree alone may not always yield accurate densities (Evans and Gregoire 2007). However, these studies did not evaluate sistens and progrediens adelgid densities throughout the crown over multiple years. Adelgid populations vary through time, and these fluctuations are influenced by tree health and physiology after the initial infestation (McClure 1991a).

This density dependent feedback of *A. tsugae* populations and tree health could indirectly influence the

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performance of predators such as *Laricobius nigrinus* Fender (Lamb et al. 2006). In a companion study, we studied the effect of low rates of imidacloprid insecticide and fertilizer on adelgid abundance and tree growth (Joseph et al. 2011). The purpose of that study was to manipulate tree health and adelgid populations to provide an adequate, uninterrupted supply of high quality adelgids to support long-term predator population growth. As part of that project, we also were interested in how *A. tsugae* populations were distributed within hemlock trees over time, which is the aim of the current study. This information will help to design monitoring strategies for evaluating insecticide efficacy and to improve deployment and sampling of biological control agents.

Materials and Methods

Study Site and Experiment 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). Sixty eastern hemlock trees between 15 and 38 cm in diameter at breast height (dbh), 7.3–24.6 m tall (mean = 15.6 m), and 25–70 yr old were selected based on their accessibility from a forest road for sample collection using a hydraulic lift.

Trees were treated with 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 (untreated check and 0.1× and 0.25× dosages, respectively) and one of two levels of fertilization; fertilized or not. Insecticide was injected into the soil in a circle around the tree ≈30 cm from the tree root collar and 5 cm deep by using a Kioritz soil injector (Kioritz Corp., Tokyo, Japan) on 14 November 2006. One injection point was made for each 2.5 cm of tree diameter by pressing the Kioritz dispensing knob six times to deliver 29.5 ml/2.5 cm dbh of insecticide solution into the soil. The insecticide rates were selected to maintain optimum populations of adelgid as a sustainable food resource for the predators in the future but also to prevent the adelgid from killing the tree. 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 <19.1, 19.1–35.6, and >35.6 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, St. Louis, MO) in total were 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 was applied on 11 June.

Sample Collection and Evaluation. Hemlock terminals from the treated trees were sampled 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, six 60-cm-long hemlock branch terminals per tree were sampled from 30 trees, representing one treatment from each block. Two branch terminals each were cut from the lower, middle, and upper tree crown so that one sample at each crown location (lower, middle, and upper) was taken from the side of the crown facing the road and the other on the opposite or forestside of the crown. In February and June 2008 and 2009, four 30-cm-long terminal branches were sampled per tree from the 60 trees. 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 roadside and the forestside of the crown. The 60-cm samples were initially collected to see whether the adelgid density varied with branch length. Samples were placed in polyethylene bags and transported to the laboratory where they were stored at −5°C.

The 60-cm terminals were subdivided into six 10-cm-long sections. We examined both the distribution of adelgids and the distribution of new tree growth within the tree crown and along the length of the 30-cm-long branches. The numbers of ovisacs, eggs, and nymphs (crawlers and settled first-instars) were counted on each 10-cm section of the 60-cm branch samples and on 30-cm branch samples. The number of new branch shoots, the length of new shoots, and the number of needles on new growth also were measured.

Statistical Analyses. The experiment was arranged in a factorial design consisting of 10 replications in five blocks (two samples per block). Trees were grouped based on proximity to one another and blocks also represented changes in elevation. We selected 60 trees (two trees per treatment per block) for treatment because the study area was scheduled for selective harvesting to remove hazardous trees along the road.

The various rates of insecticide had significant effects on numbers of adelgid ovisacs, eggs, or nymphs and tree growth parameters from the June 2008 sample onward (Joseph et al. 2011). Fertilizer treatments increased eggs per ovisac but had no demonstrable effects on other life stages. Likewise, tree growth parameters were unaffected by fertilization (Joseph et al. 2011). Therefore, we included data from trees treated with insecticide in our analyses for the June 2007 and February 2008 sample dates for which insecticide treatment was not significant. On fertilized trees, we only examined variation in adelgid eggs per ovisac associated with crown position.

