POPULATION ECOLOGY

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

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Environ. Entomol. 1–7 (2015); DOI: 10.1093/ee/nvu007

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 (progrediens) 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 progrediens 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 Carriere) 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
hatch in April and May, producing the springtime pro-
gressiens 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 parthenogenetic 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 sistenentes (plural of sistens) oviposit in March, and
eggs hatch in late spring.

The Experimental Site. The experiment was con-
ducted in a small hemlock plantation located at the
University of Massachusetts Crops Research and Edu-
cation Farm in South Deerfield, Massachusetts
(42.4769° N, 72.583° W). The plantation was estab-
lished in fall of 2007 with 1-m-tall hemlock seedlings
from Cheshire Nursery Garden Center (Wallington,
CT). The plantation comprised approximately 130
hemlock trees arranged in a grid at 2 m spacing
between trees and exposed to full sunlight on agricul-
turally 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 land-
scape fabric. All of the hemlocks in this plantation
were uninsected with hemlock wooly 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 gener-
ation 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 over-
night, and attached to experimental hemlocks the fol-
lowing day.

Experimental trees consisted of 16 uninsected hem-
locks 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 ovi-
sacs, 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 choriomns
from hatched eggs in three woolly ovisacs on each bun-
dle of inoculum twigs. These numbers were then aver-
aged across all 16 trees in the stand. The number of
progrediens crawlers produced on each experimental
branch or tree was estimated by multiplying the num-
ber of sistens ovisacs on the inoculum branches on
each tree by the mean fecundity. This procedure con-
verted the four density categories of infested inocula-
tion 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 gener-
ation, sample twigs were cut from each inoculated tree
on 24 May 2012 and placed in water picks shortly fol-
lowing the establishment of spring generation crawlers.
Populations of hemlock wooly 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 pro-
gredientes 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 proce-
dure to estimate the total cm of branches with foliage
on each tree. For each experimental tree at the planta-
tion, 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 differ-
ent trees. Plotting these two measures against each
other yielded the following equation: total
\[ \text{cm} = 0.251(\text{length} \times \text{width}) + 601.1 \quad (R^2 = 0.963); \text{ see Supp Fig. 1 [online only],} \]
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 hem-
lock 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 (42.233° 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 crawlers 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 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 NPAR1WAY, 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 sexuparae also declined with density of progrediens crawlers (Fig. 1C; Table 1) and with density of settled progrediens (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 progrediens (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 ± 25.5 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 = −5.54, P < 0.001), where the average aestivation survival on 64 trees or branches was 16.2 ± 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°C compared with 31.62°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 72–385/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 (± 0.86 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
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
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 ± 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 sistentes 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 ± 0.3%, and this corresponds to the warmer maximum summer temperature at the nearby weather station of 36.61°C in 2011 compared to 34.67°C in 2012 (Table 3). Aestivation mortality in our data thus appears to rise to values close to 99% when maximum temperatures rise above 36°C. Mech et al. (2012) have recorded similar high temperature effects on aestivating sistentes mortality in both laboratory and field studies in Georgia. Adelgids were exposed in a controlled experiment to temperatures ranging from 20–40°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°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 Ellkinton (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 Ellkinton (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 over-wintering survival of hemlock woolly adelgid recorded earlier that year (29.6%) at our forested site (Sussky and Ellkinton, 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.

Acknowledgments

We thank T. Dowling, A. Hostetter, C. Huffman, N. Manyak, and A. McKenzie for help with the field work; N. Milano for editing the text; D. Munsel, J. Pezet, and G. Boettner for planting and caring for the trees; and N. Lany, A. Mayfield, A. Mech, P. Sievert, A. Weed, B. Van Driesche, and two anonymous reviewers for discussions or for reviewing earlier drafts of the manuscript. This research was supported by grants from the U.S. Department of Agriculture Forest Service Cooperative Agreement 09-CA-11420004-360.
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Received 17 August 2014; accepted 8 October 2014.