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Impact of Predators on Hemlock Woolly Adelgid (Hemiptera: Adelgidae) in the Eastern and Western United States

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Impact of Predators on Hemlock Woolly Adelgid (Hemiptera: Adelgidae) in the Eastern
and Western United States

A Thesis Presented

by

RYAN S. CRANDALL

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ABSTRACT

IMPACT OF PREDATORS ON HEMLOCK WOOLLY ADELGID (HEMIPTERA: ADELGIDAE) IN THE EASTERN AND WESTERN UNITED STATES

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Hemlock woolly adelgid, *Adelges tsugae*, native to Asia and the Pacific Northwest of North America (Pacific Northwest), has devastated eastern hemlock (*Tsuga canadensis*) in a major portion of its range in the eastern U.S. After many years and much effort directed towards rearing and releasing biological control agents to manage HWA, one of these agents, *Laricobius nigrinus*, native to the Pacific Northwest, is now well-established in sites from the southern to the mid-Atlantic states of the eastern U.S. However, there have yet to be studies of its efficacy in lowering *A. tsugae* densities, and there has been no noticeable drop in *A. tsugae* densities. Population models for *A. tsugae* have suggested that even upwards of 90% predation on eggs laid by the overwintering generation will have minimal effect in reducing the population densities of *A. tsugae*, if *A. tsugae* are at high density, due to compensatory density-dependent survival in the progreiens generation. Additionally, no studies showing insect predators are indeed what regulate *A. tsugae* in its native range exist. We established predator exclusion experiments, and recorded *A. tsugae* densities, mortality factors, and fecundity for

multiple generations in both the native and invaded ranges. In the invaded range, we studied *A. tsugae* populations in sites with well-established populations of *L. nigrinus* to test its efficacy in reducing *A. tsugae* and tested model predictions of minimal difference in *A. tsugae* densities between treatments with and without predators. In the Pacific Northwest we tested the effect of insect predators and tree species, western (*Tsuga heterophylla*) and eastern hemlock, on populations of *A. tsugae*. In the invaded range we found that *L. nigrinus* predation was significantly higher in unbagged branches, however, model predictions were validated, and there was no effect of predation by treatment on the *A. tsugae* summer generation. In our plots in the Pacific Northwest we found that tree effects were not significant, but that summer-active predators were significantly lowering levels *A. tsugae* densities on unbagged branches. Our study demonstrates the importance of summer-active predators in reducing *A. tsugae* and suggest that summer- and winter-active predators are needed to suppress *A. tsugae* to innocuous densities.

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CHAPTER 1

REBOUND OF *ADELGES TSUGAE* (HEMIPTERA: ADELGIDAE) PROGREDIENS GENERATION FOLLOWING PREDATION BY THE INTRODUCED BIOLOGICAL CONTROL AGENT *LARICOBBIUS NIGRINUS* (COLEOPTERA: DERODONTIDAE)

1.1 Introduction

Hemlock woolly adelgid (*Adelges tsugae* Annand; Hemiptera: Adelgidae), hereafter HWA, is a major forest pest causing high mortality to eastern (*Tsuga canadensis* (L.) Carriere) and Carolina (*Tsuga caroliniana* Engelm) hemlocks in the eastern United States. HWA was first discovered in eastern North America in Richmond, Virginia in the early 1950s and it is believed to have been brought there accidentally on infested hemlock nursery stock imported from Japan (Havill et al., 2014). HWA is native to parts of East Asia and the Pacific Northwest of North America, and DNA evidence suggest that the lineage of HWA introduced to eastern North America comes from Southern Japan (Havill et al., 2006). Since its discovery in Richmond, Virginia, HWA has spread to 20 additional eastern states, as well as southeastern Ontario and Nova Scotia, Canada (USDA Forest Service, Northern Research Station Range Map) (Ellison et al., 2018).

HWA has two generations per year, both of which reproduce asexually. The sistens generation is present from early summer through early spring, and it feeds from fall through spring after going through a mid-summer aestivation period (McClure, 1991, 1987). The progrediens generation develops from eggs laid by the sistens generation in late spring. These eggs hatch into crawlers, which settle at the base of a hemlock needle and feed from early spring to early summer. At high HWA densities, which commonly occur in the eastern U.S., progrediens can either settle on a hemlock twig or develop into

winged adults called sexuparae, which in Asia then seek out tigertail spruce (*Picea torano* (K. Koch) Koehne), on which they initiate a sexual generation (Havill et al., 2006). In the U.S., however, native spruce trees are unsuitable hosts, and therefore sexuparae fail to reproduce (McClure, 1989, 1987). HWA has no natural enemies in eastern North America, and both hemlock species were found to be very susceptible to infestation and subsequent decline/mortality (McClure, 1987). The loss of hemlock as a dominant forest tree prompted the USDA Forest Service to devote vast resources to the search for natural predators, which could be released for HWA population control (Havill et al., 2014).

Several HWA predators from Asia and the Pacific Northwest were introduced to eastern North America (Onken and Reardon, 2011). To date, one species, *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), has successfully established in substantial numbers (over 400,000 released from Georgia to Maine with successful establishment in each state) at field sites in the eastern U.S. (Foley et al., 2019). *Laricobius nigrinus* is active as an adult in the fall, winter, and spring. From February through April *L. nigrinus* adults lay their eggs within the sistens' ovisacs (the "woolly" wax secretions produced by females of HWA to cover the eggs). *Laricobius nigrinus* adults feed on settled sistens nymphs and adults, and as larvae they feed primarily on progrediens eggs (Zilahi-Balogh et al., 2003). After developing through four instars, *L. nigrinus* larvae finish feeding and then drop to the soil to pupate in late spring. *Laricobius nigrinus* pupates in the spring, and aestivate during summer months as adults, synchronized with sistens aestivation, resuming activity in the fall (Zilahi-Balogh et al., 2003) when the adult beetles emerge and feed on the developing HWA of the sistens generation.

Although *L. nigrinus* established and could be reliably collected at various sites, its impact in reducing overall HWA population densities, or on hemlock health through reduction in HWA density have not been determined (Preisser et al., 2014), though some predator enclosure experiments have shown *L. nigrinus* can reduce densities of HWA when they are both inside a cage together (Lamb et al., 2005). To better understand population dynamics of HWA, a model was created by Elkinton et al. (2011) to see how various mortality factors affected HWA populations. Surprisingly, model simulations suggested that, even with upwards of 90% predation on eggs in the sistens ovisacs, there would be little or no reduction in the subsequent progrediens density the following spring. HWA populations exist at or near carrying capacity in the eastern U.S. and there is strong competition for space to settle on hemlock twigs. HWA settles at the base of the hemlock needles, and when there are more than one HWA per needle, survival decreases, likely due to intraspecific competition for a limited food source, the carbohydrates and proteins stored in xylem ray parenchyma cells (Sussky and Elkinton, 2014; Young et al., 1995). When sistens densities are high, each female can replace herself approximately once due to habitat saturation (of settling sites), yet each adult lays between 40 – 200 eggs (McClure, 1991; Paradis, 2011). The vast majority of crawlers emerging from such ovisacs, therefore, die before reaching maturity because there is not enough space for them on the infested hemlock twigs. Sussky and Elkinton (2014) recorded strong density-dependent mortality, including dispersal of progrediens crawlers that die before settling and density-dependent production of winged adults (called sexuparae), which subsequently die because there is no appropriate *Picea* hosts for them in North America (McClure, 1987). This density-dependent survival may completely compensate for any

effect of predation on progrediens eggs (Elkinton et al., 2011). The model left open the possibility that predation by *L. nigrinus* adults on the sistens generation of HWA in the fall might have a significant impact on HWA densities.

However, if the prediction of the Elkinton et al. (2011) model is accurate, then *L. nigrinus* may have little or no significant impact in reducing HWA progrediens densities if sistens population densities are high. In this study, we established predator exclusion experiments at field sites with populations of *L. nigrinus* to test its efficacy as a biological control agent and to test the predictions of this model. We used mesh cages to restrict access to HWA by *L. nigrinus*, and we recorded densities of HWA. The impact of *L. nigrinus* on sistens ovisacs has been reported in a companion paper (Jubb et al., 2019). The goal of our study was to test whether or not *L. nigrinus* is having an impact in lowering HWA densities in field sites with high densities of HWA and established populations of *L. nigrinus*. Using the Elkinton et al. (2011) model, which helps us to understand complex population dynamics of the HWA, we parameterize it with our field collected data from the sistens generation and then compare the model predictions of progrediens density to our field collected progrediens density and in doing so, determine the accuracy of model predictions and whether or not *L. nigrinus* predation is having an impact on the progrediens generation. We hypothesize that densities of progrediens on branches open to predation by *L. nigrinus* would be slightly lower than those on branches that excluded *L. nigrinus*, and that density-dependent survival in the progrediens generation will at least partially compensate for predation by *L. nigrinus*.

1.2. Methods

1.2.1 Field site locations

In 2016 we selected sites in Maryland, New Jersey, North Carolina, and Virginia that, had significant infestations of HWA (i.e. trees had been infested for several years with densities of around 2-3 HWA per cm or greater), as well as established populations of *L. nigrinus* (i.e. *L. nigrinus* has been recovered in multiple years following its release) (Table 1). These sites were chosen in collaboration with research partners from the southern U.S. who have been rearing and releasing *L. nigrinus* since 2003. They represent a subset of the sites used in concurrent, companion study (Jubb et al., 2019) examining the impact of *L. nigrinus* predation on the HWA sistens generation.