The 10- or 30-cm-long sections of each branch were measured along the main branch stem, so they had varying lengths of side branches. Therefore, all adelgid counts and growth parameters were standardized by dividing them with the respective total hemlock branch lengths (main stem plus side branches) within a 10- or 30-cm-long sample and expressed on a per

Table 1. Analysis of variance of effect of tree crown position (lower or upper) on adelgid life stages per cm branch on 30-cm-long *T. canadensis* branches sampled from trees (30 trees in 2007 and 60 trees in 2008–2009) treated with 0, 10, or 25% of 1.5 g imidacloprid per cm tree diameter in spring 2006

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*
All treatments															
Ovisacs	9.3	1, 54	0.003***	6.7	1, 109	0.011**	5.9	1, 109	0.016**	0.0	1, 109	0.931	0.9	1, 109	0.336
Nymphs	28.8	1, 54	<0.001****				21.9	1, 109	<0.001****				0.0	1, 109	0.924
Untreated control															
Ovisacs	4.2	1, 14	0.059*	2.7	1, 29	0.111	1.3	1, 29	0.263	0.3	1, 29	0.543	2.5	1, 29	0.118
Nymphs	14.9	1, 14	0.002***				27.4	1, 29	<0.001****				0.5	1, 29	0.460
Imidacloprid 0.1×															
Ovisacs	1.2	1, 14	0.277	2.0	1, 29	0.165	19.9	1, 29	<0.001****	0.6	1, 29	0.416	1.4	1, 29	0.247
Nymphs	7.1	1, 14	0.018**				28.0	1, 29	<0.001****				0.6	1, 29	0.422
Imidacloprid 0.25×															
Ovisacs	5.6	1, 14	0.033**	2.3	1, 29	0.139	0.0	1, 29	0.762	0.0	1, 29	0.928	1.2	1, 29	0.282
Nymphs	15.6	1, 14	0.001***				2.1	1, 29	0.156				0.0	1, 29	0.781

Analysis performed on log-transformed data. The notations indicate the significant difference (*, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.01$; and ****, $P < 0.001$) of log-transformed data.

centimeter of branch length basis. The data obtained from 10-cm-long subsampling of 60-cm branches in June 2007 was used to shape the sampling procedure in the later dates. Standardized independent variables which included number of ovisacs, eggs, and nymphs (crawlers and settled first instars), number of new shoots, total length of new growth (centimeters), needles on new growth, and nymphs on new growth, were log transformed (ln[x + 1]) to accomplish homogeneity of variances. The transformed data for each sample date were examined using the PROC GLM procedure of SAS (SAS Institute 2003), and means were separated using the least significant difference (LSD) method ($\alpha = 0.05$). We looked for correlations in log-transformed ovisac densities between the upper and lower tree crown by using PROC REG of SAS. Also, we were interested in determining how well adelgid counts from 10- or 30-cm branch sections correlated with adelgid counts from entire 60-cm branch cuttings, so we used PROC CORR of SAS to calculate Pearson's correlations using log–transformed ovisac counts. Untransformed means and standard errors are reported in tables and figures.

Results

Effects of Tree Crown Position on Adelgid Counts. When all the trees were included in the analysis, *A. tsugae* ovisacs per centimeter of branch were more abundant in the upper tree crown than in the lower crown between summers 2007 and 2008 (Table 1; Fig. 1a). However, trees that did not receive insecticide

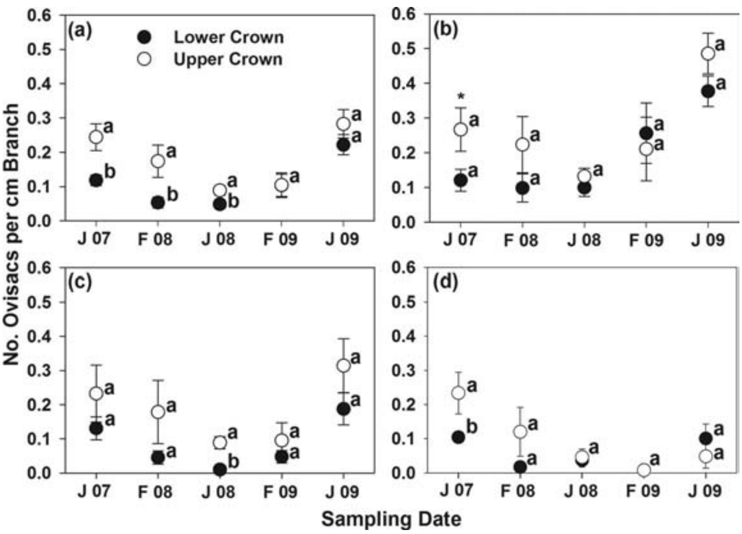

Fig. 1. Effects (means ± SE) of tree crown position on abundance of ovisacs per cm branch from June 2007 to 2009. Graphs are all treatments combined (a), untreated check (b), 0.1× imidacloprid (c), and 0.25× imidacloprid (d). In a, $N = 30$ for 2007 data and $N = 60$ for 2008–2009 data; in b–d $N = 10$ for 2007 data and $N = 20$ for 2008–2009 data. J, June; F, February. Within the same date, circles with the same-case letters are not significantly different ($\alpha = 0.05$; LSD) and asterisks (*) above a pair of circles are significantly different at $\alpha = 0.1$.

