Top-Down Regulation of Hemlock Woolly Adelgid (Adelges Tsugae) in Its Native Range in the Pacific Northwest of North America

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Top-down regulation of hemlock woolly adelgid (*Adelges tsugae*) in its native range in the Pacific Northwest of North America

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Abstract

The density of insect herbivores is regulated by top-down factors (e.g., natural enemies), bottom-up effects (e.g., plant defenses against herbivory), or a combination of both. As such, understanding the relative importance of these factors can have important implications for the establishment of effective management options for invasive species. Here, we compared the relative importance of top-down and bottom-up factors on the abundance of hemlock woolly adelgid (HWA), *Adelges tsugae*. HWA is invasive in eastern North America, but its native range includes the Pacific Northwest of North America where it has co-evolved with western hemlock, *Tsuga heterophylla*. Eastern hemlock, *Tsuga canadensis*, can also be found planted in city and park settings in the Pacific Northwest and the presence of both host species allowed us to directly compare the importance of predators (top-down) and host plant resistance (bottom-up) on HWA abundance by placing mesh exclusion bags on branches of both species and monitoring HWA abundance over two years. We found no evidence for bottom-up control of HWA on western hemlock (a native host). HWA established more readily on that species than on eastern hemlock on which it is a major pest in eastern North America. We found strong evidence for top-down control in that both summer and winter-active predators significantly reduced HWA densities on the branches of both tree species where predators were allowed access. These findings support the validity of the biological control program for HWA, the goal of which is to reduce outbreak populations of HWA in eastern North America.

Keywords

Predator exclusion, insect predators, artificial infestation, *Tsuga canadensis*, *Tsuga heterophylla*

Introduction

The densities of many herbivores are regulated through a combination of factors that include mortality caused by natural enemies (top-down effects), and reductions in fitness mediated by plant defenses against their feeding (Hunter and Price 1992; Power 1992). Recent studies of herbivorous insects have shown that top-down control usually has a larger effect than bottom-up control, although there is considerable variation related to factors such as diet breadth (e.g., specialists versus generalists) and feeding guild (Vidal and Murphy 2018). The view that densities of terrestrial herbivores are mainly regulated by top-down factors was argued by Hairston et al. (1960) and supported by others including Lawton and Strong (1981) and Strong et al. (1984). Murdoch (1966) challenged this idea and Denno et al. (1995) provided many counterexamples. Fretwell and Barach (1977) and Oksanen et al. (1981) argued that number of trophic levels in a community may determine the relative importance of top-down versus bottom-up regulation of herbivore density. The densities of natural enemies of herbivores are often held at low densities by their own natural enemies in the trophic level above. Clear examples of herbivores attacking forest trees that are regulated by bottom-up forces include bark beetles (Scolitinae) whose densities are typically determined by the availability of host trees whose defenses are weakened by environmental factors (Biedermann et al. 2019). As a result, ecologists have long sought to understand the relative importance of these top-down versus bottom-up effects: identifying and quantifying the relative importance of these factors is critical to understanding and implementing effective management strategies for invasive pest species (Hovick and Carson 2015).

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a sap-feeding insect on hemlock (*Tsuga*) species native to eastern Asia and the Pacific Northwest region of North America (hereafter Pacific Northwest), where multiple distinct lineages of HWA have coevolved with different species of hemlock trees found across its range (Havill et al. 2016). In the Pacific Northwest, HWA is associated with western hemlock, *Tsuga heterophylla* [Rafinesque] Sargent (Pinaceae: Pinales), a species commonly found across this region (McClure 1992). However, while western hemlock is a dominant understory tree in this region, HWA typically occurs at low densities (McClure 1992). In contrast, HWA in eastern North America is a non-native pest that was introduced from Japan (Havill et al. 2016). This Japanese lineage of HWA can reach high densities in its introduced range where it is responsible for the widespread mortality of eastern hemlock, *T. canadensis* [L.] Carriere, and Carolina hemlock, *T. caroliniana* Engelmann, across much of eastern North America (Havill et al. 2014). Various studies suggest that HWA populations in eastern North America are primarily regulated by bottom-up factors in the form of competition for space on hemlock twigs and HWA-induced reduction of new hemlock shoots that the insect depends on for the next generation of HWA populations (McClure 1991; Elkinton et al. 2011; Sussky and Elkinton 2014). There are no known parasitoids of HWA (Cheah et al. 2004) and natural enemies native to eastern North America play an insignificant role in regulating HWA densities (McClure 1987; Montgomery and Lyon 1996; Wallace and Hain 2000).