Table 1.1. Field site names and GPS Coordinates

Site	Location	Coordinates
DEWA, NJ	Delaware Water Gap National Park, Walpack Township, NJ	41.12 N, -74.91 W
Rocky Gap, MD	Rocky Gap State Park, Gross, MD	39.70 N, -78.67 W
James River, VA	James River State Park, Gladstone, VA	37.64 N, -78.80 W
Kentland, VA	Kentland Farm, McCoy, VA	37.21 N, -80.59 W
Celo, NC	Celo Community, Burnsville, NC	35.82 N, -82.21 W

1.2.2 Establishment of predator exclusion cages

Before *L. nigrinus* became active in the fall, 5-15 trees in each site (20 trees at DEWA, NJ) which had branches with moderate to high densities of HWA (2-3 HWA/cm) were chosen as sample trees. Pairs of 1-m-long branches that were within arm's reach were marked to represent the treatments "cage," and "no cage." In sites that had less than 15 trees, some trees had multiple sets of treatment branches. Trees chosen were all mature understory trees ranging from 10 to 35-cm in DBH (diameter at breast height), with some trees located on the forest edge. From each branch pair a 20-cm long

sample branchlets (one per 1-m-long treatment branch, total of 30 sample branchlets per site except at DEWA, NJ which had 40) that appeared to have similar densities of HWA were marked and the number of HWA and total length of new growth were recorded. Each of the 20-cm long sample branchlets were chosen so that both branchlets from a pair were at comparable densities to each other by counting the adelgid and recording length of growth on the branch in the field. The “cage” branch was then struck ten times to knock off any *L. nigrinus* that may be on the branch and were fitted with a predator exclusion cage (1 m long by 0.5 m wide Equinox® No-See-Um mosquito netting ~569 holes per square centimeter) to exclude *L. nigrinus* from having access to the adelgids on the branches. Self-stick vinyl foam (3.2 cm. x 48 mm.) was wrapped around the branch three to four times where the cage was attached with cable ties as padding between the cable ties and branches so as not to cut off the flow of nutrients or water.

In March of both years, coinciding with peak progrediens egg production, the predator exclusion cages were removed and both “cage” and “no cage” treatment branches were enclosed in fine-mesh cages. The fine-mesh cages were made from silk screening (1 m long by 0.5 m wide SeFar Basic 61/156-64W PW) with openings (97x97 nm) smaller than adelgid first instar nymphs, thereby preventing crawler immigration and emigration between branches. By excluding *L. nigrinus* on our caged branches, we expected to have greater HWA sistens densities than on uncaged branches. When there is a high contrast in density among branches, one would expect a higher number of HWA crawlers to emigrate away from than rather than onto the high-density branches. In contrast to this experiment, in natural populations the density contrast between adjacent branches would be minimal and thus emigration would balance immigration between

branches. Without preventing dispersal at this stage of the experiment, we would expect higher levels of dispersal away from rather than onto our caged branches, thus creating an experimental artifact that would not apply to natural populations. These fine-mesh cages were applied to ensure that the settled progrediens resulted solely from crawlers originating on the same branch to which the predator exclusion treatment was applied.

In spring of each year after the progrediens eggs had hatched, the crawlers had settled on the sample branches, and *L. nigrinus* had completed feeding, the fine-mesh cages were removed. All branches then remained uncaged for the duration of the progrediens generation (late May through June), when *L. nigrinus* was no longer active on the tree and predation by generalist insect predators is thought to be negligible (McClure, 1987).

1.2.3 Estimating fecundity

In our first year of data collection (2017), we estimated HWA fecundity by randomly selecting twigs, 10-cm-long with at least 30 ovisacs, from each sample branch (30 from all sites except at DEWA which had 40) and then placing them into petri dishes that were sealed with parafilm, allowing sistens adults to complete oviposition in the laboratory (Tobin et al., 2013). In the second year (2018), twig samples were collected randomly from each sample branch when adult HWA had completed oviposition (as indicated by dead HWA adults in ovisacs with eggs) and these twigs were brought back to the laboratory for fecundity estimates. For each site a few randomly selected ovisacs per twig sample were examined under a dissecting microscope until we had examined at

least 30 ovisacs per treatment. The number of chorions, unhatched dead eggs, and any eggs that were predated upon, (evidenced by hemolymph left behind in the chorion) were counted (Fig. 1). Overwintering mortality of maturing sistens was determined from the fecundity samples by counting the live sistens (sistens that survived to the adult stage and produced eggs) and dead sistens (sistens that produced wool but never reached maturity and therefore did not produce eggs).

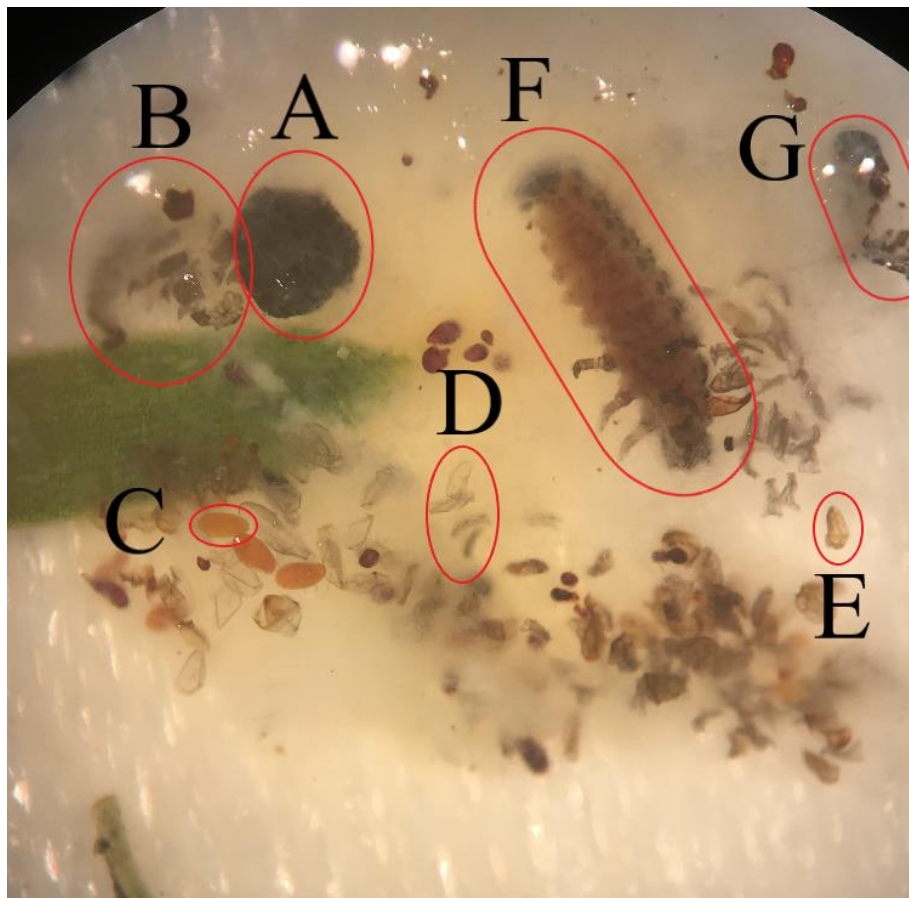


Figure 1.1. View of a dissected sistens ovisac submerged in 70% ethanol to dissolve the wax. A) Adult female hemlock woolly adelgid sistente. B) *L. nigrinus* larval exuvium left over from a molt that occurred inside the ovisac. C) Healthy progrediens egg, laid by sistens adult. D) Four egg shells or “chorions.” Chorions of successfully hatched eggs appear grey. E) Predated egg, similar in appearance to a chorion, but hemolymph left inside the egg gives it a slight orange appearance. F) *L. nigrinus* larva found inside the ovisac predated eggs. G) Sistens exuvium left over from molting.

1.2.4 Estimating adelgid density and new hemlock shoot growth

At the beginning of the experiment in mid-November, before deploying the predator exclusion cages, we estimated densities of maturing sistens (individuals secreting wool) in the field on 20-cm branchlets chosen on each 1-m study branch (160 branchlets total) (Jubb et al., 2019). On each 20-cm branchlet, we also recorded the length of 10 current-year shoots (representing new-growth from previous growing season). In April, at peak HWA egg abundance, we returned to sample branchlets and measurements were repeated. Overwintering mortality and the proportion of sistens ovisacs disturbed by predation were recorded on both caged and uncaged branches (Jubb et al., 2019).

In 2017, we also recorded data on the percentage of terminal branch tips with new growth on sample branches at all sites. This has been a standard technique for estimating new growth on hemlock branches in several previous studies of HWA impact on hemlocks (McClure, 1991; Sussky and Elkinton, 2014). Samples obtained to estimate progrediens density in the laboratory were also used to estimate current-year growth estimates in 2017. In November 2018, branch samples were taken from the Delaware Water Gap, NJ (DEWA) field site to get an estimate of the proportion of branch tips showing some new growth in the second year. Because field collections were over and the logistics of travelling to field site locations, only DEWA was sampled for proportion of new growth in 2018.

1.2.5 Modifying the predictive model

The sources of density-dependent mortality affecting HWA populations are (1) dispersal of progrediens crawlers, (2) decreased survival of settled progrediens, (3) sexuparae production, (4) reduction of progrediens fecundity, (5) dispersal of sistens crawlers, and (6) decreased survival of settled sistens (Sussky and Elkinton, 2014). In this experiment factors 4, 5, and 6 occurred after we collected data on the progrediens generation, leaving the first three factors as the focus of our study. As mentioned above, we used fine-mesh anti-dispersal cages on both treatments during hatch. The purpose of these cages was to remove density-dependent dispersal effects so the only density-dependent mortality factors left would be survival of settled progrediens and sexuparae production.