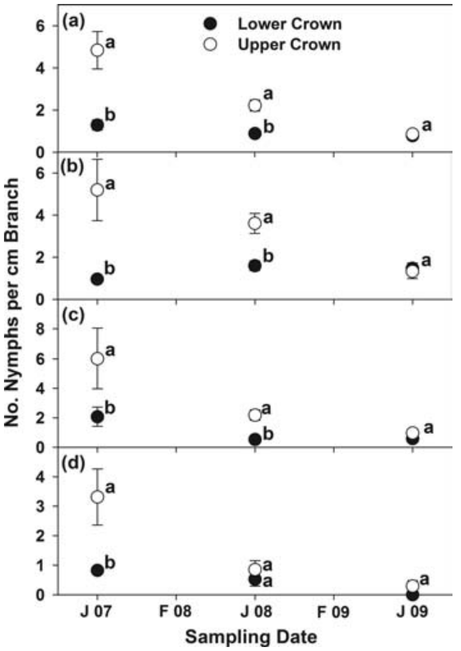

Fig. 2. Effects (means ± SE) of tree crown position (lower and upper) on abundance of adelgid nymphs per cm branch from June 2007 to 2009. Graphs are all treatments combined (a), untreated check (b), 0.1× imidacloprid (c), and 0.25× imidacloprid (d). J, June; F, February. Same-case letters are not significantly different ($\alpha = 0.05$; LSD).

had similar densities of adelgid ovisacs in both crown positions for all the sample dates except in June 2007 where we observed a marginally higher number of ovisacs ($P < 0.1$) in the upper crown (Fig. 1b). In June 2008, significantly greater numbers of ovisacs were recorded in the upper crown of 0.1× dosage trees (Fig. 1c). This is the only sample date where we found a significant difference in the adelgid distribution within 0.1× dosage trees. On 0.25× dosage trees, more ovisacs were found in the upper tree crown in June 2007, whereas later samples had similar densities at both crown positions (Fig. 1d).

Our winter sampling was targeted to sample ovisacs so very few nymphs were recorded in February samples. However, summer samples had sufficient densities of adelgid nymphs for analysis (Table 1; Fig. 2). In 2007 and 2008, the number of sistens nymphs that settled per cm branch was significantly greater in the upper tree crown than in the lower crown (Fig. 2a–d) in all cases except in the 0.25× dosage trees in June 2008. In June 2009, the upper and lower crown had similar numbers of nymphs in all treatments.

In June 2007, we also sampled branches from the mid-crown region. Progrediens ovisacs in the middle and upper crown were not different but fewer of both life stages occurred in the lower crown (Table 2). Crown position did not affect eggs per ovisac, but more nymphs were observed in the upper third of the crown, followed by the middle, and the fewest were in the lower third of the crown (Table 2).

Adelgid eggs per ovisac tended to be higher in the upper crown but not always (Table 3). In the initial sample (June 2007), no differences were found regardless of insecticide treatment or fertilization. The number of eggs laid was greater in the upper tree crown than in the lower crown in February 2008 when all trees were pooled or within untreated trees. In February and June 2009 eggs per ovisac were higher in the upper crown when all trees were combined and in the untreated trees alone. In contrast, *A. tsugae* were more fecund in June 2008 in the lower crown regardless of insecticide rate applied or whether all trees were pooled. We did not see any pattern in eggs per ovisac between tree crown strata within 0.25× dosage trees except during the June 2008 sample period when eggs per ovisac were higher in the lower crown. *A. tsugae* eggs per ovisac were significantly higher in the upper crown of fertilized trees in February 2008 and June 2009 (Table 3), whereas fertilized trees sampled during June 2008 had higher eggs per ovisac in the lower tree crown than in the upper crown. Fertilization had no effect on eggs per ovisac from the two crown positions in February 2009. In summer 2009, adelgids had higher eggs per ovisac in the upper crown regardless of fertilizer.