Given the economic and ecological importance of hemlock trees, the USDA Forest Service has devoted significant funding to the importation, mass rearing, and release in eastern North America of various insect predators of the Japanese HWA lineage. Some of these predators, such as *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) from the Pacific Northwest, have established self-sustaining populations and are spreading from many release locations (Mausel et al. 2010; Foley et al. 2019; Jubb et al. 2021). There has been very limited success with these introductions in terms of reducing HWA populations despite high predation rates (see Crandall et al. 2020; Jubb et al. 2020), suggesting that bottom-up forces might also be important in determining HWA abundance in its native and introduced ranges.

We studied the relative importance of top-down and bottom-up forces on HWA densities in its native range in the Pacific Northwest by inoculating branches of both eastern and western hemlock trees and excluding predators from half of the inoculated branches. By comparing plant host and predation simultaneously, we directly examined (1) the relative importance of summer-active and winter-active native predators, (2) whether HWA colonization and abundances differed by tree species, and (3) the relative importance of top-down and bottom-up forces on HWA feeding on native and non-native hosts in its native range.

Methods

HWA life cycle and feeding

HWA feeds by settling at the base of a hemlock needle and inserting their long thin mouthparts, called stylets, into the twig to feed on ray parenchyma cells (Young et al. 1995). HWA are parthenogenic and produce two generations per year, the overwintering sistens generation and the spring progrediens generation (McClure 1987). The sistens generation hatch in the summer, settle on freshly produced new growth, aestivate until early fall, feed through the winter, and oviposit from late winter through early spring (McClure 1987; Sussky and Elkinton 2015). In spring, the progrediens eggs hatch and the progrediens first instar nymphs, commonly referred to as “crawlers”, settle and feed on the same growth as the parent sistentes (plural for sistens), until early to mid-summer when they lay sistens eggs (McClure 1991). The progrediens generation can either become adult progredientes or winged sexuparae (McClure 1991; Sussky and Elkinton 2014), which, in the native range, seek out tigertail spruce, *Picea torano* (K. Kock) Koehne, to carry out a sexual generation (Havill et al. 2006). Sexuparae produced in eastern North America fail to reproduce because no suitable native spruce host exists; sexuparae have not been previously observed in the Pacific Norwest (Zilahi-Balogh et al. 2003).

Collection of HWA inoculum and deployment on experimental branches

Two year-long rounds of inoculations and observations of hemlock woolly adelgid-infested hemlock branches were carried out in the Washington Park Arboretum in Seattle, Washington, USA in 2015 and 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, USA (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 approximately four days until deployment.

We deployed inoculum onto experimental branches on 23-March-2015 and 27-March-2017. Inoculum branchlets deployed at Washington Park Arboretum in bundles of 1, 3, 10, and 20 on experimental branches to create varying densities 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. Branches within each pair were given equivalent densities of ovisacs. 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 nutrient and water flow. 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 June of 2015, densities of HWA were very low; >0.1 adelgid per centimeter. Branches with some HWA were kept in the study and natural infestations 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.

Density data collection

For round one of this experiment (i.e., 2015-2016), densities of HWA were estimated by removing 30-cm branchlets and counting HWA life stages. Length of branchlet growth and counts of hemlock woolly adelgid on branchlets were observed with a dissecting microscope. In June of 2015, the density of progrediens was estimated on both inoculated and naturally infested branches. Adelgid densities on naturally infested branches were sampled in November of 2015 (maturing sistens) and March of 2016 (adult sistens). During these sampling periods, HWA populations on inoculated branches were still establishing, and therefore had very low sistens densities and were not sampled until November 2016. In June 2016, progrediens densities were counted on the naturally infested branches. In November of 2016, the maturing sistens generation was quantified on both the inoculated branches and naturally infested branches. The inoculated branches were also sampled for the past 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.

