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Density-Dependent Recruitment and Diapause in the Spring-Feeding Generation of Hemlock Woolly Adelgid (Hemiptera: Adelgidae) in Western North America

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Abstract

Insect populations are affected by density-dependent and density-independent factors, and knowing how these factors affect long-term population growth is critical to pest management. In this study, we experimentally manipulated densities of the hemlock woolly adelgid on eastern and western hemlock trees in the western USA to evaluate the effects of density and host species on hemlock woolly adelgid crawler colonization. We then followed development of hemlock woolly adelgid on each hemlock species. Settlement of crawlers was strongly density-dependent and consistent between host species. In addition, a period of hot days that coincided with the settlement of hemlock woolly adelgid crawlers put our experimental and naturally occurring populations into diapause during April. Diapause resulted in one generation that yr in our experimental population. Analyses of long-term air temperature records indicated that diapause-inducing temperatures in April similar to those observed in our experiment have occurred rarely since 1909 and the frequency of these events has not changed over time. Prior work suggests that hemlock woolly adelgid completes two generations per yr in the western USA with a diapause occurring in the summer. This typical life history reflects the long-term influence of regional average seasonal temperature patterns on development and the timing of diapause-inducing temperatures. However, the timing of unseasonal weather, such as the hot days observed in our experiment, occasionally changes life history trajectories from this normal pattern. Our results show that density-dependent and density-independent factors have strong effects on generational mortality and life history of hemlock woolly adelgid that are important to its population dynamics and management.

Key words: *Adelges*, *Tsuga*, survival, diapause, life history

Population growth of insects is affected by the interplay of density-dependent and density-independent factors. The relative importance of these factors manifests into the diversity of dynamics characteristic of insect populations (Price et al. 2011). Negative density-dependent processes tend to regulate populations around an equilibrium abundance (Berryman 1999, Turchin 2003) and result from direct effects of predation, competition for resources, or for other reasons (Berryman 2002, Berryman 2003). In sap-sucking insects such as scales and adelgids, for example, density-dependent mortality is commonly due to intra-specific competition (Washburn et al. 1985, Itioka and Inoue 1991, McClure 1991), and this feedback may also moderate other sources of regulation (e.g., from natural enemies) affecting the life cycle (Elkinton et al. 2011).

The hemlock woolly adelgid, *Adelges tsugae* Annand, is an exotic sap-feeding insect that was documented in the eastern United States during the 1950s (McClure and Cheah 1999, Havill et al. 2016) and has caused extensive mortality of eastern (*Tsuga*

canadensis [L.]) and Carolina (*Tsuga caroliniana* Engelm.) hemlocks in the eastern United States (Ellison et al. 2005). Hemlock woolly adelgid also occurs in the northwestern United States, but it is only an occasional pest of western hemlock (*Tsuga heterophylla* [Raf.] Sarg) in urban settings and is functionally extinct within the natural forest landscape (Zilahi-Balogh et al. 2003, Mausel 2005, Kohler et al. 2008a). Apparently, hemlock woolly adelgid colonized northwestern North American hemlocks more than 10,000 yr ago (Havill et al. 2016), and it is hypothesized that the natural enemies and host resistance are major factors explaining its non-pest status (McClure and Cheah 1999; Zilahi-Balogh et al. 2003; Kohler et al. 2008b,a; Kohler et al. 2016).

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 hemlock woolly adelgids develop, a white protective wool-like wax is secreted from the female, within which it will eventually oviposit (McClure 1989, 1991). In eastern North

America, hemlock woolly adelgid has two parthenogenic generations per year. The overwintering generation lays eggs that hatch in April and May, producing the springtime generation (progredientes; McClure 1989). Some progredientes become winged sexuparae that, in their native Japan, disperse to spruce (*Picea* spp.) and initiate a sexually reproducing generation (sexuparae). In eastern North America, however, none of the offspring of these sexuparae survives on the native spruce species, so there is no sexual life cycle (McClure 1989, Havill et al. 2016). Sexuparae are not produced in western North America (Zilahi-Balogh et al. 2003). Following reproduction of the spring generation, the asexual crawlers of North American populations settle on hemlock shoots produced in the previous year. In the eastern United States, the spring generation matures and oviposits in June, and sistens eggs hatch in late June or July. Populations in the western United States display a similar phenology; however, there tends to be greater overlap of generations and life stages in the growing season in the western United States than observed in the eastern United States (Zilahi-Balogh et al. 2003, Kohler et al. 2008a, Kohler et al. 2016).The resulting crawlers of the summer generation (sistens) settle preferentially on current year shoots, and enter a period of diapause (aestivation) during the warmest months of the year, where feeding and development cease. Diapause induction is apparently facultative, being triggered by exposure of hemlock woolly adelgid ovipositing adults and crawlers to warm temperatures (Salom et al. 2001). The length of the diapause can also vary depending on temperature, length of temperature exposure, and life stage (Salom et al. 2001). In the eastern and western United States, feeding and development of hemlock woolly adelgid typically resumes in October, and continues throughout winter.

