

# Survival and Near Extinction of Hemlock Woolly Adelgid (Hemiptera: Adelgidae) During Summer Aestivation in a Hemlock Plantation

ELIZABETH M. SUSSKY<sup>1</sup> AND JOSEPH S. ELKINTON<sup>1,2,3</sup>

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**ABSTRACT** Varying densities of the spring generation of the hemlock woolly adelgid were manipulated on 16 previously uninfested eastern hemlocks in an open-field plantation in Massachusetts. In contrast to experimentally created hemlock woolly adelgid populations in a forest, as reported previously, there was no evidence of density-dependent survival on a tree-wide basis in the plantation in the spring (progre-diens) generation. There was, however, comparable density-dependent survival of settled crawlers and sexupara production when samples of the population were examined from branches with high density. Plantation hemlocks had 9.3 times more foliage and 10 times lower adelgid densities per cm than the forest hemlocks. These results show that density-dependent processes in the progre-diens generation may only be evident when hemlock woolly adelgid density reaches a critical threshold. In the sistens generation that begins in midsummer, we counted a mean of 126 settled crawlers on marked branch on all 16 trees, but none of these adelgids survived the mid-summer aestivation phase, perhaps due to higher temperatures that were recorded in the plantation compared with a nearby hemlock forest, where 16% of the adelgids survived the aestivation phase. Whole tree counts of overwintering adelgids revealed that the adelgid populations had gone extinct on 13 out of the 16 trees. Mortality in the midsummer aestivation phase often exceeds overwintering mortality that has been widely thought to be the main factor that limits adelgid population growth and spread, particularly in northern states.

**KEY WORDS** population regulation, density dependence, sexuparae, forest insect, aestivation

## Introduction

The hemlock woolly adelgid, *Adelges tsugae* Annand, is an invasive forest insect from Japan that has been killing eastern hemlocks (*Tsuga canadensis* Carrierre) by the millions in the eastern United States (Orwig and Foster 2008, Orwig et al. 2012), but the rate of hemlock mortality has slowed in central New England at the northern edge of the invasion front (Orwig et al. 2012). This fact has been widely attributed to overwintering mortality of the adelgid being much higher in New England than further south (Paradis et al. 2008, Trotter and Shields 2009, Orwig et al. 2012). Here we show a significant decline in hemlock woolly adelgid density in Massachusetts occurring during the late summer aestivation phase associated with the higher temperatures that occur in a hemlock plantation growing in full sun, in contrast to a nearby population growing beneath the shade of a forest canopy. We argue that mid-summer mortality is a poorly understood component of adelgid survival and population growth.

This study complements our recent report (Sussky and Elkinton 2014) of density-dependent survival in an

experimentally created hemlock woolly adelgid populations in a forest setting. Our results demonstrated density-dependent processes that stabilized the population densities and suggested that these processes may explain the long-term survival of adelgid-infested hemlocks in the northeastern United States. These findings were consistent with those of McClure (1991) and Paradis (2011), who documented similar density-dependent mortality in naturally occurring populations of hemlock woolly adelgid in forests in southern New England.

Here we use the same methods to create experimental populations of the adelgid in an open-grown plantation of hemlocks, and we compare the adelgid survival in this setting to that we previously reported in a forest.

## Materials and Methods

**The Study System.** Hemlock woolly adelgids feed on the ray parenchyma cells of hemlocks by inserting their stylets at the base of needles (Young et al. 1995). As the adelgids develop, a white, protective wool-like wax is secreted from the female in which it will eventually oviposit (McClure 1989, 1991). Hemlock woolly adelgids have two parthenogenic generations per year. The overwintering sistens generation lays eggs that

<sup>1</sup> Graduate program in Entomology, Department of Plant, Soil and Insect Sciences, University of Massachusetts, Amherst, MA 01003.

<sup>2</sup> Department of Environmental Conservation, University of Massachusetts, Amherst, MA 01003.

<sup>3</sup> Corresponding author, e-mail: elkinton@ent.umass.edu.

hatch in April and May, producing the springtime progrediens generation (McClure 1989).