In June 2008, we counted the numbers of sistens nymphs found on new growth to see whether crown position affected settling position. The densities of nymphs on new growth were not affected by crown

Table 2. Analysis of variance and means ± SE for effect of tree crown positions on *A. tsugae* and tree growth parameters per centimeter of branch length within 60-cm-long branches from 30 *T. canadensis* trees in 2007

Variable	*F*	df	*P*	Tree crown position		
				Lower	Middle	Upper
Adelgid counts						
Ovisacs	6.7	2, 82	0.002***	0.09 ± 0.01b	0.20 ± 0.03a	0.20 ± 0.02a
Nymphs	18.5	2, 82	<0.001****	1.24 ± 0.18c	2.50 ± 0.28b	4.44 ± 0.81a
Eggs	0.3	2, 82	0.725	2.83 ± 0.31a	3.23 ± 0.30a	3.96 ± 0.52a
Growth parameter						
New shoots	2.3	2, 82	0.103	0.003 ± 0.002a	0.009 ± 0.005a	0.021 ± 0.008a
New needles	2.9	2, 82	0.058*	0.092 ± 0.082b	0.321 ± 0.204ab	0.909 ± 0.388a
New shoot length	3.1	2, 82	0.051*	0.004 ± 0.003b	0.014 ± 0.007b	0.048 ± 0.021a

The notations indicate the significant difference (*, $P < 0.1$; ***, $P < 0.01$; and ****, $P < 0.001$) for log-transformed data. Means within rows followed by the same letter are not significantly different ($\alpha = 0.05$ or 0.1; LSD).

Table 3. Analysis of variance and means ± SE for effect of *T. canadensis* tree crown position (lower or upper) on number of eggs per *A. tsugae* ovisac on 30-cm-long branches (from 30 trees in 2007 and 60 trees in 2008–2009) for trees treated with low rates of imidacloprid insecticide in November 2006, and fertilizer in 2007 and 2008

Sample date	Tree crown position	All treatments	Eggs per ovisac				
			% highest labeled rate of imidacloprid			Fertilizer regime	
			Untreated	0.1×	0.25×	Unfertilized	Fertilized
2007 June							
	Lower	3.0 ± 0.3	2.8 ± 0.4	3.6 ± 0.7	2.6 ± 0.3	2.7 ± 0.5	3.3 ± 0.3
	Upper	4.1 ± 0.5	4.2 ± 0.8	4.3 ± 0.9	3.7 ± 1.1	3.5 ± 0.6	4.7 ± 0.8
	F (df)	0.1 (1, 53)	1.3 (1, 14)	0.0 (1, 14)	0.2 (1, 14)	0.4 (1, 23)	0.0 (1, 24)
	P	0.791	0.275	0.997	0.637	0.532	0.905
2008 Feb.							
	Lower	8.1 ± 0.9	6.9 ± 0.9	9.2 ± 2.2	8.7 ± 1.9	7.9 ± 1.1	8.2 ± 1.4
	Upper	11.4 ± 0.9	12.2 ± 1.5	11.2 ± 1.4	10.2 ± 2.2	9.1 ± 1.0	14.4 ± 1.5
	F (df)	5.4 (1, 71)	7.3 (1, 25)	1.6 (1, 15)	0.2 (1, 11)	0.4 (1, 37)	7.0 (1, 23)
	P	0.023**	0.012**	0.223	0.647	0.548	0.014**
2008 June							
	Lower	4.3 ± 0.4	3.7 ± 0.4	4.3 ± 0.7	5.9 ± 1.9	5.1 ± 0.7	3.5 ± 0.5
	Upper	2.6 ± 0.2	2.6 ± 0.4	2.1 ± 0.3	3.3 ± 0.4	2.5 ± 0.3	2.7 ± 0.3
	F (df)	13.4 (1, 75)	6.1 (1, 28)	5.4 (1, 20)	6.7 (1, 9)	1.2 (1, 31)	17.61 (1, 33)
	P	<0.001***	0.019**	0.029**	0.029**	0.287	<0.001***
2009 Feb.							
	Lower	13.6 ± 1.3	13.3 ± 1.5	14.3 ± 2.2	13.5 ± 4.9	10.5 ± 1.6	16.5 ± 1.8
	Upper	16.0 ± 1.0	17.5 ± 1.6	14.4 ± 1.1	11.0 ± 1.9	14.2 ± 1.5	17.9 ± 1.4
	F (df)	3.7 (1, 58)	5.9 (1, 29)	0.1 (1, 10)	0.0 (1, 4)	0.5 (1, 49)	0.0 (1, 49)
	P	0.059*	0.021**	0.741	0.907	0.472	0.863
2009 June							
	Lower	9.2 ± 0.6	8.6 ± 0.5	10.6 ± 1.3	8.4 ± 1.6	9.1 ± 0.9	9.2 ± 0.8
	Upper	12.9 ± 0.8	12.2 ± 1.1	13.3 ± 1.4	14.9 ± 2.1	12.7 ± 0.9	13.0 ± 1.3
	F (df)	12.0 (1, 69)	4.7 (1, 29)	3.5 (1, 16)	2.4 (1, 3)	4.6 (1, 29)	9.4 (1,29)
	P	<0.001***	0.039**	0.015**	0.219	0.040**	0.005***