Round two of this experiment was initiated in March 2017, using the same inoculation method as round 1. 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. Of the 20 paired branches inoculated for each tree species, 17 pairs were successfully inoculated for western hemlock and 13 pairs were successfully inoculated 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.

Surveying natural HWA abundance

HWA densities were quantified at 36 sites in three regions in western Washington state (12 sites per region) to compare HWA abundance in each region. Hemlock trees were checked for HWA presence in three locations: Seattle, Washington (urban), Route 90 and Route 2 (rural), and the Olympic Peninsula (rural). Sites were either forests or wooded areas in parks and were at least one kilometer from other sites. In mountainous regions, we limited sites to below 1,500 ft elevation to reduce the effect of elevation as a confounding factor. Where possible, 20 trees per site (10 trees minimum where hemlock was less abundant) were sampled which involved checking the top and bottom of one-meter-long branch and rating the HWA infestation or lack thereof. We used a 0-3 scale of HWA infestation (0 = none; 1 = 1-10 ovisacs/m branch; 2 = 11-100 ovisacs/m branch; 3 = 101-1000 ovisacs/m branch), adapted from the methods used in Preisser et al. (2008). We also added in sites from New England to compare the densities from Washington to those in the invaded range of HWA. Sites were mainly from Massachusetts, but included one site from Vermont, one site from New Hampshire, and three sites from Maine. The same techniques were used to collect HWA abundance as described for Washington.

Data analysis

To look for differences in establishment of HWA by tree species, we subset our data to look only at bag data. By looking at the bag data separately, we remove any effect of predation on HWA establishment and isolate the effect of tree species on HWA establishment success. Data for this analysis were coded as a "1" if the branch had HWA and a "0" if the branch did not have HWA and were analyzed using a binomial 'glm' model in R version 4.1.2. Only data from Round 2 were used as this data had better establishment.

Densities of hemlock woolly adelgid were compared between treatments by branch pairs for each generational life stage on each tree species using two generalized linear mixed model (GLMM). The 'glmmTMB' function (Package = *glmmTMB*, Version 1.1.2.3) was used for zero-inflated data by specifying in the model a zero-inflation factor and using a zero-inflated Gamma family of distributions. Each model had a random effect for branch pair (Bates et al. 2015). The "Maturing Sistens 2016" failed to converge using the 'glmmTMB' function due to low sample size and the data being heavily zero-inflated. Instead, we ran that data with the 'glmer' function, using the Gamma distribution, and added a small constant (0.0001) to the response variable (HWA density) to permit analysis even when HWA were absent (Zar 2010). In addition to testing our data with GLMM models, we also analyzed HWA densities with the Wilcoxon signed rank test since our data was not normally distributed. Adding a non-parametric test compliments and supports the results from our parametric GLMM tests.

Rates of survival were compared between species and treatments for the progrediens generation in spring 2017 as well as the maturing sistens generation in fall 2017. These were the only two generations for which we had a full sampling of the generation from settlement to adult or maturing stages. To analyze the proportion of HWA surviving by treatment the 'glmer' function was used for each generation on both tree species. In each model, branch pair was a random effect, the response variable was a two-column vector containing the counts of live and dead HWA for each branch sample, and the binomial distribution as used. The binomial models were checked for overdispersion, but no overdispersion was found.

Throughout the 2017-2018 experiment, we used iButtons (Maxim Integrated, San Jose, CA) to record air temperatures at two-hour intervals in both the bagged and unbagged treatments to test for between-treatment differences in air temperature between bagged and unbagged treatments that might potentially account for increased densities inside bagged treatments. We used data recorded from January 2018 to July 2018 because it was the most complete dataset from the two years. Data were summarized to daily average temperatures and analyzed using the 'lm' function (Package = "*stats*", Version = 4.1.2).