To determine the predicted outcomes for the Elkinton et al. (2011) model from our experimental results, we modified model parameters in the following way to mimic our experimental design. We removed mortality from progrediens dispersal because it was prevented by the fine-mesh cages. Although the model can predict densities of the subsequent sistens generation, here we focused on what the model predicted for adult progrediens. For each treatment, we modified the model to start with the observed mean densities of maturing sistens and imposed the percent overwintering mortality recorded at each site as reported by Jubb et al. (2019). This yielded the observed density of sistens ovisacs in the spring. We used the observed mean proportion of disturbed ovisacs on the “no cage” treatments at each site (Jubb et al., 2019) to model the predicted impact of *L. nigrinus* predation on the subsequent density of adult progrediens.

1.2.6 Data analysis

All analyses were performed in R 3.5.3 (RCoreTeam, 2019) using RStudio, version 1.2.1335. Our statistical analyses were designed to determine if there were significant differences in adult progrediens density between treatments in the paired branches with and without predator exclusion cages. We applied these analyses to the data collected across all sites in both years to maximize the statistical power of our tests. We used a generalized linear mixed effects model (GLMM) (Package = *lme4*, Version 1.1-21) and analyzed the data with the ‘glmer’ function, specifying the gamma family of distributions for our density data, and we specified both site and branch pair as random effects (Bates et al., 2015). We added the value of 0.0001 to the density value on each branch to remove zeros in the data to permit analyses with the few branches with zero adelgids (Zar, 2010).

We compared the ratio of densities in the cage treatment to the corresponding no cage treatment in each cage pair to the ratio predicted by the model. We used \log_{10} (ratio) for these comparisons so that the distribution of values greater than or less than 1.0 would be similar. These analyses were performed with ‘lmer’ function (Package = *lme4*, Version 1.1-21) assuming a Gaussian distribution. The model was fit with treatment (in this case “Predicted” and “Observed”) as a fixed effect and site as a random effect and run separately in each year.

We tested for density-dependent survival in the progrediens generation in 2018 because that was the only year we had all the relevant data needed to complete the analysis. Using the number of sistens ovisacs, percent mortality of sistens (overwintering mortality plus *Laricobius* predation) we found the predicted number of sistens maturing to the adult stage. The predicted number of surviving sistens adults was multiplied by the mean sistens fecundity to get the predicted number of progrediens crawlers that were produced. These numbers were expressed as densities per 20 cm branch sample. We then divided our counts of progrediens adults by the total number of progrediens crawlers to get the proportion surviving to the adult stage. We graphed the proportion of progrediens surviving versus the \log_{10} number of sisten adults. We analyzed these data using a logistic regression via a generalized linear model (Package = *stats*, Version 3.5.3) with a quasibinomial distribution (logit link) because the data were overdispersed (RCoreTeam, 2019). We ran a nearly identical logistic model to examine the effect of treatment (cage vs non-caged) on the proportion of progrediens crawlers surviving to the adult stage. All graphical data were displayed using ggplot2 (Wickham, 2009).

1.3. Results

1.3.1 Density, predation estimates, model predictions, and density-dependent progrediens survival

Each fall of the experiment, there was no statistical difference in sistens density between treatments across both years and sites Figs. 1.2A & 1.3A. Model predictions for progrediens density were quite similar for both years (Figs. 1.2C & 1.3C), even though overwintering mortality, sistens density, and ovisac disturbance values were unique to the

observed values recorded for each year, and they predicted minimal difference in adult progrediens density between treatments. The average density predicted in 2017 was 1.2 HWA/cm of twig growth, and 1.1 for 2018. When we tested the observed progrediens density, we found no significant difference between treatments across sites in 2017 ($z = -0.960, P = 0.3373$) or 2018 ($z = 0.933, P = 0.3508$) (Figs. 1.2D & 1.3D).

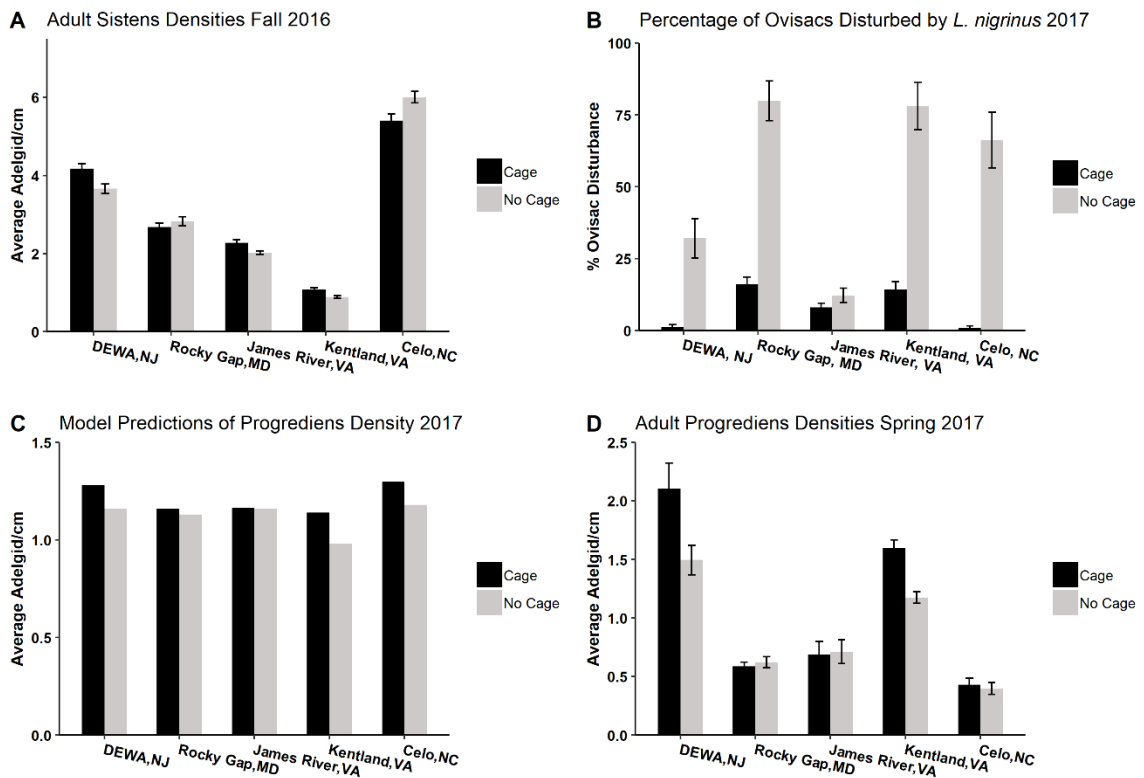


Figure 1.2. 2017 mean (\pm SE) observed and predicted HWA densities, and ovisac disturbance by treatment “Cage” and “No Cage.” A) Density estimated for sistens generation 2016. B) Percent ovisac disturbance by *L. nigrinus*. C) Model predictions of progrediens density based on the mean sistens density, overwintering mortality, ovisac disturbance, and fecundity by treatment and site. D) Observed density of the progrediens generation 2017.

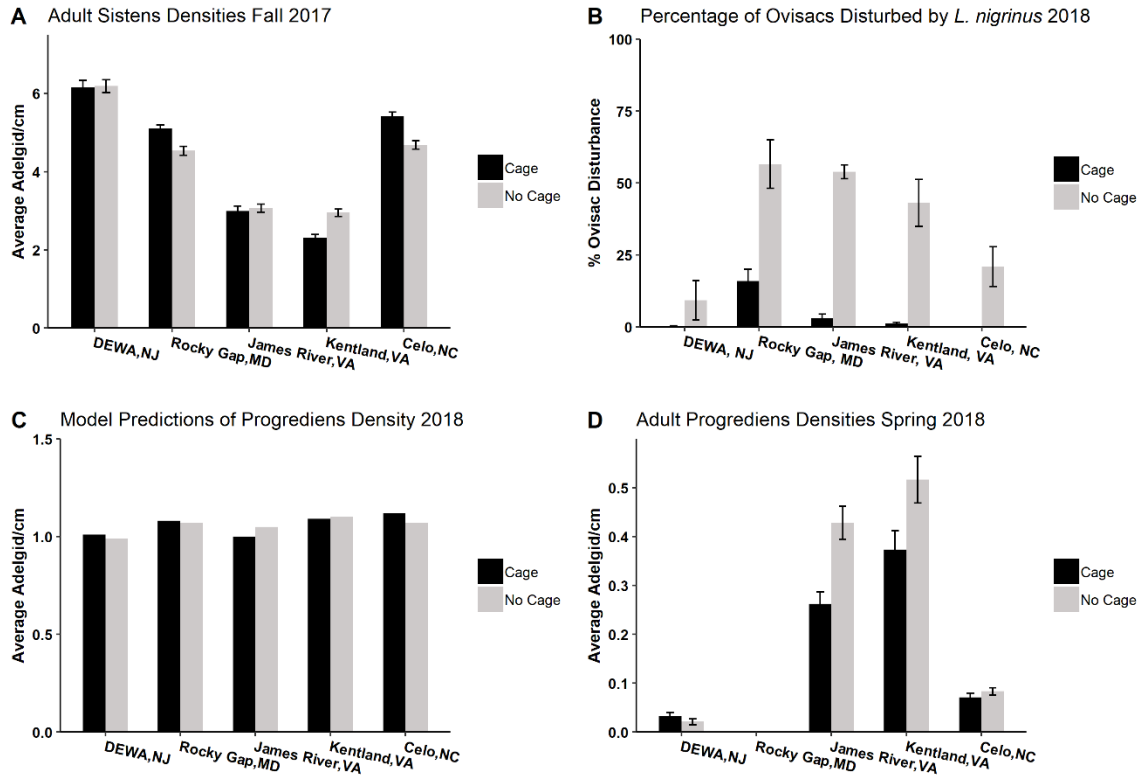


Figure 1.3. 2018 mean (\pm SE) observed and predicted HWA densities, and ovisac disturbance by treatment “Cage” and “No Cage.” A) Density estimated for sistens generation 2017. B) Percent ovisac disturbance by *L. nigrinus*. C) Model predictions of progreddiens density based on the mean sistens density, overwintering mortality, ovisac disturbance, and fecundity by treatment and site. D) Observed density of the progreddiens generation 2018.