The notations indicate the significant difference (*, $P < 0.1$; **, $P < 0.05$; and ***, $P < 0.01$) of log-transformed data within dates and treatments.

position (all trees: $F = 0.7$; df = 1, 89; $P = 0.394$, untreated check: $F = 2.7$; df = 1, 19; $P = 0.112$, 0.1× dosage: $F = 0.0$; df = 1, 23; $P = 0.911$, and 0.25× dosage: $F = 1.6$; df = 1, 25; $P = 0.217$). Moreover in February 2009, sistens ovisacs ($F = 0.1$; df = 1, 83; $P = 0.812$), progrediens eggs ($F = 0.2$; df = 1, 83; $P = 0.663$), and eggs per ovisac ($F = 1.1$; df = 1, 30; $P = 0.301$) of females that developed on the previous-year's shoots (in 2008) were not significantly affected by crown position.

Ovisac densities in the lower crown exhibited a significant correlation with ovisacs in the upper crown in June 2008 ($r^2 = 0.09$; $t = 2.4$; df = 1, 58; $P = 0.019$), February 2009 ($r^2 = 0.31$; $t = 5.1$; df = 1, 58; $P < 0.001$), and June 2009 ($r^2 = 0.37$; $t = 5.8$; df = 1, 58; $P < 0.001$). In June 2007 ($r^2 = 0.01$; $t = 0.4$; df = 1, 28; $P = 0.634$) and February 2008 ($r^2 = 0.06$; $t = 1.8$; df = 1, 58; $P = 0.069$), no correlations were found.

Effects of Tree Crown Position on Tree Growth. Crown position had relatively little effect on the number of new shoots produced on branches, and no consistent trends were evident (Table 4; Fig. 3). The largest differences were noted on untreated trees that had significantly more new shoots in the lower crown in 2008 and 2009 (Fig. 3b). Trees treated with 0.1× dosage insecticide had similar amounts of new growth in the upper and lower crown for all summer sample dates (Fig. 3c), whereas the 0.25× dosage trees had slightly more new shoots in the upper crown in 2009 (Fig. 3d). Other growth measurements (new needles and new shoot length) were similar to the numbers of new shoots, i.e., untreated trees had more new needles and longer new shoots in the lower crown in June 2009, whereas 0.25× dosage trees had more needles and longer shoots in the upper crown (Table 4; Fig. 4).

Effects of Distance From Branch Tip. In June 2007, numbers of progrediens ovisacs per cm branch were greater on branch tips as compared with regions of the branch nearer the tree trunk when all tree sample positions were combined ($P < 0.1$) ($F = 2.0$; df = 5, 170; $P = 0.082$) and for the lower ($F = 1.9$; df = 5, 164; $P = 0.091$) and middle ($F = 2.2$; df = 5, 170; $P = 0.057$) crown positions (Fig. 5a–c) but not for the upper crown position (Fig. 5d). Density of settled nymphs and adelgid eggs per ovisac were unaffected by distance from the branch tip regardless of crown position. Likewise, numbers of new shoots, new needles, and length of new growth were similar regardless of location along the branches ($P > 0.1$). Ovisac densities in the 60-cm-long hemlock branch exhibited a significant correlation with ovisacs in the terminal 10 cm ($R = 0.75$, $P < 0.001$; $n = 30$) or the terminal 30 cm ($R = 0.95$, $P < 0.001$; $n = 30$) when all the samples per tree were combined.

Discussion

Adelgid population estimates are more accurately acquired through sampling of several hemlock tree crown strata. Our results show that adelgid ovisacs and nymphs tend to be higher in the upper crown than in the lower crown, so sampling only the lower crown may not provide accurate estimates of adelgid densities. We did observe significant correlations between

Table 4. Analysis of variance of effects of crown positions (lower or upper) on *T. canadensis* tree growth parameters per cm of branch from 2007 to 2009 for trees treated with untreated check, 0.1×, or 0.25× of the recommended rate of imidacloprid insecticide in November 2006 and fertilizer in 2007 and 2008