HWA survey abundance data was analyzed using a non-parametric Kruskal-Wallis test because the data were non-normally distributed. The Kruskal-Wallis test allowed us to test for differences in average abundance between each of the regions that we sampled, acting analogously to a one-way ANOVA test. We also looked for differences in the percentage of branches with HWA by region using a 'glm' model with a quasibinomial distribution.

All analyses were performed in R 4.1.2 (R Core Team 2021). All graphs were prepared using ggplot2 (Wickham 2009).

Literature review of HWA fitness on *T. canadensis* and *T. heterophylla*

The Web of Science database and Google Scholar were queried to find publications that compared HWA density, survival, and/or fecundity on both *T. canadensis* and *T. heterophylla*. Search terms included combinations of the words "*Adelges tsugae*", "*Tsuga canadensis*", "*Tsuga heterophylla*", "host resistance", "bottom-up" "top-down", "predator exclusion experiment". The main findings of each of the studies were recorded in a table with either a ">", "<", or "=" sign to show the direction of the results. Symbols ">" and "<" denote significant effects while "=" denotes no significant effect.

Results

Effect of tree species on HWA establishment and survival

HWA settled more readily on western hemlock than eastern hemlock (Table 1). Splitting the data by HWA generation, we found that initial establishment rates were lower on eastern hemlock, and that HWA populations declined on eastern hemlock after the completion of the founding progrediens generation (Table 1).

HWA densities and survival exclusion treatment

In the first experiment (Round one, 2015-2016 sampling period) (Figs. 1A-B), HWA densities in both bagged and unbagged treatments were generally low (<1 HWA per cm). HWA densities on bagged and unbagged western hemlock branches were only significantly different for the progrediens adults 2016 (Table S1D); all other sample periods were not significant (Table S1A-C and S1E). On eastern hemlock, densities of progrediens adults 2015 and progrediens adults 2016 were not significantly different between the bagged and unbagged treatments (Table S2A and S2D) but there was a significant difference for the maturing sistens in November 2016 (S2E; Fig. 1A). Densities of HWA were too low for collection on eastern hemlock for the maturing sistens in 2015 and for the adult sistens in 2016.

In the second experiment (Round two, 2017-2018 sampling period) (Figs. 1C-D), there was no significant difference between treatments on either hemlock species for the density of established progrediens nymphs following inoculation at the start of the experiment, as we had intended. On western hemlock there were statistically significant differences in density between bagged and unbagged treatments at all the subsequent sampling points (5 life stages, 4 consecutive generations) (Table S1G-K). On eastern hemlock (Table S2F-K), only the adult sistens in March 2018 (Table S2I) had a significant difference between treatments. The overall lower establishment (Table 1) of HWA on eastern hemlock compared to western hemlock reduced the number of replicates we had on that species and thus compromised our statistical power.

The results of the Wilcoxon signed rank test were mostly in agreement with those from our GLMM models (Table S1 and S2). Results did differ for western hemlock sistens adults 2016 (Table S1C) with the Wilcoxon signed rank test finding a significant difference where our GLMM model did not. For eastern hemlock, the Wilcoxon test did not find the maturing sistens 2016 to be significantly different (Table S2E), and it did find the maturing sistens 2017 to have a significant treatment effect (Table S2H). For all other generations, the Wilcoxon signed rank test agreed with the results from our GLMM models.

The difference between treatments for the survival data was highly significant for both tree species in the progrediens generation in 2017, with higher survival in the bagged treatment (eastern hemlock: $Z = -4.17$, $P = <0.001$; western hemlock: $Z = -23.97$, $P = <0.001$) (Table 2A). For the maturing sistens in November 2017, we found that there was significantly higher survival in bagged treatments on eastern hemlock, however, there were no significant differences in survival on western hemlock (eastern hemlock: $Z = -2.362$, $P = 0.0182$; western hemlock: $Z = -1.158$, $P = 0.2467$) (Table 2B). Due to destructive sampling and difficulty in telling sistens nymphs and progrediens nymphs apart when settled together, we only analyzed this data for the progrediens in 2017 and the maturing sistens in 2017.