Our ratio data also found minimal difference between observed and predicted ratios between treatments (Table 1.2). For 2017 and 2018, we found no significant difference between the observed and predicted ratios (2017: $t = -0.180$, $df = 56.5$, $P = 0.8576$; 2018: $t = 1.872$, $df = 127$, $P = 0.0635$). We determined the mean ratios by site for both years and transformed them back to the natural scale using antilog (10^x). Mean values by site and treatment can be seen in Table 1.2. The average observed and predicted ratio densities of “no cage”/ “cage” across sites in 2017 were 0.88 and 0.92, respectively, and for 2018 they were 1.33 and 0.99, respectively. For our logistic model,

we also found a significant negative trend for progrediens survival by density of sistens per 20 cm sample branch ($df= 166$, $\text{pseudo}R^2 = 0.49$, $P<0.001$), as the density of sistens increased there was a significant drop in the proportion of progrediens surviving to adult (Fig 1.4A). Figure 1.4B shows the difference in survival by treatment at each site as well as a total across sites. Our logistic model for proportion of progrediens surviving by treatment showed a that there were significantly more progrediens surviving in the uncaged treatment across sites ($df = 166$, $t = 3.721$, $P<0.001$).

Table 1.2. Mean ratio of HWA densities (No Cage /Cage) for observed and predicted values in 2017 and 2018.

Site	Year	Predicted	Observed
Celo, NC	2017	0.91	1.09
DEWA, NJ	2017	0.91	0.70
James River, VA	2017	1.00	1.02
Kentland, VA	2017	0.86	0.74
Rocky Gap, MD	2017	0.97	1.07
Celo, NC	2018	0.96	1.15
DEWA, NJ	2018	0.98	1.13
James River, VA	2018	1.05	1.68
Kentland, VA	2018	1.01	1.87
Rocky Gap, MD	2018	0.99	ND

*Rocky Gap has the greatest drop in density in the progrediens generation with no HWA recorded from samples. ND = “No Data”.

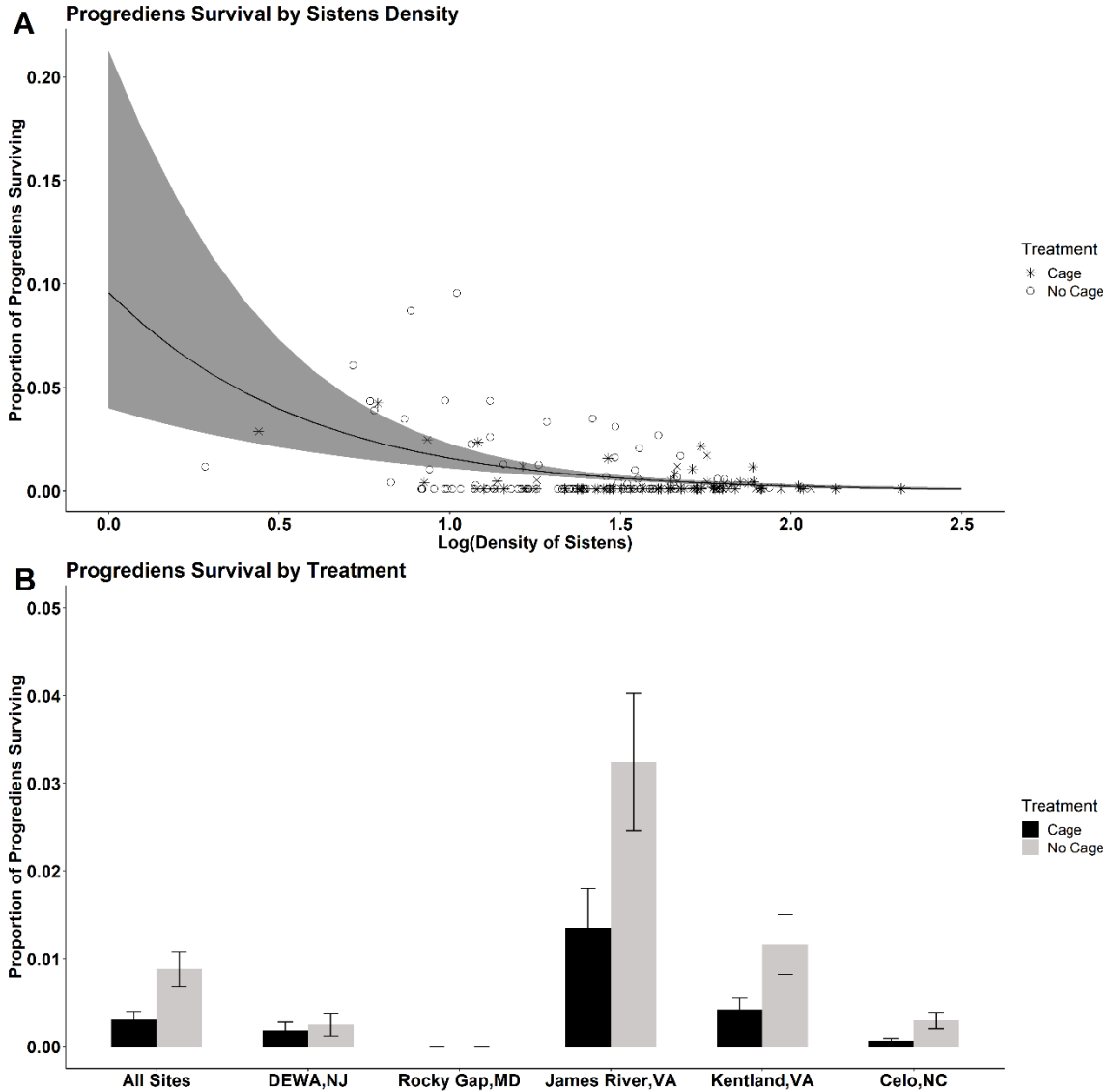


Figure 1.4. Survival of the progrediens to adult. A) Logistic regression of the proportion of progrediens surviving to adult by versus density of sistens surviving to adult by treatment across sites in 2018. Each point represents data from a single branch, the solid line represents the fit model and the grey shading show the 95% confidence interval. B) 2018 mean (\pm SE) proportion of progrediens surviving to adult by treatment, site and across sites.

1.3.2 Estimates of sistens fecundity and new growth

In 2017, our estimates of fecundity from samples of adult HWA held in petri dishes were compromised due to fungal contamination, except for our New Jersey site (DEWA). In 2018, we were able to record fecundity for each site (Table 1.3), which shows a sharp decline in fecundity for DEWA from 2017 to 2018. We found that in both years there were very low percentages of new growth on sample branches, all below 15% (Table 1.4).

Table 1.3. Mean (\pm SE) Sistens fecundity estimates for sites in 2017 and 2018.

Site	Year	Eggs per female	# Ovisacs Sampled
DEWA, NJ	2017	101.7 \pm 4.45	110
DEWA, NJ	2018	22.2 \pm 2.36	96
James River, VA	2018	46.7 \pm 2.77	110
Rocky Gap, MD	2018	46.7 \pm 2.69	117
Kentland, VA	2018	83 \pm 4.35	126
Celo, NC	2018	41.7 \pm 2.88	111

Table 1.4. Mean (\pm SE) percentage of hemlock twigs with new growth on sample branches across sites. Low new growth production in spring 2017 is the result of high densities from that year and effects the adult sistens and progrediens of 2018. Increasing new growth production in 2018 occur at the end of the study and would effect the adult sistens and progrediens in 2019.

Site	Year	Treatment	n	New Growth %
Celo, NC	2017	Cage	13	7.21 \pm 2.95
Celo, NC	2017	No Cage	14	20.86 \pm 5.89
DEWA, NJ	2017	Cage	22	6.11 \pm 3.46
DEWA, NJ	2017	No Cage	21	5.13 \pm 3.21
James River, VA	2017	Cage	11	7.33 \pm 4.60
James River, VA	2017	No Cage	15	8.33 \pm 2.69
Kentland, VA	2017	Cage	15	13.80 \pm 4.55
Kentland, VA	2017	No Cage	15	9.45 \pm 3.58
Rocky Gap, MD	2017	Cage	15	0.00 \pm 0
Rocky Gap, MD	2017	No Cage	15	0.00 \pm 0
DEWA, NJ	2018	Cage	21	14 \pm 6.67
DEWA, NJ	2018	No Cage	22	11 \pm 4.02