Some progredientes (plural of progrediens) become winged sexuparae, which in their native Japan, disperse to spruce (*Picea torano*) and initiate a sexually reproducing generation. In North America, however, none of the offspring of these sexuparae survive on the native spruce species, so there is no sexual life cycle and only parthenogenic reproduction occurs (McClure 1989). Thus sexupara production in North America results in mortality. The asexual progrediens crawlers settle on hemlock shoots produced in the previous year. They mature and oviposit in June, and eggs hatch in late June or July. The resulting sistens crawlers (fall or winter generation) settle preferentially on current-year shoots, and enter a period of summer aestivation, where feeding and development cease. Feeding and development resume in October, and continue throughout winter. In the northeastern United States, the sistentes (plural of sistens) oviposit in March, and eggs hatch in late spring.

**The Experimental Site.** The experiment was conducted in a small hemlock plantation located at the University of Massachusetts Crops Research and Education Farm in South Deerfield, Massachusetts (42.4769° N, 72.583° W). The plantation was established in fall of 2007 with 1-m-tall hemlock seedlings from Cheshire Nursery Garden Center (Wallingford, CT). The plantation comprised approximately 130 hemlock trees arranged in a grid at 2 m spacing between trees and exposed to full sunlight on agriculturally modified soils. For the next four winters (December–March), the trees were protected from winter wind damage with a 1.5-m-tall barrier of landscape fabric. All of the hemlocks in this plantation were uninfested with hemlock woolly adelgid when the experiment was launched in May 2012.

**Experimental Design.** Trees in the plantation were inoculated with adelgids by attaching 30-cm cut branches of eastern hemlock heavily infested with mature ovisacs of the overwintering sistens generation to experimental hemlocks just before the spring generation crawlers began to hatch. Infested inoculum branches of eastern hemlock were collected from a nearby forest on 17 April 2012, grouped into bundles of 1, 3, 10, or 30, placed in water-saturated foam overnight, and attached to experimental hemlocks the following day.

Experimental trees consisted of 16 uninfested hemlocks growing in a 4 × 4 block in the southeast corner of the plantation. All the trees were each inoculated with a total of 30 twigs, with 1, 3, 10, or all 30 of the twigs being heavily infested with healthy adelgid ovisacs, and the remaining twigs free of adelgids. The twigs were all attached to one large branch at the top of each experimental tree.

**Estimating Adelgid Density.** After successful establishment of the spring generation progrediens crawlers, on 18 May 2012 the twigs used as the source of adelgid inoculum were removed and brought to the laboratory in order to count the number and estimate the fecundity of the sistens ovisacs used to inoculate

experimental trees. Fecundity of these sistens ovisacs was determined by the average number of chorions from hatched eggs in three woolly ovisacs on each bundle of inoculum twigs. These numbers were then averaged across all 16 trees in the stand. The number of progrediens crawlers produced on each experimental branch or tree was estimated by multiplying the number of sistens ovisacs on the inoculum branches on each tree by the mean fecundity. This procedure converted the four density categories of infested inoculation twigs (1, 3, 10, 30 twigs) to an estimated number of progrediens crawlers released (a continuous variable) on each experimental tree. We observed no settlement of the hatching crawlers on the inoculation twigs, which were not supplied with water after they were attached to the experimental trees.

To estimate sexupara production in the spring generation, sample twigs were cut from each inoculated tree on 24 May 2012 and placed in water picks shortly following the establishment of spring generation crawlers. Populations of hemlock woolly adelgid on these cut twigs were reared in a laboratory rearing room (mean temperature 21°C) in small Berlese funnels (BioQuip Products, Rancho Dominguez, CA) to collect emerging sexuparae. Settled nymphs were counted under the microscope, and the survival and fecundity of those adelgids that lived long enough to secrete wool and reproduce was determined. The total number of progredientes that reached maturity was also recorded on each experimental hemlock in the field.

In early August, a branch containing visible settled sistentes from each experimental tree was selected and the terminal 30 cm were marked off. On each branch terminal, the proportion of twigs with new growth, the total length of new growth (cm), and the number of settled sistentes were recorded in field observations. Aestivation survival was determined by recording the number of sistentes that survived to produce wool in December on both the 30-cm sample branches and on the entire experimental tree.