Tree growth parameter	2007 June			2008 June			2009 June		
	F	df	*P*	*F*	df	*P*	*F*	df	*P*
All treatments									
New shoots	3.1	1, 54	0.079*	3.6	1, 109	0.060*	0.3	1, 109	0.568
New needles	4.3	1, 54	0.041**	0.0	1, 109	0.789	0.6	1, 109	0.434
New shoot length	3.7	1, 54	0.059*	1.6	1, 109	0.199	0.0	1, 109	0.929
Untreated control									
New shoots	1.6	1, 14	0.225	5.3	1, 29	0.028**	12.1	1, 29	0.002***
New needles	1.8	1, 14	0.194	2.9	1, 29	0.096*	11.8	1, 29	0.002***
New shoot length	1.2	1, 14	0.282	0.4	1, 29	0.530	11.5	1, 29	0.002***
Imidacloprid 0.1×									
New shoots	1.0	1, 14	0.335	2.5	1, 29	0.119	0.7	1, 29	0.402
New needles	1.6	1, 14	0.215	0.7	1, 29	0.392	0.7	1, 29	0.401
New shoot length	1.9	1, 14	0.182	0.0	1, 29	0.777	0.1	1, 29	0.667
Imidacloprid 0.25×									
New shoots	1.2	1, 14	0.282	0.1	1, 29	0.757	4.0	1, 29	0.054*
New needles	1.4	1, 14	0.256	2.7	1, 29	0.111	6.6	1, 29	0.015**
New shoot length	1.1	1, 14	0.296	4.1	1, 29	0.050*	8.4	1, 29	0.007***

Analysis performed on log-transformed tree growth parameters data. Growth parameters were only measured on hemlock branches during summer. The notations indicate significant differences (*, $P < 0.1$; **, $P < 0.05$; and ***, $P < 0.01$).

adelgid ovisac densities in the upper crown with densities in the lower crown of the same trees for three of the sample dates. However, on the other two dates the relationships were not significant and the R^2 values were low so predicting upper crown densities from lower crown samples would be imprecise. Gray et al. (1998) sampled both upper and lower crown positions, but they did not report differences in adelgid densities between them. Past research using randomized branch sampling has shown that *A. tsugae* infestation levels may influence its distribution within the tree crown, at least for sistens populations (Evans and Gregoire 2007). They observed that sistens densities were higher in the upper crown when trees had low level infestations but this pattern was reversed for high levels of infestations. They also suggested that adelgid densities were likely to be greater in the upper strata of the crown on trees that have low or new infestations. Our data support this idea. When all trees were combined, ovisac densities were higher in the upper crown of the trees until February 2008 and nymphs were higher through June 2008. After that date differences in adelgid densities were no longer evident between the upper and lower crown. However, in a hemlock stand with an established infestation the density of *A. tsugae* might vary depending on tree health (McClure 1991a, Pontius et al. 2006), minimum winter temperature, seasonal temperatures (Trotter and Shields 2009), latitude (Orwig et al. 2002), and their interactions. Therefore, an accurate estimate of *A. tsugae* populations can only be achieved by quantifying their density throughout the crown. Sampling the upper and lower crown regions and pooling the subsamples into an estimate for the tree is likely to be the best approach to obtain accurate assessments of adelgid densities in a given tree.

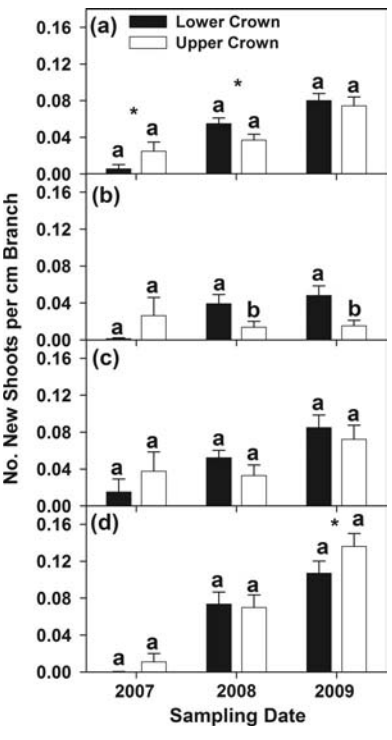

Fig. 3. Effects (means ± SE) of tree crown position (lower and upper) on abundance of new shoot growth per cm branch in *T. canadensis* from June 2007 to 2009. Graphs are all treatments combined (a), untreated check (b), 0.1× imidacloprid (c), and 0.25× imidacloprid (d). In a, $N = 60$ for 2007 data and $N = 120$ for 2008–2009 data and in b–d, $N = 20$ for 2007 data and $N = 40$ for 2008–2009 data. Same-case letters are not significantly different at $\alpha = 0.05$ (LSD), and asterisks (*) above a pair of bars are significantly different at $\alpha = 0.1$.