1.4. Discussion

This study's main goal was to explain why after over 10 years of *L. nigrinus* releases (Mausel et al., 2010) does HWA remain at high density and continue killing hemlock trees (Trotter et al., 2013) despite significant rates of predation by *L. nigrinus* (Jubb et al., 2019). The Elkinton et al. (2011) model helps illuminate the reason for minimal effect of predation on overall HWA densities, which is that the strong compensatory density-dependent survival affecting the progrediens generation largely overcomes the effect of predation. A companion publication lead by graduate student Carrie Jubb from Virginia Tech (Jubb et al., 2019) working in the same field sites focused on assessing the impact of *L. nigrinus* on overwintering sistens ovisacs and found that there was significantly higher rates of ovisac disturbance on uncaged treatments. Each spring mean rates of ovisac disturbance by *L. nigrinus* differed significantly between treatments at all sites, with higher levels of ovisac disturbance on "no cage" branches that were exposed to predators (Jubb et al., 2019). As reported by Jubb et al. (2019) in 2017, the average percent ovisac disturbance across sites was 1-16% for "cage" and 12-80% for "no cage." The 2018 ovisac disturbance estimates were 0-16% for "cage", and 9-57% for "no cage." The reported differences were all highly significant. Ovisac disturbance by site and treatment can be seen in figures 1.2B and 1.3B. Despite the significant predation occurring on uncaged ovisacs, model predictions of minimal difference in the progrediens generation densities between treatments (Figs. 1.2C & 1.3C) were validated by our observed progrediens densities (Figs. 1.2D & 1.3D). Our analysis of the ratio data also showed that there was only a small difference between the exclusion treatments in the observed data and the model prediction. Elkinton et al. (2011) suggest

that even with upwards of 90% predation on the progrediens eggs, there would not be a significant effect on the progrediens densities. In this study, there were two “no cage” treatments at separate sites (Rocky Gap and Kentland) in 2017 that both experienced about 80% sistens ovisac disturbance. At those two sites, the progrediens generation density showed minimal differences between treatments. These results support our hypothesis and model predictions that predation by *L. nigrinus* on sistens ovisacs will have minimal impact on the density of the subsequent progrediens generation in high-density HWA populations. Predation having a minimal impact on progrediens density is largely due to subsequent density-dependent survival factors that compensate for most of the predation on sistens ovisacs caused by *L. nigrinus*, which we documented with our analysis of progrediens survival vs. log sistens density. The data suggest a significant drop in progrediens survival with increasing density of sistens (Fig. 1.4). Low rates of progrediens survival in association with high densities of sistens suggest that predation by *L. nigrinus*, even with high rates of predation seen in our study, will not have a noticeable effect on the progrediens generation densities because predation is largely compensated for by density-dependent survival of 10% or less of progrediens at these high HWA densities.

Under the conditions in this experiment, the model predicted that progrediens density in the uncaged treatment would be approximately 10% lower than the caged treatment. The model predicted lower progrediens density in the uncaged treatment because the high levels of predation on HWA in the uncaged treatment would only be partially compensated for by the density-dependent progrediens survival which results in a rebound in density. However, our predictions for each cage were based on the observed

sistens density in the fall (Figs. 1.2A & 1.3A), the observed ovisac disturbance rate (Figs. 1.2B & 1.3B), and the observed overwintering mortality on each branch, as reported in (Jubb et al., 2019). In 2018, in particular, Jubb et al. (2019) reported higher overwintering mortality inside the cages than outside at some sites. As a result of the branch to branch variation in these data, sometimes our model predicted higher progrediens densities in the no cage treatment than the caged treatment, despite the higher predation rates on the no-cage branches. That pattern explains the variation in predicted densities for the two treatments evident in (Figs. 1.2C & 1.3C). We recorded differences consistent with model predictions for density (Fig. 1.3D) where the “no cage” treatment had higher densities than the “cage,” treatment, which is opposite of what would be expected given that *L. nigrinus* caused significant predation. Interestingly, and for reasons that are unclear, there was significantly higher overwintering mortality in the caged branches than in uncaged branches at these sites in 2018 as reported by Jubb et al. (2019), which may be causing this difference in the model, as well as in the observed data. When establishing the experiment, branch pairs were chosen so that conditions experience by HWA populations on both cages, so it is hard to know exactly what happened to the caged branches with higher winter mortality. A companion paper by Jubb et al. (2019) which collected the overwintering mortality data used to parameterize the Elkinton et al. (2011) model describe potential reasons for higher overwintering mortality. In another bag experiment working with HWA in the PNW we have found no significant difference in air temperature between bags (Crandall et al., unpublished data), additionally Nelson and Rieske (2014) also found that there was no difference between air temperature and humidity inside cages compared to ambient measurements for uncaged branches.

Progreiens density in 2018 was lower than predicted by our model across both predator exclusion treatments. This unpredicted drop in density is likely due to environmental factors, such as tree condition and weather, factors not included in our model. Tree health is an important factor in HWA performance in that HWA prefer to settle and have better performance on healthy trees that are producing lots of new growth (McClure, 1991; Sussky and Elkinton 2015). Weather factors such as low or high temperatures in the winter and summer respectively would also impact HWA mortality (Sussky and Elkinton, 2015; Mech, 2015). However, including tree health and climactic factors is beyond the scope of the Elkinton et al. (2011) model and were never the purpose of the model anyway. The low proportion of new growth produced at the Delaware Water Gap, NJ (DEWA) in 2018 (Table 1.4) suggests that we may have had a decline in survival of HWA due to deterioration of host health (McClure, 1991). In both years, the trees produced new growth on less than 15% of terminal branchlets, which is similar to the tree growth reported by both McClure (1991) and Paradis (2011) on hemlock branches following outbreak phase of HWA densities. Similar reductions in new growth production (the number of terminal buds producing new growth on sample branches) were observed by (Sussky and Elkinton, 2014) on branches following inoculation of branches with high densities of HWA the previous year. In our study, we suspect that the low production of new growth at the end of the 2017 sampling period was the result of host decline. The data also suggest that with the 2018 reduction in HWA fecundity and progrediens density, the trees were beginning to rebound as shown by the doubling in the percentage of tips with new growth in 2018 compared with 2017 at the DEWA site (Table 1.4). This new growth occurred at the end of our experiment and

would have been available to the following sistens generation, not the final progrediens generation for which we measured.

In 2018 lower than average sistens fecundity was observed and, as a result, progrediens density also declined. In 2018, sistens mean fecundity across all sites was 48.1 which is much lower than reports of about 100-150 eggs per female from sistens settling on new growth of healthy hemlock (McClure, 1991; Paradis, 2011). Since only a low proportion of new growth shoots were produced by our sample trees, we would therefore expect that fecundity would also be lower due to deteriorated host health. However, because reduced fecundity was observed across all sites, we suspect that weather may also have played a role. Population densities of many forest insects fluctuate in synchrony across large regions due to shared weather impacts (the Moran effect) even though these weather events do not directly cause the fluctuations (Liebhold et al., 2004). McClure (1991) showed that population fluctuations of HWA are characterized by a two-year boom and bust cycle governed by the interaction of HWA with its hemlock host. Other recent research has documented the impact of recent cold winter events that have decimated HWA populations over the entire eastern U.S. in certain years (Cheah, 2017; Elkinton et al., 2017; McAvoy et al., 2017; Tobin et al., 2017). These events may cause the boom and bust cycle to synchronize over the region, even though the low density in 2018 may not have been directly caused by winter freeze events or other weather conditions in that year.

The branches we selected for this experiment were heavily infested with HWA and thus near carrying capacity (two or more HWA per needle base) and the simulations we present were run at those HWA densities in the model. Previous studies by Lamb et

al. (2005) suggest that *L. nigrinus* can have significant impacts on sistens and resulting progrediens generation. However, a major difference here was that the Lamb et al. (2005) experiment was a predator enclosure experiment and ours was a predator exclusion experiment which allows for a more “natural” interaction between predator and prey rather than restricting the tested *L. nigrinus* access to a single HWA infested branch. Data from a predator exclusion experiment, similar to the one reported here, in the native range of HWA in the Pacific Northwest of the U.S. suggest that summer-active predators may be playing an important role in reducing the numbers of progrediens; in a separate study, we found large and significant differences in HWA density between caged and uncaged branches in the Pacific Northwest (Crandall et al., unpublished). The data suggest that if summer-active predators, such as the two species of *Leucopis* (Diptera: Chamaemyiidae), *Leucopis argenticollis* Zetterstedt and *Leucopis piniperda* Malloch, HWA specific predators that are abundant on HWA in the Pacific Northwest (Motley et al., 2017; Kohler et al., 2016), could be established in the eastern U.S. to help reduce HWA densities well below carrying capacity, then the effect of predation by *L. nigrinus* might not be subject to the rebound effect we document here. Crandall et al. (unpublished) data suggest that summer-active predators, along with *L. nigrinus* may be able to convert HWA to non-pest status and indicate the viability of the ongoing biological control effort for HWA.

Ecological interactions are complex and may not be intuitively obvious. Using field-collected data to parameterize mathematical models allows us to explore the ecology of an organism and suggest experiments that can help us better understand these complexities. Here we used the predictions of a simulation model to design a predator

exclusion experiment to test whether an introduced biological control agent can lower densities of HWA. Our results suggest that predation by *L. nigrinus* is not having a major impact on progreiens densities because of the strong density-dependent survival affecting HWA progreiens stage (Fig 1.4), which largely compensates any effect of predation by *L. nigrinus* is on HWA ovisacs.

CHAPTER 2

WHY ISN'T THE HEMLOCK WOOLLY ADELGID KILLING TREES IN ITS NATIVE RANGE? THE ROLE OF INSECT PREDATORS IN MANAGING HEMLOCK WOOLLY ADELGID POPULATIONS IN THE PACIFIC NORTHWEST OF NORTH AMERICA

2.1 Introduction

The era of globalization threatens biodiversity, human health, and economies worldwide through the importation of species from geographically distinct regions. (Early et al. 2016, Hulme, 2009). Global commerce and trade have facilitated introduction of exotic species, some of which may have the potential to become invasive in these novel environments, damaging biodiversity and ecosystem function (Charles and Dukes, 2008). Many insects are prominent examples of invasive species, many of which have devastating impacts where they invade through direct effects, such as herbivory of native plants, predation, parasitism, and hybridization of native insects, and indirect effects, such as vectoring disease and the cascading effects of habitat fragmentation and loss (Kenis, 2009).