In order to estimate adelgid density (numbers per cm) on a whole tree basis, we used the following procedure to estimate the total cm of branches with foliage on each tree. For each experimental tree at the plantation, the length and width of each limb at its widest point was measured, in combination with the total branch length of an individual branch from eight different trees. Plotting these two measures against each other yielded the following equation: total cm = 0.281(length × width) + 601.1 ( $R^2 = 0.963$ ; see [Supp Fig. 1](#) [online only]), which we used to obtain an estimate of total twig length on the entire tree from length × width measurements of all main branches (those attached to the stem) of the 16 experimental trees. The regression equation is strongly linear because the number of cm within a branch increases linearly with branch area (length × width) as the branch grows.

Beginning in early July, air temperature data were collected every 2 h using iButtons (Maxim Integrated, San Jose, CA). Two iButtons were placed at the hemlock plantation in South Deerfield, and for comparison,

we placed four more in a forested site (Quabbin Reservoir) where we had similar ongoing experiments reported in Sussky and Elkinton (2014). Daily maximum and average temperatures were also obtained for May through September 2012 from the nearest NOAA weather stations, namely the South Deerfield weather station, South Deerfield MA (Lat/Long: 42.480° N 72.579° W) located 425 m from the hemlock plantation, and the Belchertown weather station (42.253° N 72.359° W): in Belchertown MA, located 20.78 km from our forest site and 30.65 km from the hemlock plantation.

**Statistical Analyses.** The effects of hemlock woolly adelgid progrediens crawler and settled progrediens densities, as well as the length of hemlock new growth, on adelgid survival in the progrediens life stage were analyzed by logistic regression (Proc LOGISTIC, SAS Institute 2012). All densities were  $\log_{10}$  transformed for these analyses. The effects of adelgid density on fecundity or length of new growth were analyzed by simple linear regression (Proc REG, SAS Institute 2012). Wilcoxon's nonparametric rank sum test (Proc NPARIWAY, SAS Institute 2012) was used to compare survival during the summer aestivation phase of the adelgids with that recorded simultaneously in our forest experiment (Sussky and Elkinton 2014).

## Results

The survival of the inoculated spring (progrediens) generation on the entire tree was not density dependent (Fig. 1A; Table 1). In contrast, survival of settled progrediens crawlers declined with density (Fig. 1B; Table 1). The proportion becoming wingless progredientes instead of winged sexuparae also declined with density of progrediens crawlers (Fig. 1C; Table 1) and with density of settled progredientes (Fig. 1D; Table 1). There was no significant effect of settled crawler density on adult progrediens fecundity (Fig. 1E; Table 2). The length of new growth on hemlocks was unrelated to progrediens fecundity (Fig. 1F; Table 2) or the survival of settled progredientes (Fig. 1H; Table 1). We show these two graphs because the amount of new growth is a measure of hemlock health, which might influence progrediens survival or fecundity, even though the progrediens individuals settle exclusively on old growth tissue (McClure 1991). Likewise, settled progrediens density also had no effect on the amount of new growth produced later that summer by the infested hemlocks (Fig. 1G; Table 2).