There are notable differences, however, in the seasonality of hemlock woolly adelgid life stages on *T. heterophylla* in northwestern North America compared with populations in Virginia and Connecticut colonizing *T. canadensis* (Zilahi-Balogh et al. 2003, Kohler et al. 2008a). As mentioned above, there is greater overlap of hemlock woolly adelgid generations in western North America (Zilahi-Balogh et al. 2003, Kohler et al. 2008a, Kohler et al. 2016). As synchrony of cohort development and timing of diapause are strongly temperature-dependent (Salom et al. 2001, Salom et al. 2002), variation in hemlock woolly adelgid seasonality between western and eastern North America is probably linked to regional differences in average temperature conditions, which could have notable effects on long-term population growth in each region. For example, hemlock woolly adelgids can suffer high mortality during diapause (Sussky and Elkinton 2015) and winter (Trotter and Shields 2009), and natural enemy mortality in the western United States appears to be strongly linked to phenological synchrony with particular hemlock woolly adelgid life stages (Zilahi-Balogh et al. 2003, Kohler et al. 2008a, Kohler et al. 2016). Variation in developmental trajectories due to seasonal temperatures has been shown to affect population dynamics of other species (Tobin et al. 2008, Powell and Bentz 2009).

In this study, we experimentally manipulated densities of hemlock woolly adelgid on eastern (*T. canadensis*) and western hemlock (*T. heterophylla*) trees in the western United States to evaluate the effects of conspecific density and host species on hemlock woolly adelgid crawler colonization. In eastern North America, Sussky and Elkinton (2014) demonstrated a strong density-dependent recruitment of hemlock woolly adelgid on eastern hemlock. Here we tested whether this effect varied by host species. We then followed seasonality of settled hemlock woolly adelgid on each hemlock species in the field and laboratory.

Materials and Methods

Experimental Inoculation of Hemlock Woolly Adelgid Onto Branches

Experimental Design

We experimentally manipulated densities of hemlock woolly adelgid on co-located mature eastern and western hemlock trees growing at the University of Washington's Washington Park Arboretum in Seattle, Washington, USA (N 47.64°, W 122.29°), to evaluate the effects of density and host species on hemlock woolly adelgid colonization. Western hemlocks selected for the study were all naturally occurring within the park; eastern hemlocks were all planted during the 1950s to the 1970s from sources in New Jersey, North Carolina, Maryland, and Pennsylvania. Hemlock woolly adelgid naturally occurs and is more abundant on *T. heterophylla* than *T. canadensis* in the park (Mausel 2005).

In late March 2014, just before hatch of hemlock woolly adelgid crawlers, western hemlock branchlets (∼30-cm-long) heavily infested with healthy hemlock woolly adelgid ovisacs were collected from nearby hemlock stands as experimental inoculum. Ovisac dissections from these collections revealed that each ovisac contained 61.8 ± 7.6 eggs (mean$\pm$SE, $n = 40$) and crawler hatch was imminent, as evidenced by the few crawlers observed during dissections. The number of ovisacs was counted on each inoculum branchlet (mean $= 131 \pm 75$ ovisacs per branchlet; range $= 13 - 435$; $n = 880$) and then branchlets were grouped in bundles of 1, 3, 10, or 20. Inoculum was held in water-saturated floral foam prior to deployment in the field. The number of ovisacs in each bundle was summed to estimate the number of ovisacs and eggs being inoculated on each branch.

Ten mature trees (> 8 cm dbh) within the Washington Park Arboretum of western and eastern hemlock (20 trees total) were selected for experimental inoculation, all occurring within an area of about 5 ha. Four lower branches were selected from each tree (80 branches total) for inoculation; branches on each tree were grouped into pairs by physical proximity. We did our best to select branches without current hemlock woolly adelgid populations by visually scanning branches for sistens ovisacs. Varying densities of hemlock woolly adelgid were inoculated onto each branch using the infested branchlets similar to Sussky and Elkinton (2014). One of four different density treatments (1, 3, 10, or 20 inoculum branchlets) was then randomly assigned to each pair of experimental branches by tree species, and one of the branches in the pair was later randomly covered with a fine mesh bag following hemlock woolly adelgid colonization to serve as a predator exclusion treatment. This setup resulted in two density treatments per tree, and 10 replicate pairs (mesh bag and no bag) of each density treatment.

Inoculum bundles were transferred to branches on 28 March 2014 by laying inoculum across branches and securing with twist ties (Sussky and Elkinton 2014). Once inoculum was in place, we covered the entire branch with fine mesh bags to reduce predation on the inoculum. Thermochron iButtons (DS1921G, Dallas Semiconductor Corp, Dallas, TX) were placed inside and directly outside the mesh bags on trees at the extreme northern and southern portions of the site to monitor hourly air temperature. The iButtons were shielded from direct exposure to sunlight to avoid artificially elevating the recorded temperatures (Hibbard and Elkinton 2015).