In invaded ecosystems, exotic herbivorous insects often attack host plants that have little resistance, reducing bottom-up impacts on the invaders, in contrast to what the insects may experience with their native hosts. Invasive herbivorous insects often lack effective natural enemies (predators, parasitoids, pathogens, etc.) in their introduced range, and as a result the invaders have higher survival rates due to reduced top-down impacts (Keane & Crawley, 2002). These reductions in top-down and bottom-up restraints can allow the invader to achieve much higher densities than occur in their

native range. The two most common methods for control of invasive insects attacking forest trees are application of pesticides, and biological control. Pesticides are highly effective at the tree level but are not feasible at the forest scale due to their high cost and effects on native and beneficial insects. Biological control through the introduction of the invasive pest's natural enemies can be effective on a forest level scale, but may require years to implement, moving through several steps including finding natural enemies in the native range, assessing their host specificity, mass rearing the agents for release, and obtaining establishment. Even after establishment of new natural enemies, some considerable time may be required before population level effects on the target occur. However, when biological control works, it provides self-sustaining control with little impact on non-target species and little effort from land managers. For all of the above reasons, biological control has been the main focus of management efforts against the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), since its introduction into the eastern United States.

Hemlock woolly adelgid, which is native to Asia and the Pacific Northwest of the North America (hereafter Pacific Northwest), is a devastating invasive insect that was accidentally introduced to the eastern United States from Japan in the early 1900s. Subsequently, it has since caused widespread mortality of eastern (*Tsuga canadensis* [L.] Carriere) and Carolina (*Tsuga caroliniana* Engelm) hemlocks in much of their range (Havill et al., 2014). Where hemlock species occur, they often exist in dense stands that create unique habitats on which several species depend (Orwig and Foster, 1998, Adkins and Rieske, 2013). This dense-shade habitat cannot be replaced by other native trees in the eastern United States, and therefore widespread hemlock mortality results in

significant loss of this special habitat (Preisser et al., 2014). The threat of this invasive adelgid to hemlock has prompted extensive research on hemlock woolly adelgid in both its native and invaded ranges to better understand its population dynamics, host plant interactions, and natural enemies (Gonda-King et al., 2012, Gouli et al., 2013, Kohler et al. 2008a, Sussky and Elkinton, 2014). There are many generalist predators and several specialists attacking HWA in its native range, and some of these have been released in the eastern U.S. (Onken and Reardon, 2011).

HWA feed on hemlocks by inserting their piercing-sucking mouthparts, called stylets, into the base of needles to remove starch from the ray parenchyma cells (Young et al., 1995). There are two generations of HWA per year (Figure 2.1). The overwintering sistens generation occur from early summer to mid spring and the progrediens generation is present from late spring to early summer (McClure, 1989). Both generations reproduce parthenogenetically, laying their eggs under wool-like wax forming an ovisac that provides protection from some predators (McClure, 1987). When hemlock twigs become overcrowded with HWA and tree health declines, some individuals of the progrediens generation can develop as winged adults called sexuparae capable of migrating to an alternate host. However, the alternate spruce host, *Picea torano* (K. Koch) Koehne, is not found in North America, and sexuparae die without reproducing. Sexuparae are thought to no longer be produced by this adelgid in the Pacific Northwest (Zilahi-Balogh et al., 2003, Havill et al., 2006).

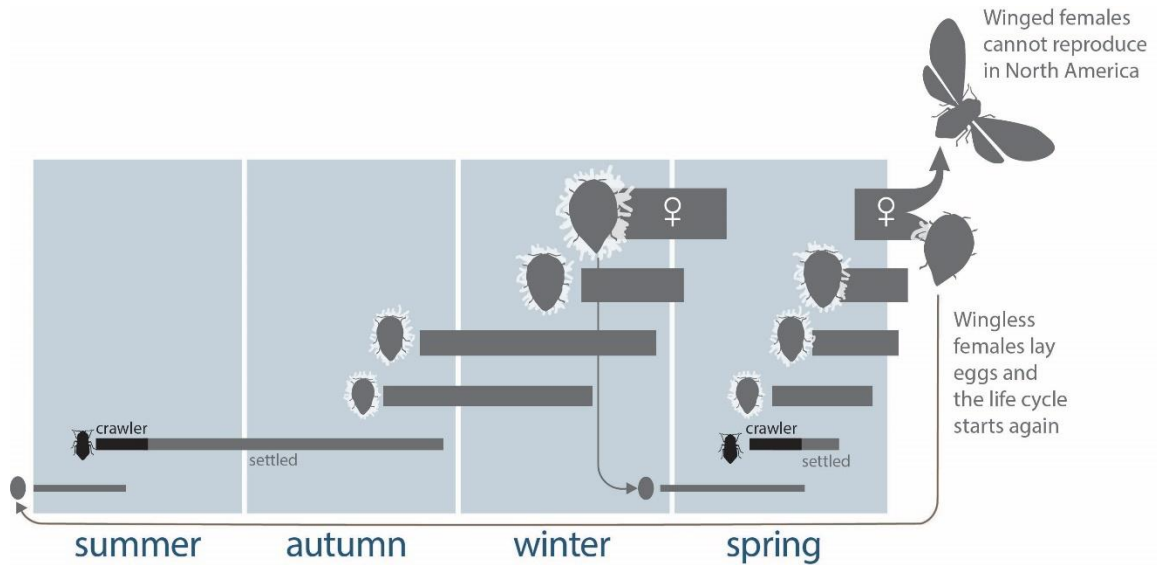


Figure 2.1: Hemlock woolly adelgid North American lifecycles. The sistens generation, sometimes referred to as the “overwintering” generation, are present from summer through early spring. Sistens adults lay eggs in late winter which give rise to the progrediens generation. Progrediens are active from early spring through early summer and can produce eggs which become the sistens generation. In high-density situations, common to the invaded range, progrediens can choose to become winged adults called sexuparae which carry out a sexual generation on Asiatic spruce species. In North America there are no suitable spruce species, therefore sexuparae fail to reproduce. Used through permission by Nathan Havill and Vince D’Amico.

In the Pacific Northwest, HWA is not considered a pest and typically occurs at low densities and can be hard to find, even though western hemlock (*Tsuga heterophylla* [Rafinesque] Sargent) is a very common species. Havill et al. (2016) suggest that hemlock woolly adelgid has been present in the Pacific Northwest for tens of thousands of years, if not longer. In that time, western hemlock may have evolved chemical resistance to hemlock woolly adelgid or tolerance to its damage. Western hemlocks rarely die due to HWA infestation, which has been attributed to the suite of native predators present in the Pacific Northwest. It is also assumed that western hemlock may have defenses to deter hemlock woolly adelgid feeding, unlike eastern hemlock, although studies remain unclear whether trees are resistant or tolerant of feeding damage or some combination of both (Oten et al., 2014). In the Pacific Northwest hemlock woolly adelgid

is associated with a group of native generalists and some specialist insect predators that suppress hemlock woolly adelgid to innocuous levels; however, the effect of these predatory insects on hemlock woolly adelgid populations has not been experimentally assessed.

In the eastern United States, several predatory beetles from the native range have been released, but only *Laricobius nigrinus* Fender (Coleoptera: Derodontidae), collected from the Pacific Northwest, has clearly established (Cheah et al., 2004; Foley et al., 2019). *Laricobius nigrinus* is univoltine and is synchronized with the hemlock woolly adelgid seasonal life pattern, undergoing summertime aestival diapause at the same time as sistens nymphs. Adults of *L. nigrinus* feed on sistens nymphs and adults, and lay eggs on sistens ovisacs. When *L. nigrinus* eggs hatch, the larvae feed primarily on eggs of the progrediens generation (Zilahi-Balogh et al., 2003). Other specialist adelgid predators of interest as potential biocontrol agents include two *Leucopis* species (Diptera: Chamaemyiidae) (*Leucopis argenticollis* [Zetterstedt] and *Leucopis pinniperda* [Malloch]). These flies are native to the Pacific Northwest and are the second most abundant predator, after *L. nigrinus*. They are bivoltine and well synchronized with the hemlock woolly adelgid life cycle, feeding on eggs, nymphs and adults of both the progrediens and sistens generation (Kohler et al., 2016).

The USDA Forest Service has devoted significant funding to the importation, rearing, and release of various insect predators of HWA from its native range. *Laricobius nigrinus* from the Pacific Northwest has been successfully established in many sites across the invaded range in the eastern U.S. (Mausel et al., 2010), but it has not yet been shown to provide significant regulatory pressure on HWA (Chapter 1), despite high rates

of predation (Jubb et al., 2019). Currently, two *Leucopis* species, also from the Pacific Northwest, are being studied as summer predators that might compliment the action of *L. nigrinus*. Host range testing and predator enclosure experiments are being conducted in preparation of their potential release in the eastern United States (Grubin et al., 2011, Motley et al., 2017). Despite release of predators from the Pacific Northwest in the eastern United States, no empirical evidence exists to show they are indeed what keeps hemlock woolly adelgid densities in the Pacific Northwest below damaging levels.

To evaluate predation of hemlock woolly adelgid by native predators in the Pacific Northwest, we conducted a predator exclusion experiment at the Washington Park Arboretum in Seattle, WA, using both eastern and western hemlock. Having both tree species in a common garden plot allowed us to look for differences in predation rates on hemlock woolly adelgid and evaluate host tree effects, while controlling for environmental factors. For this study, we had two goals: (1) to determine if insect predators were responsible for maintaining HWA densities below carrying capacity and examine the relative importance of summer-active versus winter-active predators and (2) to determine if HWA colonization and densities differed by tree species. The potential for resistance or tolerance of western hemlock to HWA could account for the vastly lower densities of HWA on western hemlock in the Pacific Northwest, compared to densities of HWA on eastern hemlock in the invaded range of HWA.

2.2 Methods

2.2.1 Collection of HWA inoculum and deployment on experimental branches

Two rounds of inoculations and observations of hemlock woolly adelgid-infested hemlock branches, with each round lasting one year, were carried out in the Washington Park Arboretum in Seattle, Washington, one in 2015 and another in 2017. In March of 2015 natural infestations of HWA were found in Green Lake Park, Seattle, WA (47.671072, -122.344422). In March of 2017, natural infestations of HWA were found in the Olympic Peninsula in the town of Sequim, WA (48.078056, -123.101389). Hemlock woolly adelgid-infested branchlets were chosen that had no or few signs of predation and 20 to 200 ovisacs. Branchlets were clipped, the number of ovisacs counted, and branchlets randomly grouped into bundles of 1, 3, or 5 and placed in bricks of water-soaked floral foam (Smithers-Oasis Kent Co. OH, USA) to prevent desiccation. Branchlets were stored in bins that were kept indoors at room temperature for about four days until deployment.