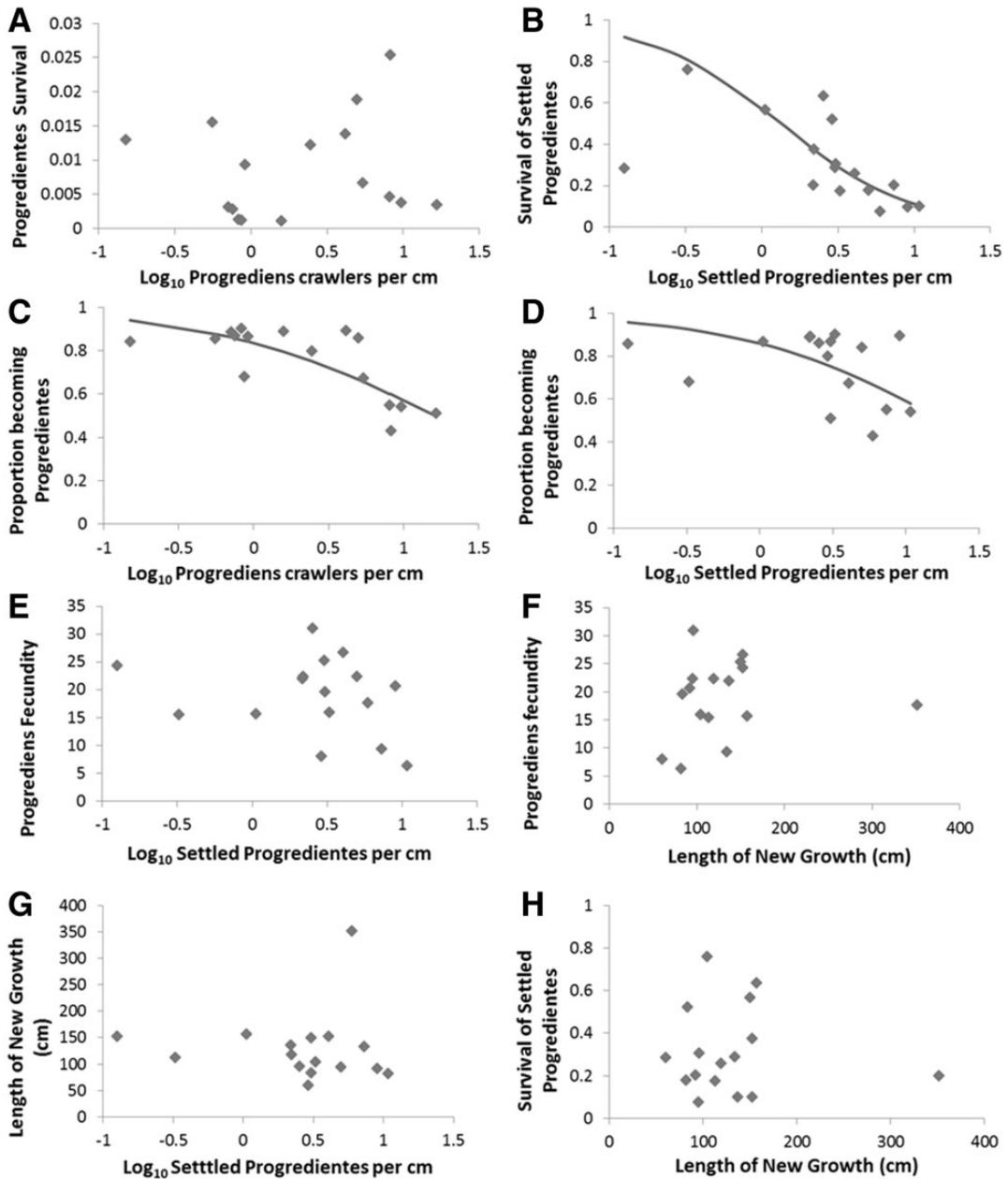
In early August, the number of settled sistentes recorded on a terminal 30-cm sample branch averaged  $126.1 \pm 28.5$  SE sistentes per branch ( $n = 16$ ). By early December, none of the aestivating sistentes on our sample branches had produced wool, indicating that aestivation mortality was 100% across all treatments. All 16 trees were thoroughly searched, and no hemlock woolly adelgids were found on 13 trees. Small clusters of adelgids that were putting on wool (had broken aestivation) were found on the shaded inner portions of foliage near the bole of three trees. The aestivation survival of adelgids in the plantation was significantly less

than the survival during the same period in our forest experiment (Wilcoxon's approximate  $Z = -5.84$ ,  $P < 0.001$ ), where the average aestivation survival on 64 trees or branches was  $16.2 \pm 2.0\%$  SE.

The monthly maximum temperatures and average temperatures at the two nearest weather stations (Table 3) were comparable with one another, but the temperatures recorded by the iButtons in the plantation were several degrees warmer than at the nearby weather stations, whereas the temperatures recorded by iButtons in the forest understory were several degrees cooler than the nearby weather stations (Table 3). The maximum iButton temperature recorded in the South Deerfield plantation was  $36.75^\circ\text{C}$  compared with  $31.62^\circ\text{C}$  in the forest (Table 3).