Hemlock branch terminals are often sampled to determine the insecticide efficacy by using a pole pruner to reach branches that are otherwise inaccessible from the ground, and probably this is the most feasible procedure for sampling trees in the forest interior (Gouger 1971; Webb et al. 2003; Doccola et al. 2005, 2007; Cowles et al. 2006; Cowles 2009). We sug-

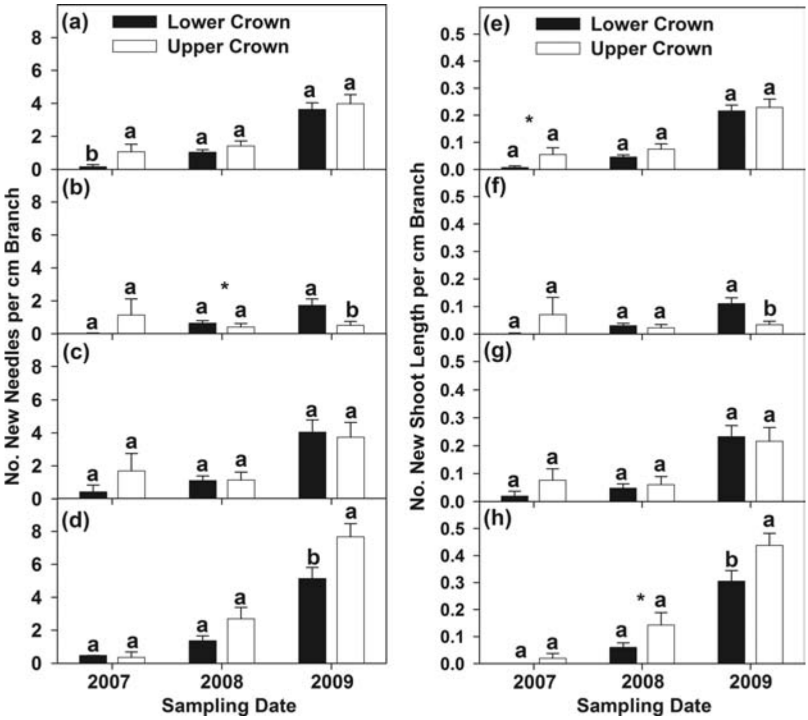

Fig. 4. Effects (means ± SE) of tree crown position on new needles and shoot length per cm branch from June 2007 to June 2009. Graphs are all treatments combined (a and e), untreated check (b and f), 0.1× imidacloprid (c and g), and 0.25× imidacloprid (d and h). In a and e, $N = 60$ for 2007 data and $N = 120$ for 2008–2009 data and in b–d and f–h, $N = 20$ for 2007 data and $N = 40$ for 2008–2009 data. Within the same date and graph, bars with the same case letters are not significantly different at $\alpha = 0.05$ (LSD), and asterisks (*) above a pair of bars are significantly different at $\alpha = 0.1$.

gest that, wherever possible, including samples from the upper tree crown would provide a better appraisal of the adelgid density but recognize the current limitations of sampling the upper crowns in forests.

Sampling procedures vary in their purpose. Costa and Onken (2006) and Fitzpatrick et al. (2009) provide methods for early detection and monitoring of infested stands, whereas Fidgen et al. (2006) estimated relative levels of infestation for individual trees. In their nondestructive binomial sequential sampling only branches from the lower third of the crown were examined (Fidgen et al. 2006). An in situ visual observation of adelgid woolly ovisacs on a certain number of trees can provide an estimate of the general infestation level in an area (Costa and Onken 2006, Faulkenberry et al. 2009). However, Fitzpatrick et al. (2009) reported a high incidence of biased misidentifications between trained observers and volunteers in the monitoring program developed by Costa and Onken (2006), especially when *A. tsugae* infestation on a given hemlock tree was low. Based on our observations, we suggest that sistens woolly ovisacs might be adequately visible to record infestation, but it might be difficult to distinguish progrediens woolly ovisacs because they occur along with the dead sistens or even with the previous-year progrediens woolly mass in a cluster on hemlock branches leading to population overestimates.

We found that adelgids tend to be more abundant in the upper crown, especially early in the infestation. If adelgid predators seek higher prey densities for oviposition or establish better at higher densities, then this study suggests that predator releases and subsequent recovery efforts should focus on the upper crown, if possible, where higher prey densities are more likely. Most predator releases have been made on the lower third of the hemlock tree crown (McClure and Cheah 1998, 1999; McClure et al. 2000; Flowers et al. 2006; Lamb et al. 2006). Although adult predators can fly, their ability to locate and colonize branches based on adelgid density is not well understood. Because larval mobility is limited, predator larvae might have a greater chance of survival if placed on shoots having a patch of high adelgid density. Future research should examine the pattern of predator establishment within the tree crown to improve open release of adult predators in the forest.