The daily mean air temperature data from both bagged and unbagged treatments were indistinguishable ($t = -0.062$, $df = 398$, $P = 0.951$).

Predator collections

Predator samples, collected opportunistically from progrediens ovisacs while estimating density, were identified using cytochrome oxidase subunit I (CO1) DNA barcoding. Fly larvae found foraging on progrediens ovisacs included three species of chamaemyiid flies (*Leucotaraxis piniperda* Malloch*, Le. argenticollis* Zetterstedt and one *Neoleucopis* sp.), two species of cecidomyiid flies (not identified to genus), and syrphid flies (not identified to genus). We also frequently observed predatory true bugs (Hemiptera) including Lygaeidae (*Kieidocerys resedae* (Panzer)), Anthocoridae, Reduviidae, and green lacewing nymphs (Chrysopidae) (all three not identified to genus) and much less frequently (3-4 individuals over the course of the experiment) the coccinellid *Harmonia axyridis*.

Survey for natural HWA abundance

HWA abundance and percentage of branches with HWA were higher in New England than Washington (Fig. 2). Within Washington, HWA abundance (Fig 2A) and percentage of branches with HWA (Fig. 2B) were significantly higher in Seattle than the Cascades (Route 90 and Route 2) and the Olympic Peninsula; there was no difference between the Cascades and the Olympic Peninsula.

Findings from HWA literature review

Our HWA literature review (Table 3) found mixed conclusions regarding HWA fitness when feeding on *T. canadensis* and *T. heterophylla*. Results also suggest a possible difference in HWA fitness on the two tree species depending on the HWA lineage used, i.e., Japanese lineage: Table 3A-E, and North American lineage: Table 3F)

Discussion

We had no difficulty inoculating western hemlock branches with HWA and achieving within-bag densities comparable to those in outbreak populations of HWA on eastern hemlock in eastern North America (Fig. 1) (McClure 1991; Jubb et al. 2020). We thus have no evidence that the low HWA densities found on western hemlock in the Pacific Northwest are due to bottom-up interactions between HWA and its hemlock host. Our findings instead suggest that the western lineage of HWA is better suited to settling on western hemlock than eastern hemlock (Table 1).

Our predator exclusion results provided strong support for the hypothesis that predator-related mortality maintains the low HWA densities found on western hemlock throughout the Pacific Northwest (Table 2A). These findings support the validity of the biological control program for HWA, which has devoted significant resources to the importation and release of predator species from the Pacific Northwest, to reduce outbreak populations of HWA in eastern North America. Until now, there have been no studies investigating whether these insect predators suppress HWA to low densities in the Pacific Northwest.

In our data, survival was significantly lower on unbagged branches during the progrediens generation in 2017 (Table 2A) implying summer-active predator feeding on HWA as the direct cause. There was also significantly lower survival in the maturing sistens nymph stage in November 2017 on eastern hemlock (Table 2B). Generalist summer-active predators, as well as *Leucotaraxis* spp., are active during the aestivating sistens stage up until about October (Kohler et al. 2016), when *La. nigrinus* adults are active beginning in September (Zilahi-Balogh et al. 2003). This could explain the lower HWA survival outside of bags during the maturing sistens nymph stage (data taken in November). These data support the recent decision of the USDA Forest Service to refocus the HWA biological control effort on introducing two species of silver flies, *Le. argenticollis* and *Le. piniperda*, that feed on both the sistens and progrediens generation of HWA in the Pacific Northwest (Kohler et al. 2016). Previous efforts focused on introducing *La. nigrinus* that feeds on the overwintering sistens generation on HWA. That species has been widely established in the eastern U.S. (Mausel et al. 2010; Foley et al. 2019; Jubb et al. 2021) and has caused significant mortality to HWA ovisacs (Jubb et al. 2020), but the mortality is insufficient to regulate densities of HWA (Crandall et al. 2020). Our results imply that a combination of summer-active and winter-active predators will be required to suppress densities of HWA in eastern North America.