## Discussion

Sussky and Elkinton (2014) described pronounced density-dependent mortality occurring primarily in the spring progrediens generation in experimentally created adelgid populations on understory hemlocks in a mixed hardwood-hemlock forest in Massachusetts. Here we present data from a parallel study conducted using the same methods and in the same year as Sussky and Elkinton (2014), but in a hemlock plantation growing in full sunlight 15.8 km from the forested site. When compared with the total amount of foliage (cm) on hemlock saplings in the forested site, the plantation hemlocks were on average 9.3 times larger. The differences in tree size resulted in substantial differences in progrediens crawler density which ranged from 0.32 to 334.58 crawlers/cm (mean = 33.81) on forested hemlocks but only 0.10 to 10.25 crawlers/cm (mean = 2.54) on the plantation hemlocks. Paradis (2011) reported densities of progrediens crawlers that ranged from 72–388/cm (mean = 167) in her study of natural infestations of hemlock woolly adelgid in six populations from forested sites in southern New England over a four-year period. These densities were approached in the forest experiment (Sussky and Elkinton 2014) only at the highest levels of inoculation, but the densities in the plantation experiment described here were lower by an order of magnitude at least. The mean density of hemlock needles/cm was  $14.89 (\pm 0.86 \text{ SE})$ , for an average of 2.21 progrediens crawlers per needle on forest hemlocks, and 0.14 crawlers per needle on plantation hemlocks. There were on average 0.20 settled progrediens crawlers per needle on the forest hemlock samples, and 0.28 settled crawlers per needle on the plantation hemlock samples. As explained above, the settled crawler densities in the plantation were from branches with higher settled crawler densities than were typical for the tree as a whole. As hemlock woolly adelgid crawlers settle and feed at the base of hemlock needles, it may well be competition for space at the needle bases that limits the number of settled crawlers and the resulting maximum density adelgid adults. However, both experiments have shown that density-dependent processes can affect the survival of settled crawlers at densities well below one adelgid per



**Fig. 1.** Survival and fecundity of the progrediens generation as a function of hemlock woolly adelgid density, fecundity, survival, and new hemlock growth per cm on sample branches. (A) Total survival in the progrediens generation versus progrediens crawler density; (B) Survival of settled progrediens crawlers versus their density; (C) Proportion of settled crawlers that became progrediens instead of sexuparae versus progrediens crawler density or (D) settled crawler density. (E) Progrediens fecundity versus density of settled crawlers or (F) length of new growth on hemlock on sample branches; (G) Effect of progrediens crawler density on mean length of new hemlock growth (cm); (H) Effects of length of new growth on progrediens survival. Regression lines are plotted only when they are statistically significant ( $P < 0.05$ , Table 1).

needle. The much lower overall densities in the plantation setting probably explains why the same density-dependent processes over the entire progrediens stage that had been documented in Sussky and Elkinton (2014), or that Paradis (2011) reported from her study

of natural hemlock woolly adelgid populations, were not observed in this experiment.

In the Sussky and Elkinton (2014) forest experiment, density-dependent competition for resources, or some other facet of interaction with the host tree, probably

**Table 1. Logistic regression analysis of effects of progrediens density and length of hemlock new growth on progrediens survival and sexupara production in a hemlock plantation (graphs shown in Fig. 1)**

Fig. 1	Dependent variable <i>P</i>	Independent variable <i>X</i>	Logistic parameter $\pm$ SE		Test of significance	
			A	B	Wald $\chi^2$	$P > \chi^2$
A	Total progrediens survival	Progrediens crawler density	-4.66 $\pm$ 0.02	0.023 $\pm$ 0.02	0.87	0.35
B	Settled progrediens survival	Settled progrediens density	0.29 $\pm$ 0.12	-2.35 $\pm$ 0.19	150.7	<0.001 <sup>a</sup>
C	Proportion becoming progredientes	Settled progrediens density	1.81 $\pm$ 0.14	-1.44 $\pm$ 0.19	60.61	<0.001
D	Proportion becoming progredientes	Progrediens crawler density	1.63 $\pm$ 0.09	-1.34 $\pm$ 0.11	149.8	<0.001
H	Settled progrediens survival	Length of new growth (cm)	-1.31 $\pm$ 0.001	0.001 $\pm$ 0.001	1.71	0.19

Logistic regression:  $\ln(p/(1-p)) = a + bx$ , where  $p$  is the proportion surviving or becoming progredientes (or not becoming sexuparae),  $a$  and  $b$  are fitted parameters, and the independent variable  $x$  is  $\log_{10}$  (hemlock woolly adelgid density) or length of new growth (cm).

<sup>a</sup> Bold text indicates  $P \leq 0.05$ .