Hemlock Woolly Adelgid Colonization of Experimental Branches

Inoculum and mesh bags were removed from experimental branches on 25 April 2014 (∼190 degree days above a 3.9°C base), when we were confident that all crawlers had hatched and had ample time to

settle on the experimental branches. A small branch sample, approximately 20-cm-long, was clipped from each branch and all of the newly settled crawlers were counted from the branch sample. Although branches might have been infested with hemlock woolly adelgid previously, no living hemlock woolly adelgid life stages other than the inoculated crawlers were evident on these branches at the time of the sample. Settled crawlers were easily distinguished from other hemlock woolly adelgid life stages (e.g., brick-red in color, visible penetration of stylet, and reactive to probing). The inoculum was also removed from branches and brought back to the laboratory to examine crawler settlement and evaluate extent of egg hatching on inoculum. At this time, when hemlock woolly adelgids were confirmed to have settled onto branches, one mesh bag was replaced on one of the randomly chosen experimental branches in each pair to act as the predator exclusion.

We evaluated the effect of tree species and inoculation density on crawler colonization survival in the samples taken on 25 April. The four inoculation density treatments were converted into a continuous variable by multiplying the number of ovisacs on the inoculum branches by the mean number of eggs per ovisac reported above (Sussky and Elkinton 2014). Crawler survival on each branch was calculated as the total number of crawlers observed divided by the estimated number of eggs inoculated on each branch. Survival data were logit transformed prior to analysis of covariance examining the effects of tree species and estimated inoculation egg density on hemlock woolly adelgid crawler survival. As mesh bags to exclude predators were present on all branches until crawler settlement, this variable was not evaluated in the model.

Development of Hemlock Woolly Adelgid

Eleven (4 no bag, 7 bagged) eastern hemlock and 13 (6 no bag, 7 bagged) western hemlock 20-cm branchlet samples were collected from randomly chosen experimental branches on 12 June and brought to the laboratory to assess hemlock woolly adelgid survival and age structure under a microscope. Branch samples were transferred from the field in a cooler and held in a refrigerator until inspection. All hemlock woolly adelgids were carefully inspected to assess the life stage, survival, and whether they were of the sistens or progrediens generation. Alive hemlock woolly adelgids were those clearly developing wax and moved notably when poked. Hemlock woolly adelgids that were dehydrated and did not exude hemolymph when poked with a pin were recorded as dead. Life stage was determined by counting the shed exuviae. During the assessment of survival, it was found that the majority of hemlock woolly adelgid individuals on branch samples showed no signs of movement or development and were not obviously dehydrated, so it was suspected that these went into dormancy (diapause). Logistic regression was used to evaluate whether host type, presence of a mesh bag, or hemlock woolly adelgid density (hemlock woolly adelgids cm^{-1} branch) affected the probability that live (developing and putatively diapausing) hemlock woolly adelgid (1,494 individuals on 24 branches) remained in the crawler (N1) stage versus developing to later developmental stages. Individual trees were treated as a random effect in the model.

As we were unable to definitively distinguish using a microscope whether hemlock woolly adelgid were dead or in diapause on branches, we placed the infested branches in an incubator to later evaluate whether hemlock woolly adelgids were dead or had entered a developmental delay. We monitored hemlock woolly adelgid in the laboratory to reduce potential field-related mortality factors and standardize temperature conditions across all samples. Branch samples were inserted into floral water pics and held in clear, plastic cups with ventilated lids and first transferred to a refrigerator (∼4.5°C) for 2 wk to expose hemlock woolly adelgid to cool temperatures, potentially acting as cue to break diapause. Cups were then transferred to an incubator (Percival model I-36VL, Perry, IA) set at 12°C and a long-day photoperiod of 16:8 (L:D) h for 48 h to acclimatize. We gradually increased the temperature from 12°C every 2 to 3 wk until reaching 18.5:15.5°C (16:8 h) to accelerate development. Variable temperature conditions were programmed to simulate daily temperature fluctuations; however, these temperatures were not based necessarily on local Seattle conditions, but were set to temperatures conducive to development Salom et al. 2002. Branch samples were inspected weekly for health and hemlock woolly adelgid development. The number of alive or dead hemlock woolly adelgid in each life stage was counted from each branch sample on 18 August once hemlock woolly adelgid development became obvious and before branch health started to decline. Seven of the 24 branches showing notable signs of poor health (e.g., dead needles and fungal growth) were discarded at the end of the trial. Hemlock woolly adelgid survival and development was assessed on 10 eastern and 7 western hemlock branches. We evaluated the proportion of individuals in the N1 versus N2 to adult stages, where a change in the proportion of hemlock woolly adelgids from the N1 stage to later developmental stages would indicate that hemlock woolly adelgids went into diapause and were not dead on field samples.