We deployed inoculum onto experimental branches on March 23 in 2015 and March 27 in 2017. Inoculum branchlets were taken to the test field site (Washington Park Arboretum) and bundles of 1, 3, 10, and 20 branchlets (bundles of 5 branchlets were grouped to make the 10 and 20 bundles) were placed on experimental branches (branch pairs were given equivalent densities of ovisacs) to create varying densities, between branch pairs, of ovisacs with a range of 100 to 1,400 ovisacs per bundle. Twenty hemlock trees (10 eastern and 10 western) were identified and two pairs of uninfested branches per tree were labeled to be used as experimental branches. Inoculum densities were randomly assigned to branch pairs. The pairs were then given bundles of 1, 3, 10, or 20 branchlets, corresponding to their assigned inoculum density, that were then fixed onto the branches with plastic cable ties. Predator exclusion bags (1 m long by 0.5 m wide Equinox® No-

See-Um mosquito netting, ~569 holes per square centimeter) were then placed over all branches and tied on using strips of self-stick vinyl foam insulation (3.2 cm. x 48 mm) between the branch and the cable tie so that ties did not cut off flow of nutrients and water. The sample branches were monitored over the next few weeks to confirm that nymphs had settled by examining inoculum ovisacs with a hand lens for the presence of eggs, as well as checking the sample branches visually for settled nymphs. In April of 2015 and 2017, the mesh bags were removed from one branch in each pair and the inoculation bundles were removed from all branches. The mesh bag remained on the second branch in each pair to restrict predators from reaching the HWA on that branch. In 2015, densities of HWA in June were very low; >0.1 adelgid per centimeter. Branches with some hemlock woolly adelgid were kept in the study and natural infestations, that were found in the arboretum, were incorporated into the study by choosing branch pairs with similar densities of HWA and bagging one of the branches. Before bagging, branches were shaken about 20 times to remove any predators that may have been on the branch.

2.2.2 Data collection

For round one of this experiment, in all sampling periods in 2015-2016, densities of HWA were estimated by removing a 30 - cm long sample branchlets which were then shipped overnight to our laboratory in Massachusetts for counting hemlock woolly adelgid life stages. Sample branchlets were observed with a dissecting microscope and length of growth segments and counts of hemlock woolly adelgid on growth segments were recorded. In June of 2015, the density of progrediens was estimated on both inoculation branches as well as naturally infested branches. Densities were sampled in

November of 2015 (maturing sistens) and again in March 2016 (adult sistens) for the naturally infested branches. The experimentally inoculated branches still had only very low sistens densities and were not sampled again until November 2016. Finally, in June 2016, progrediens densities were counted on the naturally infested branches. In November of 2016 both the inoculation branches and naturally infested branches were sampled for the maturing sistens generation. The inoculation branches were also sampled for the already completed progrediens generation as their ovisacs were still present on the tree and were clearly distinguishable from the smaller fresh ovisacs of the maturing sistens generation.

For round two in this experiment, March 2017, the initial number of sistens used for inoculation was estimated by counting the number of ovisacs in each inoculum bundle. From mid-June through mid-July of 2017, sample branches were checked weekly to monitor the progress of the progrediens generation to measure settlement on sample branches. For western hemlock, 17 of the 20 paired branches were successfully inoculated, progrediens nymphs had colonized large portions of sample branches, compared to the 8 for eastern hemlock.

On each sample branch, 30-cm branchlets, were chosen and marked with twist ties for later, non-destructive sampling. In the field, the numbers of progrediens nymphs and adults per centimeter on the marked branchlets were counted using a hand lens and headlight. The 30-cm long branchlets were again sampled in November 2017 and in March 2018 to record the number of maturing and adult sistens, respectively. In July 2018, progrediens densities were recorded.

2.2.3 Data Analysis

All analyses were performed in R 3.5.3 (RCoreTeam 2019) using RStudio, version 1.2.1335. All graphs were prepared using ggplot2 (Wickham, 2009).

Densities of hemlock woolly adelgid in each life stage for both generations on each tree species were compared between treatments by branch pairs with a generalized linear mixed model (GLMM) (Package = *lme4*, Version 1.1-21), using the ‘glmer’ function with a gamma distribution and a random effect for branch pair (Bates et al., 2015). In order to run the analyses, we added a small constant (0.0001) to the response variable (HWA density) to permit analysis even when counts of HWA were zero (Zar, 2010).

Throughout the 2017-2018 round 2 experiment, we used iButtons (Maxim Integrated, San Jose, CA) to record air temperatures at two-hour intervals in both the bagged and unbagged treatments. The purpose was to test for difference in air temperature between bagged and unbagged treatments that might potentially account for increased densities inside bagged treatments. We used data recorded from January to July 2018 because it was the most complete dataset from the two years. Data were summarized to daily average temperatures and analyzed using a paired t-test.

2.3 Results

2.3.1 HWA densities

In the first experiment (Round one, 2015-2016 sampling period), HWA densities were generally low (<1 HWA per cm) in both treatments. Densities were not significantly different between the bagged and unbagged treatments on eastern hemlock (Table 2.1A-

E). For western hemlock, densities of HWA between bagged and unbagged treatments were not significant in 2015 (Table 2.2A-B) but became significant by 2016 for the sistens adults (in March), the progrediens adults (in July), and the next generation of sistens nymphs (in November) (Table 2.2C-E; Figs. 2.2A-B).

In the second experiment (Round two, 2017) (Figs. 2.2C-D), there was no statistical difference between treatments (bagged and unbagged) in the number of established progrediens nymphs following inoculation on either eastern or western hemlock (Tables 2.1F and 2.2F). On eastern hemlock, the density of progrediens adults and sistens nymphs in 2017 (the following generation) also did not differ significantly between treatments (Table 2.1G-H). However, in the following two generations there were significant differences in density between the bagged and unbagged treatments for adults sistens in March 2018 (Table 2.1I) and for the subsequent progrediens generation in July 2018 (Table 2.1J). However, this difference did not persist to the next generation, i.e., in the sistens nymphs in November 2019 (Table 2.1K).

In contrast to events on eastern hemlock, for western hemlock, we found that after the initial progrediens nymph stage (reflecting success of inoculation) (Table 2.2F), there were statistically significant differences in density between bagged and unbagged treatments in all the following generations (5 life stages, 3 consecutive generations) (Table 2.2G-K).

Table 2.1. Model outputs of densities of hemlock woolly adelgid between treatments for each generation of HWA for eastern hemlock (*Tsuga canadensis*)

Generation	Sample Period	df	Test Statistic S	P value
A. Progrediens Adults 2015	July 2015	9	-20	0.0625
B. Sistens Nymphs 2015	November 2015	No Data	No Data	No Data
C. Adult Sistens 2016	March 2016	No Data	No Data	No Data

D. Progrediens Adults 2016	July 2016	5	-4	0.625
E. Sistens Nymphs 2016	November 2016	4	-6	0.25
F. Progrediens Nymphs 2017	June 2017	12	1.5	0.946
G. Progrediens Adults 2017	July 2017	12	-0.5	1
H. Sistens Nymphs 2017	November 2017	7	-13.5	0.0781
I. Adult Sistens 2018	March 2018	5	-10.5	0.0313
J. Progrediens Adults 2018	July 2018	4	-7.5	0.0625
K. Sistens Nymphs 2018	November 2018	6	-8.5	0.2188

Significant effects at $P < 0.05$ are in bold

The line in the middle of the table separates experiment 1 from experiment 2

Table 2.2. Model outputs of densities of hemlock woolly adelgid between treatments for each generation of HWA for western hemlock (*Tsuga heterophylla*)

Generation	Sample Period	df	Test Statistic S	P value
A. Progrediens Adults 2015	July 2015	17	-21.00	0.2661
B. Sistens Nymphs 2015	November 2015	11	-12.50	0.3652
C. Sistens Adults 2016	March 2016	11	-29.50	0.0137
D. Progrediens Adults 2016	July 2016	9	-26.50	0.0039
E. Sistens Nymphs 2016	November 2016	8	-17.00	0.0469
F. Progrediens Nymphs 2017	June 2017	16	14.50	0.5171
G. Progrediens Adults 2017	July 2017	16	-54.50	0.0079
H. Sistens Nymphs 2017	November 2017	16	-60.50	0.0026
I. Sistens Adults 2018	March 2018	19	-84.00	0.0009
J. Progrediens Adults 2018	July 2018	17	-85.50	<0.0001
K. Sistens Nymphs 2018	November 2018	13	-37.50	0.0166