**Table 2. Linear regression analysis of factors influencing progrediens fecundity or new growth of hemlocks (graphs shown in Fig. 1)**

Fig. 1	Dependent Variable	Independent Variable	Intercept	Slope	R <sup>2</sup>	Test of significance	
						T	P
E	Progrediens fecundity	Settled progrediens density	17.02 $\pm$ 2.2	4.64 $\pm$ 3.4	0.11	1.84	0.20
F	Progrediens fecundity	Length of new growth (cm)	17.19 $\pm$ 4.0	0.01 $\pm$ 0.03	0.02	0.48	0.64
G	Length of new growth (cm)	Settled progrediens density	134.07 $\pm$ 19.63	-13.17 $\pm$ 30.78	0.013	-0.43	0.68

Linear regression on data presented in Fig. 1 (Proc REG, SAS Institute 9.2). Adelgid densities transformed to  $\log_{10}$ .

**Table 3. Monthly average and maximum air temperatures recorded in summer months 2012 at NOAA weather stations or from iButtons placed in the hemlock plantation in S. Deerfield MA and at a nearby forest understory site close to the Quabbin Reservoir (15 km away) from which we recorded summer aestivation mortality (Sussky and Elkinton 2014)**

Month	Absolute monthly maximum				Monthly average			
	Hemlock Plantation		Forest understory		Hemlock plantation		Forest understory	
	Weather Station <sup>a</sup>	i-Button	Weather Station <sup>b</sup>	i-Button <sup>c</sup>	Weather Station	i-Button	Weather Station	i-Button
May	31.67		33.22		16.00		16.67	
June	35.56		34.67		18.44		18.72	
July	34.72	36.75	34.11	31.62	23.00	23.87	23.17	20.86
Aug.	33.89	36.00	33.06	29.20	21.56	22.93	22.22	19.45
Sept.	30.17	31.25	30.00	24.38	16.56	16.82	16.89	14.45
Max	35.56	36.75	34.67	31.62				

Data from—<sup>a</sup> NOAA weather stations, May–September 2012, South Deerfield, MA,

<sup>b</sup> Belchertown, MA, and

<sup>c</sup> from air temperatures recorded every 2 h by iButtons deployed at each site from July–September.

caused the overall density-dependent mortality during the progrediens stage. That same density-dependent survival is evident in our branch samples of settled progredientes in the plantation experiment (Fig. 1B), as well as in the forest experiment (Sussky and Elkinton 2014). Similarly, sexupara production on these branches (Fig. 1C, D) was density dependent in both experiments. In the plantation experiment reported here, however, branches used to estimate sexupara production, as well as those selected for aestivation survival, were chosen among those with observable progredientes and sistentes and thus did not represent a random sample among all branches in the tree, most of which contained no observable adelgids. On the smaller trees in the forest experiment, all branches contained observable progredientes and sistentes, and the samples were selected at random.

In a similar hemlock woolly adelgid inoculation experiment in a hemlock plantation in West Virginia, Tobin et al. (2013) also found no evidence of

density-dependent survival during the progrediens stage. The size of the trees in that study was comparable with those in this plantation experiment, but the number of inoculation ovisacs was much lower, and also the fecundity of adelgid sistentes on the inoculation branches they used was about 10-fold lower. They found that even when hemlocks were inoculated with only one ovisac per tree, progredientes established successfully on 35% of their trees and the subsequent sistens generations established on all of these trees.

On each tree, we had significant numbers of sistentes that hatched and settled successfully, but this generation went nearly extinct during the late summer or early fall aestivation period. Crawlers of the overwintering sistentes settle preferentially on new growth in late June and enter an aestivation period during late summer through early fall (McClure 1991, Young et al. 1995, Butin et al. 2007). A likely purpose of this dormancy period is avoidance of activity during the hottest

summer months (Salom et al. 2001). Intact branch counts confirmed that all 16 trees had established populations of settled aestivating sistentes with a mean of  $126.8 \pm 28.5$  SE per terminal 30-cm sample per tree. Whole tree estimates of settled sistentes were not taken during this period, because they are extremely small and difficult to count in the field. Instead, settled sistentes on 30-cm sample branches were counted in August, and the branch was marked so the same adelgids could be counted again in December to estimate aestivation survival.