Effects of Temperature on Hemlock Woolly Adelgid

Physiological experiments suggest that temperatures above 17°C can induce diapause in hemlock woolly adelgid (Salom et al. 2001). To evaluate the hypothesis that the timing of warm temperatures at our site was responsible for putting the experimental populations of hemlock woolly adelgid into spring diapause, hourly air temperature data were downloaded from the Thermochron iButtons placed in the arboretum, and daily maximum/minimum temperatures were accessed from a weather station located 6.2 km away (Seattle Sand Pt, N 47.70°, W −122.25°, elevation = 18.3 m asl). From these data, we constructed the average daily temperature from the date of inoculation (28 Mar. 2014) to the second branch sample (12 June 2014). Two-sample permutation tests (5000 resamples) evaluated whether minimum, maximum, and mean daily temperatures recorded by the iButtons differed inside and outside of the mesh bags. Air temperature data from the weather station were also used to estimate the accumulated number of degrees days (double sine method) from 28 March to 12 June 2014 based on development data of Salom et al. (2002) to evaluate whether air temperatures were permissive to hemlock woolly adelgid development.

We then evaluated how common diapause-inducing temperatures have occurred in the past during the period that likely coincides with spring hemlock woolly adelgid ovisac production and crawler settlement in the Seattle region. Historical daily air temperatures from two weather stations (Seattle Sand Pt: N 47.70°, W −122.25°, elevation = 18.3 m asl and Seattle Portage Bay: N 47.68°, W 122.33°, elevation = 38.1 m asl) with measurements from 1909 to 2015 were accessed from the National Climate Data Center (National Oceanic and Atmospheric Administration). We had to combine weather station data sets because each station did not have a complete air temperature record from 1909 to 2015. To evaluate whether we were introducing a bias into the analysis by using air temperatures recorded at two weather stations, we used regression analysis to assess the relationship between daily mean air

temperatures during the period when observations at each station overlapped (April 1987 to December 1997, $n = 4018$). The regression analysis indicated that mean daily air temperatures were strongly related between stations during this period (T_{avg} Portage Bay = $-0.14[\pm0.03SE] + 0.99[\pm0.001SE]\cdot T_{avg}$ Sand Point; RMSE < 0.001, $R^2 = 0.98$; $P < 0.001$), so we predicted missing daily temperatures (1,500 values between 1998 to 2015) at the Portage Bay weather station from Sand Point observations. In each year, we calculated the daily mean temperature during March, April, and May, which covered the period of hemlock woolly adelgid egg laying and crawler settlement during this study and at other western locations (Zilahi-Balogh et al. 2003, Kohler et al. 2008a, Kohler et al. 2016). To evaluate how common diapause-inducing temperatures have been in the past, we determined the frequency of days recording mean temperatures $\geq 17°C$ from 1909 to 2015 in each month, and assessed whether this frequency has changed over time using the Mann-Kendall test (Mann 1945).

Natural Phenology of Hemlock Woolly Adelgid in Arboretum

We monitored hemlock woolly adelgid density and development under natural conditions on non-experimental branches by collecting branchlet samples from eastern and western hemlocks from mid-April until the end of May. On 14 April, a branchlet sample, approximately 10-cm-long, was taken from eight trees of each hemlock species. After 14 April, samples were collected approximately every 150 degree days (according to the Seattle Sand Pt temperature observations as described above) on May 2nd, 16th, and 30th. Because hemlock woolly adelgid densities were found to be very low in 14 April samples, three replicate branchlet samples, each approximately 10-cm-long, were clipped from each tree with branches visibly infested with hemlock woolly adelgid on May 2nd, 16th, and 30th. Each of the replicate branchlet samples was collected from one of eight randomly chosen cardinal directions on each tree and never collected from or directly under the inoculated, experimental branches. Samples were returned to the laboratory to determine hemlock woolly adelgid life stage structure, survival, and generation as described above. The proportion of live hemlock woolly adelgid in each life stage was calculated per tree and sample date (three replicate branch samples data combined for May 2nd, 16th, and 30th samples). Analysis of variance was used to evaluate whether the probability of hemlock woolly adelgid in the crawler (N1) stage changed over the course of sampling and varied by host species. Post hoc linear contrasts were constructed to assess whether life structure differed statistically among dates on each host plant ($\alpha = 0.05$).

Results

Experimental Inoculation

Hemlock Woolly Adelgid Colonization

Hemlock woolly adelgid crawlers settled on all of the inoculated branches, and there was a positive relationship between inoculation branch density and crawler abundance (*settled crawlers per branch* = $0.08\pm0.02SE\cdot ovisacs$ *per branch* + $114.6\pm35.4SE$, RMSE = 206.7; $R^2 = 0.11$, $P = 0.001$). Over 16,000 hemlock woolly adelgids were observed on 80 branch samples of approximately the same size taken in late April. There was strong density-dependent mortality associated with crawler establishment ($F = 29.3$; df = 1, 76; $P < 0.001$; Fig. 1), which was similar on eastern and western hemlocks ($F = 0.9$; df = 1, 76; $P = 0.341$; Fig. 1). All hemlock woolly adelgids were determined to be in the first-instar (crawler) stage, as
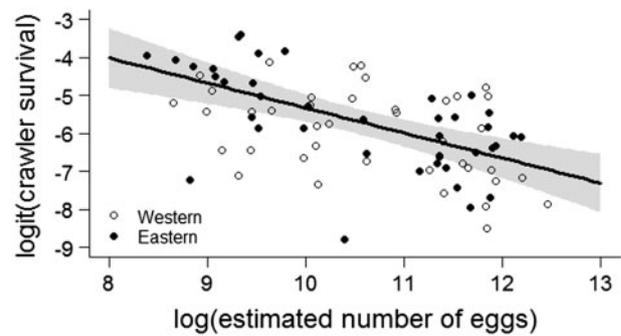