Interestingly, the new growth, which presumably adelgid nymphs prefer and settle on more frequently (McClure 1991a, Lagalante et al. 2006), was not more abundant in the upper crown. We did not find any difference in new growth within the tree crown of the control trees initially in summer 2007; however, new shoots were more abundant in the lower crown in summers 2008 and 2009. One potential explanation may be tree stress caused by the adelgids, particularly

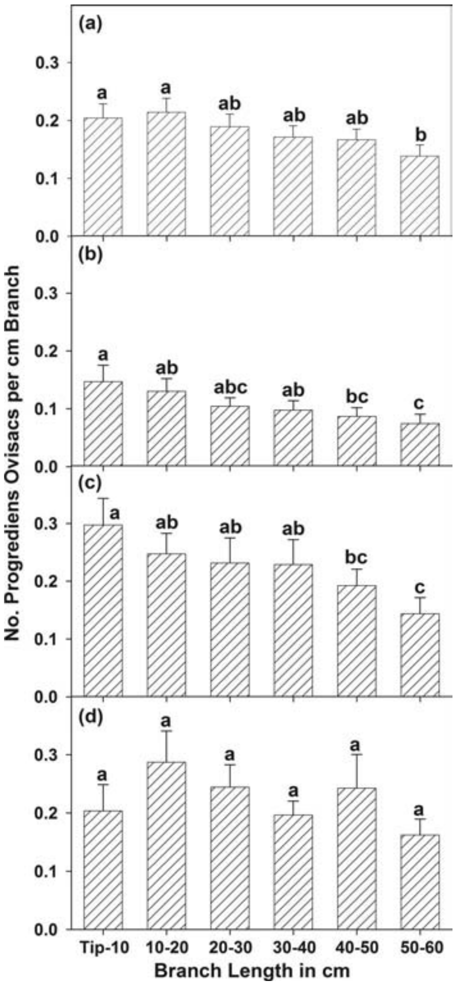

Fig. 5. Effects (means ± SE) of distance from branch tip of 60-cm-long *T. canadensis* branches on hemlock woolly adelgid ovisac abundance ($N = 180$) in June 2007. The graphs represent tree crown position: whole tree (a), lower (b), middle (c), and upper (d). Bars of the same fill color with the same letters are not significantly different (α = 0.1; LSD).

in the upper crown where adelgid densities were higher in 2007 and winter 2008, which impacted the upper crown more, resulting in a reduction in new growth in that region. Throughout the study, trees that received insecticide had a uniform density of new shoots between tree crown positions. Previously, enhanced plant growth has been reported in hybrid poplar, *Populus nigra* L. (Chiriboga 2009), and cotton, *Gossypium hirsutum* L. (Gonias et al. 2007, Hundley 2004) after imidacloprid treatment. Thus, it is also possible that imidacloprid insecticide improved the tree health (Rebek et al. 2008) and facilitated a uniform distribution of new growth within the crown.

First-instars were abundant in the upper crown of trees regardless of insecticide treatments on all sample dates. Warmer conditions in the upper tree crown might have caused early progrediens egg laying and egg hatch. Therefore, if nymphal stages are included in assessments of adelgid densities, samples should be taken from the same crown position. Because new growth is not evenly distributed and the nymphs are distributed throughout the branches, not just on new growth, it may not be reliable to estimate sistens nymphal density by merely counting them on the new growth.

McClure (1991b) found a two-fold increase in adelgid eggs per ovisac on fertilized *T. canadensis.* Likewise, fertilization had a small but significant effect on eggs per sistens ovisac on our trees in 2008 and 2009 (Joseph et al. 2011). However, this study shows that eggs per ovisac were not consistently higher in a particular crown region. For example, adelgids were more fecund in the upper tree crown in February 2008 and June 2009, but adelgids in that region were less fecund in June 2008. In June 2009, eggs per ovisac were higher in the upper crown regardless of fertilizer treatment.

Because most adelgid stages remain stationary and consume stored reserves from hemlock branches, quality of these branches might be important for their survival or reproduction. Active allocation, reallocation, or metabolism of nutrients occurs within the tree to generate new shoot growth during the summer (Nommik 1966, Miyazawa et al. 2004). Progrediens tended to be denser on the terminal tip region of previous-year branches than on the region furthest away from the branch tip on untreated or 0.1× dosage trees. However, branches sampled from trees treated with 0.25× insecticide had a similar distribution of progrediens ovisacs over the entire branch. These results suggest that sampling terminal tips of hemlock branches may be adequate for estimating adelgid density regardless of crown position.

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