In December, these branches were revisited to see how many adelgids had survived aestivation to resume development and begin producing wool. None of these adelgid had produced wool, and thus, 100% of the aestivating sistens on these branches had died. Furthermore, there was no detectable survival on 13 of our 16 trees as determined by whole tree counts of maturing adelgids. At the forested site from Sussky and Elkinton (2014), overall survival during this period was 16.2%. The lower summer temperatures in the forest setting (Table 3) may account for the higher survival of hemlock woolly adelgid in this stage. Interestingly, aestivation survival at the forested site the previous year (2011) was much lower, averaging  $1.8 \pm 0.3\%$ , and this corresponds to the warmer maximum summer temperature at the nearby weather station of  $36.61^\circ\text{C}$  in 2011 compared to  $34.67$  in 2012 (Table 3). Aestivation mortality in our data thus appears to rise to values close to 99% when maximum temperatures rise above  $36^\circ\text{C}$ . Mech et al. (2012) have recorded similar high temperature effects on aestivating sistens mortality in both laboratory and field studies in Georgia. Adelgids were exposed in a controlled experiment to temperatures ranging from  $20\text{--}40^\circ\text{C}$  for a duration of 2 to 8 d. Their results indicate that the effect of high temperatures is cumulative and that the adelgids ability to survive markedly decreases above  $30^\circ\text{C}$ . Temperatures of insects exposed to sunlight are often several degrees warmer than air temperature (e.g. Lance et al. 1987). This phenomenon is challenging to measure or estimate, especially for very small insects (Bakken 1992).

Host tree defenses may play a role in the survival of hemlock woolly adelgid. Lagalante et al. (2006) measured the terpene content of hemlock foliage across the growing season and showed that it was highest and most variable in late summer. They speculated that the aestivation phase of adelgids occurred as a means to avoid these late-summer concentrations of terpenes. Furthermore, McKenzie et al. (2014) have reported that trees in a stand from New Jersey, which have been shown to partially resist hemlock woolly adelgid infestation (Ingwell and Preisser 2011), have higher concentrations of terpenes than susceptible control trees. However, in a companion study to this one, McKenzie (2014) has shown that the terpene concentration in twigs and needles of the plantation hemlocks at S. Deerfield was several-fold lower than in the trees at our forest site, a finding consistent with that reported by Pezet and Elkinton (2014) comparing trees from our forest site with those in a hemlock plantation at the University of Rhode Island. Thus, it appears that the higher mortality we recorded at

the plantation site was not caused by higher concentrations of terpenes. What role terpenes play in adelgid survival remains unknown.

There was no significant relationship between adelgid survival, fecundity, or adelgid density on the length of the current year's new growth, a measure of tree health. In one sense, this result was to be expected, because the progrediens generation settles on old growth before the new growth in the current year elongates. The length of new growth, however, is a measure of overall health of the tree, and we thought it was important to test whether it was related to either progrediens fecundity or survival, which it was not. These results matched those from the forested site reported in Sussky and Elkinton (2014). That study showed that it is the previous year's infestation of hemlock woolly adelgid that influences the length and proportion of the current year's new growth, as well as adelgid survival. In the experiment reported here, none of our experimental trees had been infested with adelgid in the previous year.

The near-extinction of hemlock woolly adelgid on plantation trees during the aestivation sistens stage in late summer, despite many surviving adelgids from the preceding progrediens generation, may help explain why hemlock woolly adelgid has spread so slowly in our region. Hemlock woolly adelgid invaded central Massachusetts in the early 1990s, yet many trees remain uninfested nearly 20 yr later (Orwig et al. 2012, J. E., unpublished). This fact has usually been attributed to the high overwintering mortality that typically occurs in our region. Here, summertime aestivation survival ( $<1\%$ ) was far lower than the overwintering survival of hemlock woolly adelgid recorded earlier that year (29.6%) at our forested site (Sussky and Elkinton, 2014) or in previous years by Paradis et al. (2008) or Trotter and Shields (2009).

Our results, however, do not explain how or why the hemlock woolly adelgid persists and has spread rapidly in southern states, where summer temperatures are presumably much higher. Possibly adelgids have adapted to the warmer temperatures in the south in the same way that they have adapted to colder winter temperatures in the northern states, as shown by Butin et al. (2005). Studies of regional differences in adelgid performance have focused on differences in overwintering mortality and cold-hardiness (Butin et al. 2005, Trotter and Shields 2009). Our results suggest that summer mortality may be just as important to adelgid population dynamics, and further studies of this phenomenon are needed, particularly in the southern states.

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