Fig.1. Density-dependent survival of settling hemlock woolly adelgid (*Adelges tsugae*) crawlers onto eastern (*Tsuga canadensis*) and western (*T. heterophylla*) hemlock branches. Shaded area around regression line denotes 95% CI.
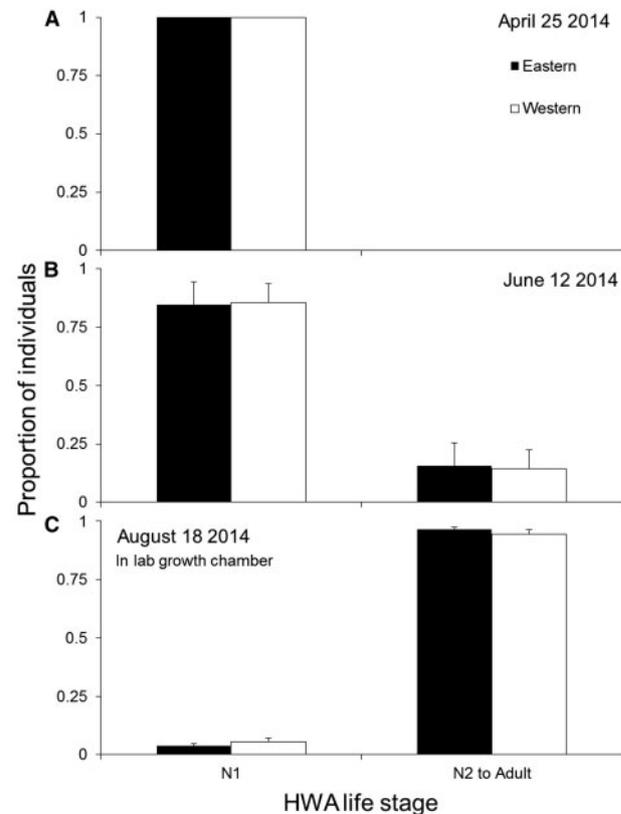

Fig. 2. Life stage structure of hemlock woolly adelgid, *Adelges tsugae*, on experimental branches (A) just after inoculation, (B) about two mo after inoculation, (C) and after development in incubator.

having recently settled (Fig. 2A). The average number of settled hemlock woolly adelgid crawlers was 203.2 ($\pm$222.4SD) hemlock woolly adelgids per sample.

Development of Hemlock Woolly Adelgid

Branch samples taken on 12 June, roughly 2 mo following hemlock woolly adelgid settlement, revealed that the majority of live hemlock woolly adelgids, which averaged 91.8 ($\pm$20.0SE) hemlock woolly adelgids branch^{-1}, were still in the first instar (Fig. 2B). This was despite local air temperatures exceeding the temperature necessary for normal nymphal development (543 accumulated degree days from 25 April to 12 June 2014), suggesting they were in diapause.
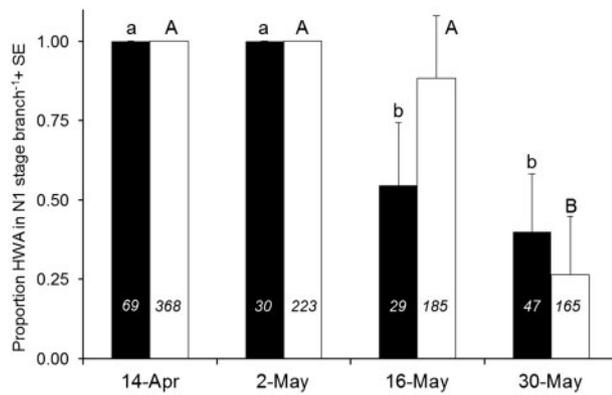
Fig. 3. Proportion of hemlock woolly adelgid, *Adelges tsugae*, in the crawler (N1) stage on non-experimental branches sampled across the Washington Park Arboretum during 2014. Numbers within bars denote the numbers of hemlock woolly adelgid examined. Letters denote results of linear contrasts.
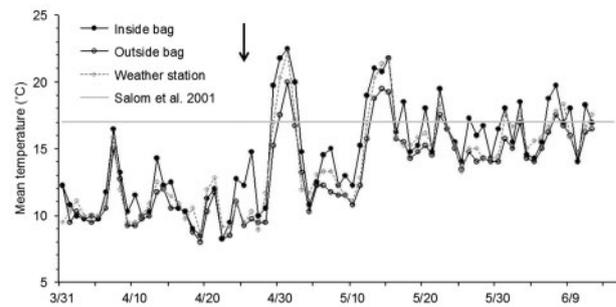

Fig. 4. Mean daily air temperatures recorded from iButtons (solid black lines) and at a weather station (dashed gray) near the University of Washington's arboretum during the study period showing hot spring temperatures that coincided with the presence of newly settled hemlock woolly adelgid crawlers. Arrow indicates date when hemlock woolly adelgid crawlers were confirmed to have just settled. Solid gray line indicates temperature inducing diapause in hemlock woolly adelgid in a previous laboratory study (Salom et al. 2001).