Significant effects at $P < 0.05$ are in bold

The line in the middle of the table separates experiment 1 from experiment 2

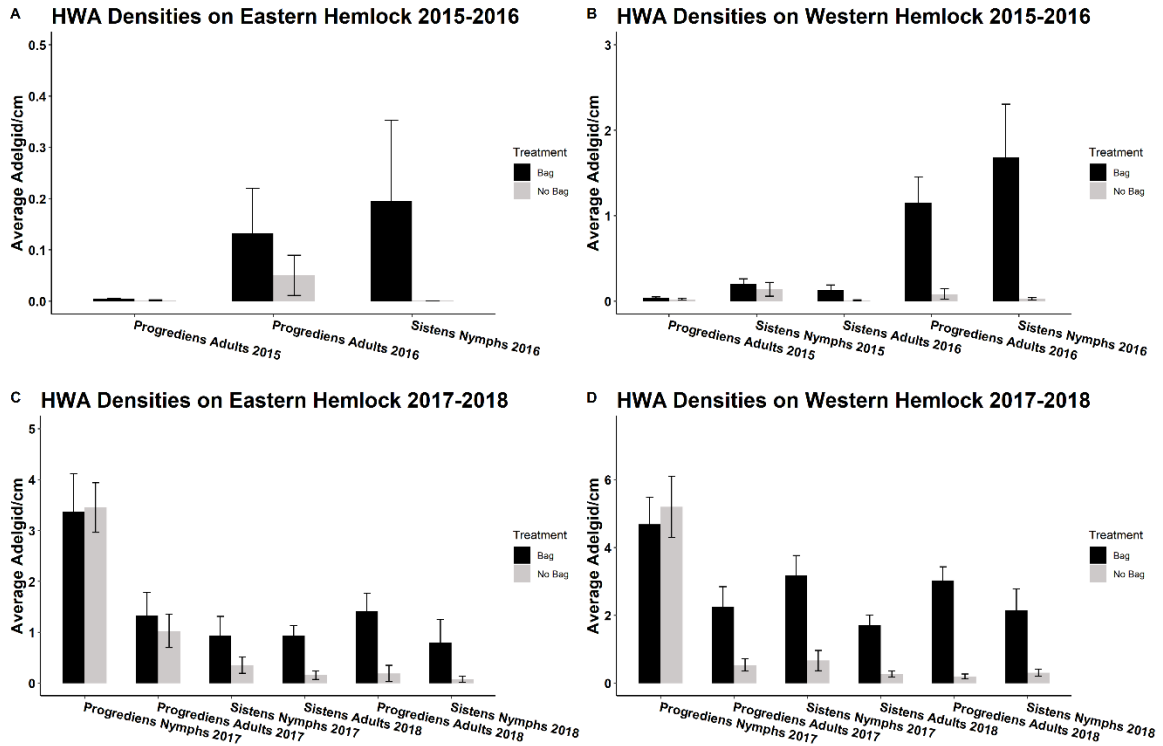


Figure 2.2. Mean (\pm SE) density of HWA life stages on bagged and unbagged branches Washington Park Arboretum in Seattle, Washington. A) Eastern hemlock 2015-2016 B) Western hemlock 2015-2016 C) Eastern hemlock 2017-2018 D) Western hemlock 2018

2.3.2 Investigating potential bag effect on air temperature

We found that there was no significant difference in temperature between bagged and unbagged treatments ($df = 4794$, $t = -0.189$, $P = 0.85$), in terms of the daily mean air temperature (Figure 2.3).

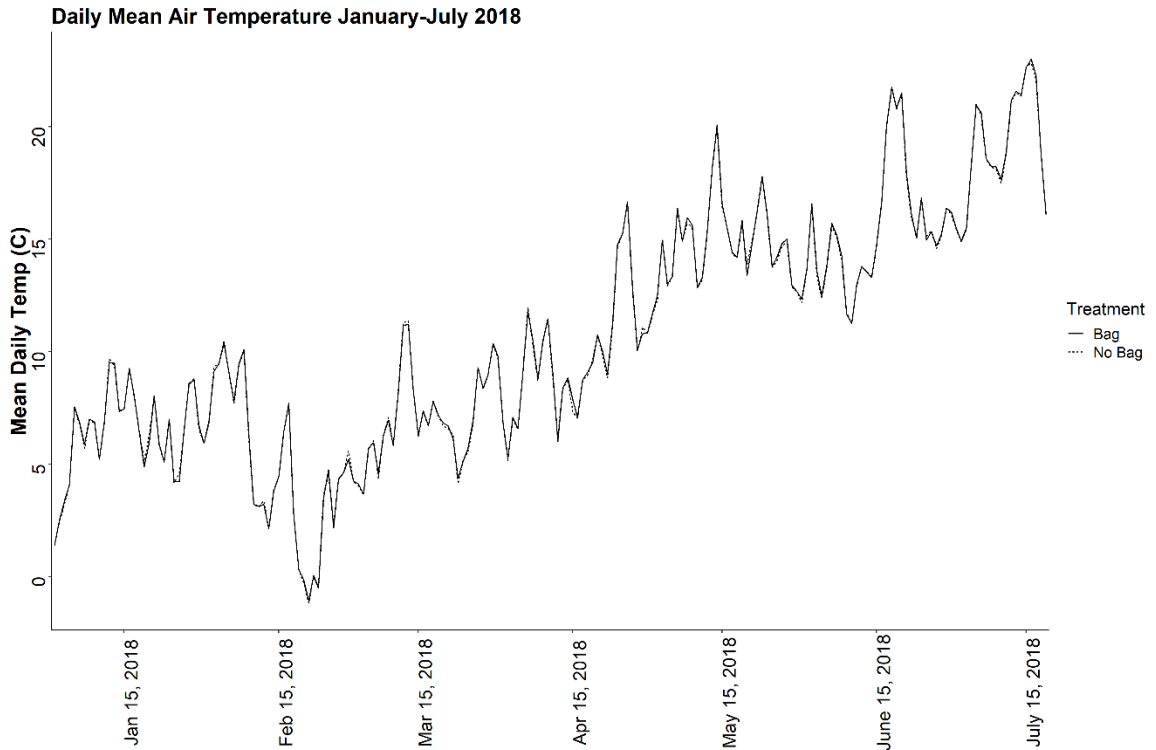


Figure 2.3 Mean daily air temperature recorded with iButtons in bagged (solid line) and unbagged (dotted line) branches in the Washington Park Arboretum Seattle, WA from January – July 2018.

2.4 Discussion

Our study reveals the importance of summer-active predators and suggests that such predators are playing an important role in regulating HWA populations and indicates that this group may be an important component needed for the biological control efforts in the invaded range of HWA. Studies in the eastern U.S. have already shown that *L. nigrinus* predation significantly affects the ovisacs of the sistens generation, nearly 80% predation (Jubb et al., 2019). However, we have shown that at high HWA density, that progrediens already low survival, >10% survive to adult, can compensate for this egg mortality allowing the progrediens generation to rebound HWA populations (Chapter 1). We believe that summer-active predators are important in

reducing the progrediens rebound effect and if released in the eastern U.S., could work in tandem with *L. nigrinus* with the potential to drastically reduce HWA densities to much lower levels.

In both years we successfully inoculated trees with HWA. In 2015 the inoculations were less successful, and it took time for the HWA densities to build on bagged branch treatments. The 2017 inoculation of sample trees was much more successful, indicated by the higher starting densities of HWA (Figs 2.2A&C) between treatments on both eastern and western hemlock in the settled progrediens nymphs. In both years, establishment of HWA on eastern hemlock was less successful. Poor establishment of HWA on eastern hemlock and successful establishment on western hemlock suggest that western hemlock is not suppressing HWA through tree resistance, otherwise we would expect that HWA would settle more successfully on eastern rather than western hemlock. It is possible that western HWA are better suited to western hemlock as their host as a result of their long coevolutionary history and therefore this is why they are not able to colonize eastern hemlock as easily.

The initial decline in density on unbagged branches during the progrediens generation in 2017 implies summer-active predator feeding on HWA as the direct cause. Unfortunately, our data do not tell us the relative impact by predator species or by combination of predators. However, we did collect and identify (using CO1 DNA barcoding), fly larvae found foraging on sample branch ovisacs and results included three species of chamaemyiids (*Leucopis piniperda*, *L. argenticollis*, and one *Neoleucopis* sp.), two species of cecidomyiids (not identified to genus), and syrphids (not identified to genus). We also frequently observed predatory true bugs (Homoptera) including

Lygaeidae (*Kieidocerys resedae* (Panzer)), Anthocoridae, Reduviidae, and green lacewing nymphs (Chrysopidae) (all three not identified to genus) and much less frequently (maybe only 3-4 individuals over the course of the experiment) found coccinellids, namely *Harmonia axyridis*.

We found that the densities inside the bag were high enough that many HWA were settling on all parts of the branch, even the mainstem of the sample branch. These conditions were enough to encourage sexuparae production, which, until now, was thought not to occur in the Pacific Northwest (Zilahi-Balogh et al., 2003). Sexuparae samples were tested (using CO1 DNA barcoding) and they matched with western North American lineage of HWA. At the time of our sampling in June of 2018 it appeared that a large number of sexuparae were produced in some bagged branches, as evidence by dead sexuparae on some needles as well as vacant messy wool, characteristic of sexuparae and distinctly different from the neat wool produced by progrediens adults (personal observation), left behind on the branches. We were unable to record the proportion of progrediens becoming sexuparae as we did not expect to find them in the first place.

Our data suggest a further impact of predators on the sistens generation because the difference in densities between bagged and unbagged treatments increased in most years between the progrediens generation and the subsequent sistens generation. We expect that much of this predation may be due to *L. nigrinus*, which re-emerges from summer aestivation in early fall and starts feeding on developing nymphs (Zilahi-Balogh et al., 2003). However, it is also possible that some of this treatment effect on sistens densities is due to a bag effect on limiting the dispersal of sistens crawlers inside the bag and artificially increasing the densities inside the bags. We plan to test for this in a

separate experiment, but for our current results we reiterate that our initial drop in density from the progrediens nymphal stage to the progrediens adult stage in 2017 would not be effected by this, as both branches were bagged during the settlement of the progrediens nymphs and potential bag effects wouldn't happen until the settlement of the 2017 sistens nymphs. We also addressed the possibility of a temperature related bag effect with our iButtons, and after analyzing that data, we found that there was no difference in air temperature between treatments. A separate study which leaves treatments bagged throughout the summer and are taken down from half the branches as summer-active predators become less active and *L. nigrinus* starts feeding is currently underway to test for any bag effects on dispersal.

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