Moreover, there was no evidence that hemlock woolly adelgids completed a full life cycle prior to the 12 June sample (i.e., woolly progrediens ovisacs absent). The probability of individuals remaining in the first instar was not affected by host (coefficient $= -0.9 \pm 1.1$SE; $z = -0.8$; $P = 0.42$; Fig. 2B), marginally negatively affected by the presence of a mesh bag (coefficient $= -1.0 \pm 0.5$SE; $z = -1.8$; $P = 0.07$), and increased with density (coefficient $= 0.2 \pm 0.1$SE; $z = -0.8$; $P = 0.04$). After transfer to the incubator, hemlock woolly adelgid development was apparent on these same branch samples within 39 d. After roughly 2 mo in the incubator, there was a notable shift in the life stage structure of hemlock woolly adelgids from the N1 stage (Fig. 2C), indicating that the adelgids underwent a developmental delay on the experimental branches after settlement in April.

Natural Phenology of Hemlock Woolly Adelgid in Arboretum

Roughly 1,400 hemlock woolly adelgids were inspected for survival and development from over 400 samples taken from non-experimental branches from mid-April to the end of May. Overall, densities of hemlock woolly adelgids were lower on eastern hemlock (0.04[$\pm$0.01SE] hemlock woolly adelgids cm^{-1}·branch^{-1}) compared with western hemlock (0.18[$\pm$0.04SE] hemlock woolly adelgids cm^{-1}·branch^{-1}). The lack of progrediens ovisacs or adults in these samples indicated that hemlock woolly adelgid did not complete a full life cycle from mid-April to the end of May; cohorts on these samples resulted from the overwintering (sistens) generation. In general, the proportion of hemlock woolly adelgid individuals in the N1 crawler stage on these samples decreased from mid-April to the end of May ($F = 19.2$; df $= 3, 39$; $P < 0.01$; Fig. 3) but did not vary by host ($F = 0.08$; df $= 1, 39$; $P = 0.78$). The interaction between date and host was not significant ($F = 1.9$; df $= 3, 39$; $P = 0.14$). Linear contrasts indicated that nearly all hemlock woolly adelgids on western hemlock were in the N1 stage on samples taken on 14 April to 16 May (Fig. 3), despite an estimated accumulation of 295 degree days between these dates. On eastern hemlock, age structure of hemlock woolly adelgid was dominated by the N1 stage in 14 April and 2 May samples (147 degree days between dates). The proportion of hemlock woolly adelgids in the N1 stage decreased significantly from 2 May to 16 May (147 degree days) on eastern hemlock; however, roughly half of the hemlock woolly adelgids examined were still in the N1 stage, and there was considerable variation in age

structure among branches (range $= 0$ to 1; averaged 0.55[$\pm$0.20SE] hemlock woolly adelgid individuals in N1 stage per branch; Fig. 3). Finally, there was a significant decrease in the N1 stage on both hemlocks from 16 May to 30 May (Fig. 3; 159 degree days between dates).

Effects of Temperature on Hemlock Woolly Adelgid

Evaluation of iButton and weather station data during the experiment indicated that temperatures warm enough to induce diapause (17°C; Salom et al. 2001) coincided with the settlement of crawlers on our experimental branches. Just after hemlock woolly adelgids were confirmed to have settled onto all experimental branches (25 April), the region was subjected to temperatures above 20°C for 3 d (Fig. 4). We suggest that the timing of this hot weather, and not the presence of mesh bags, induced diapause in the settling crawlers. First, mesh bags were fixed on all experimental branches up until crawler settlement (25 April), so branches were exposed to approximately the same conditions for the majority of the time prior to the hot weather. Any bag effect would have most likely arisen after hemlock woolly adelgid crawlers had already settled. iButton data indicated that daily maximum (mean difference: 2.7°C; $Z = 2.7$; $P < 0.01$) and average (mean difference: 1.4°C; $Z = 2.3$, $P = 0.02$), but not daily minimum (mean difference: -0.12°C; $Z = -0.27$; $P = 0.79$), temperatures were higher inside mesh bags (Fig. 4). However, it appears that the few days where a bag effect could have arisen (26 to 30 April), the presence of bags was largely unimportant in affecting diapause, as life stage data suggest that diapause was widespread on branches not associated with any experimental treatment. Second, the extended presence of the N1 stage on non-experimental branches into late May also suggests that hemlock woolly adelgids likely went into diapause across the entire arboretum (Fig. 3).

Daily mean air temperatures recorded from weather stations near the study site from 1909 to 2015 averaged 8.0$\pm$2.5°C (mean$\pm$SD), 10.4$\pm$2.7°C, and 13.5$\pm$2.9°C in March, April, and May, respectively. Mean daily temperatures equal to or above 17°C were never recorded in March during 1909 to 2015. Air temperatures ≥ 17°C have been infrequently observed in April since 1909 (1.6$\pm$1.0 d yr^{-1}), and the frequency of these warm days has remained constant since 1909 (Kendall's tau $= -0.21$; $P = 0.16$; Fig. 5). The number of days in May recording air temperatures ≥ 17°C
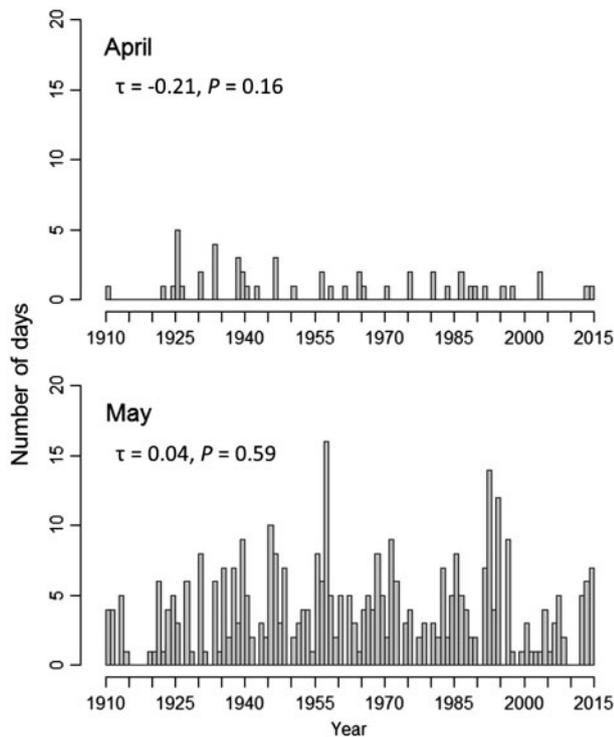
Fig. 5. Annual number of days per year in April and May where the observed mean daily temperatures were ≥ 17°C from 1909 to 2015 near the study site in Seattle, WA. Test statistics denote results of the Mann–Kendall trend test.

$(4.3 \pm 3.0 \, \text{d yr}^{-1})$ were more common than in April and have also remained constant since 1909 (Kendall's tau = 0.04; $P = 0.59$; Fig. 5). The probability that daily mean temperatures from 1909 to 2015 were ≥ 17°C in March, April, and May was <0.001, <0.01, and 0.11, respectively. In sum, although mean daily temperatures ≥ 17°C during April (when we observed hemlock woolly adelgid settlement) were rare, they have been recorded on 50 d near this site from 1909 to 2015. Diapause-inducing temperatures were more common in May.

Discussion

We experimentally manipulated densities of hemlock woolly adelgids on co-located eastern and western hemlock branches to evaluate the effects of density and host species on hemlock woolly adelgid colonization. Our inoculation using hemlock woolly adelgid-infested western hemlock branches was 100% successful on 80 branches. Settlement of crawlers was strongly density-dependent, which is similar to the findings reported by Sussky and Elkinton (2014) on eastern hemlock in Massachusetts. Similar findings have been reported for scale insects (Washburn et al. 1985, Itioka and Inoue 1991) and other adelgids as well (e.g., *A. cooleyi* Gillete; Parry 1973). Importantly, the strength of negative density-dependent settlement was consistent between western and eastern hemlock. Although a previous study reported higher hemlock woolly adelgid fecundity of the winter generation (sistens) on western compared with eastern hemlock (Mausel 2005), competition for space has been shown to be the most important factor affecting the colonization success of sessile insects (Washburn et al. 1985, Itioka and Inoue 1991). Our finding of higher natural densities of hemlock woolly adelgids on western hemlock compared with eastern hemlock corroborates the finding by Mausel (2005) that performance of

hemlock woolly adelgids is for some reason lower on eastern than western hemlock in the western United States.

Density-dependent mortality during crawler settlement of hemlock woolly adelgid has important implications for biological control of hemlock woolly adelgid in eastern North America because of its stabilizing effect on population size (Berryman 1999). Density effects on hemlock woolly adelgid crawler establishment may completely compensate for heavy egg depredation (Elkinton et al. 2011), suggesting that natural enemy pressure may be required across the entire hemlock woolly adelgid life cycle to decrease equilibrium density. The presence of multiple natural enemies that occur year-round with hemlock woolly adelgid in the Pacific Northwest may be one factor contributing to its non-pest status there (Zilahi-Balogh et al. 2003; Kohler et al. 2008a,b; Kohler et al. 2016).

Our study also demonstrated that the onset of aestivation in hemlock woolly adelgid is not necessarily restricted to the summer generation, but can occur whenever sensitive life stages are exposed to warm air temperatures; in other words, diapause is facultative (Salom et al. 2001). Samples taken from experimentally manipulated populations during this study in mid-June, approximately 2 mo following hemlock woolly adelgid settlement, indicated that hemlock woolly adelgids underwent a developmental delay in April and most remained in the first instar despite air temperatures conducive to development (as indicated by the accumulated degree days). Our results from the incubator study confirmed this developmental delay because after about 60 d, hemlock woolly adelgids began developing and by the termination of the experiment, the majority of hemlock woolly adelgids molted at least to the second instar. A previous laboratory experiment with hemlock woolly adelgids on eastern hemlock found that aestivation in the sistens generation can be induced by warm temperatures (Salom et al. 2001). Hemlock woolly adelgid nymphs entered diapause in these experiments after gravid adults, eggs, or settling crawlers were exposed to 17°C (Salom et al. 2001). Temperature data collected at the branch level and from a local weather station in Seattle confirmed that a period of warm days above 20°C coincided with the settlement of hemlock woolly adelgids on our experimental branches (Fig. 3).

Samples taken to monitor natural hemlock woolly adelgid phenology of non-experimentally manipulated populations also suggest that diapause occurred throughout the arboretum and was not linked to the experimental treatments. According to other studies, hemlock woolly adelgid normally completes a spring generation by the end of May into early June (Kohler et al. 2008a, Kohler et al. 2016); however, we did not find a notable shift in the age structure from the N1 stage on these samples until the middle or the end of May depending on the host species. In these samples, the majority of hemlock woolly adelgids remained in the N1 stage into the middle of May (136 JD), and there was no indication that hemlock woolly adelgid had completed a complete life cycle by 30 May (150 JD). This is despite exposure to temperatures that were conducive to normal development. The sustained presence of the N1 stage and lack of a complete life cycle suggests that hemlock woolly adelgids went into a developmental delay across the arboretum. Although diapause was widespread across the arboretum, apparently some hemlock woolly adelgids did not enter diapause, as evidenced by the few branches with hemlock woolly adelgids that had developed past the N1 stage. This was likely due to microsite variation in the exposure of hemlock woolly adelgids to abiotic conditions that could induce diapause. Such variation could be caused by exposure of branches to shade versus direct sunlight or because natural variation in hemlock woolly adelgid phenology resulted in the exposure of only a portion of the population to temperatures warm enough to induce diapause.

The observation of spring diapause in this study provided motivation to evaluate how common these events may have occurred in the past and whether the frequency of diapause-inducing temperatures in the spring has changed over time. Analysis of long-term (1909 to 2015) air temperatures observed near the study site in March until May indicated that mean daily temperatures never exceeded and only rarely exceeded 17°C in March and April, respectively, suggesting that the occurrence of diapause in April was possible but probably rare. Diapause-inducing temperatures are much more common in May. We do not know if diapause induction changes as the adelgids age. The frequency of days in April and May where the recorded daily mean temperature was ≥ 17°C has remained constant since 1909. This suggests that the likelihood of diapause induction in these spring months has, thus far, not increased as a result of climate change.

Variation in hemlock woolly adelgid seasonality and broad overlap of life stages have been observed among Pacific Northwest hemlock woolly adelgid populations in past studies (Zilahi-Balogh et al. 2003, Kohler et al. 2008a, Kohler et al. 2016), and likely reflect the influence of site-level variation in air temperatures on development, including the onset and possibly duration of diapause. Variation in life stage structure and voltinism is apparently common among populations of other adelgids too (e.g., *A. piceae*; Amman 1962). The typical life history of hemlock woolly adelgid that has been reported in the Pacific Northwest (two generations with summer diapause) reflects the influence of regional average seasonal temperatures on development and timing of diapause-inducing temperatures. However, the timing of unseasonal weather, such as the hot days observed in our study, occasionally changes life history trajectories from the average pattern in a way that can synchronize the timing of hemlock woolly adelgid life stages and change voltinism.

There is still much to be learned about the role of diapause in hemlock woolly adelgid life history and its consequences on population dynamics. For instance, does diapause sometimes occur in the spring generation in the eastern United States and, if so, how may this affect long-term population growth? As far as we know, spring diapause has never been recorded in eastern populations of hemlock woolly adelgid. The exact minimum temperature or thermal conditions necessary to induce diapause in hemlock woolly adelgid have not been estimated specifically, but according to the experiments by Salom et al. (2001), 14.5°C did not induce diapause but 17°C did. Other factors that may affect the induction and duration of the developmental delay (e.g., duration of exposure) need study (Tauber et al. 1986, Danks 1987, Hansen et al. 2011). Future work that can estimate the temperature or accumulation of thermal energy needed to induce diapause would permit an evaluation of how likely spring diapause may have occurred in the past and if this will change in the future in both North American ranges of this insect. Moreover, branch samples from our experimental populations examined later in the summer and fall of 2014 confirmed that spring diapause resulted in high levels of hemlock woolly adelgid mortality and that most individuals completed only one generation, not two as commonly observed (Kohler et al. 2008a, Kohler et al. 2016). High hemlock woolly adelgid mortality during diapause has also been acknowledged in the eastern United States (Sussky and Elkinton 2014). Future work is needed to understand how temperature-mediated changes in hemlock woolly adelgid life history may affect its population dynamics, as changes in voltinism may be consequential to population growth over time (Tobin et al. 2008, Klapwijk et al. 2012). These insights will have important ramifications for monitoring hemlock woolly adelgid spread and associated hemlock mortality and management, such as prioritizing classical biological control agents.

Acknowledgments

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