One alternative explanation for higher HWA densities in bagged versus unbagged treatments is that bags may inhibit crawler dispersal and thus artificially inflate the bagged treatment densities. This effect would not influence the data we took on HWA survival (Table 2A), which was measured as a proportion of the HWA nymphs settled on hemlock twigs post-dispersal. Furthermore, it would not have affected the difference between treatments in HWA density in the 2017 progrediens generation, because bags were placed on both bagged and unbagged treatments until progrediens crawlers had finished dispersing and settling on new branches. However, it might have affected the density of the subsequent sistens generation. In a separate study conducted in Deerfield, MA in 2020 (unpublished data) we tested for a mesh bag effect on sistens crawler dispersal. We compared the ratio of observed to expected sistens nymphs between bag treatments and found that there was significant but small effect of bag ($T = -2.78$, $df = 28$, $P = 0.0096$) with a ratio of 3.14 (±0.25) for bag vs 3.02 (±0.35) for no bag (Crandall unpublished data). These small but significant differences were expected because the holes in the predator exclusion bags are larger than the size of a first instar HWA crawler and we have witnessed them moving through the bag. We also addressed the possibility of a temperature related bag effect by measuring temperature inside and outside of predator exclusion bags and found no between-treatment differences in air temperature (Fig. A1).

The large differences in HWA abundance and percentage of HWA-infested branches between New England and Washington (Fig. 2) confirm what we and others (McClure 1992; Mausel 2005) have long observed: HWA is rarely found on western hemlock in western forests. Higher HWA densities at the urban site (Seattle, WA) are likely explained by the lower densities of HWA predators in such non-forest settings. When we and previous investigators (e.g., Weed et al. 2016) searched for high-density HWA populations to obtain inoculum for these experiments, we nearly always found them in urban areas (Seattle) or on isolated trees, such as in parking lots, along the side of the road, and elsewhere. When we returned to those same trees in the following years, we nearly always found low densities of HWA. It may be that HWA populations on trees at such sites can occasionally 'escape' predator control, reaching high densities until predators aggregate to their location and decrease their numbers.

Previous artificial hemlock inoculation studies have reported conflicting results regarding the relative performance (i.e., ability to settle, survive and reproduce) of HWA in the eastern U.S. on eastern hemlock versus western hemlock (Table 3). It is worth noting that the studies from Table 3A-E used the Japanese HWA lineage collected in the eastern U.S.; this strain has no evolutionary history with *T. heterophylla* (Havill et al. 2016). In contrast, the western HWA lineage used by Mausel (2005) (Table 3F) has a long evolutionary history with *T. heterophylla*. Chinese hemlock, *Tsuga chinensis* (Franch.) E. Pritz, is widely regarded as being completely resistant to HWA (McClure 1992; Bentz et al. 2002; Del Tredici and Kitajima 2004; Hoover et al. 2009; Weston and Harper 2009; Joseph et al. 2011; Lappanen et al. 2019) in studies involving infestation with the Japanese HWA lineage in the eastern U.S. However, Havill and Montgomery (2008) described finding *T. chinensis* in its native range infested with HWA and even having "dense populations", likely the result of coevolution between *T. chinensis* and its associated lineage of HWA possessing the ability to establish. These conflicting results along with ours suggest that resistance of any *Tsuga* species to HWA likely depends upon whether the lineage of HWA being tested has co-evolved with that species.

In summary, we have provided strong support for the hypothesis that western HWA is well-evolved to infest western hemlock and we have no evidence for bottom-up controls of HWA by its native host. In contrast, we provide strong support for top-down control of HWA by a suite of summer-active and winter-active predators. These results suggest that a suite of predators feeding on both generations of HWA may be necessary to reduce HWA populations in the eastern U.S and support the validity of the HWA biological control program.

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Declarations

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Author contributions: JE obtained funding and provided overall management of the project. JE and JL formulated the study design. JL and RC collected the data. RC conducted statistical analyses and wrote the manuscript. JE and JL edited drafts of the manuscript

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Figure Captions

Fig. 1 Mean (± SE) density of *Adelges tsugae* life stages on bagged and unbagged branches Washington Park Arboretum in Seattle, Washington on a) eastern hemlock 2015-2016, b) western hemlock 2015-2016, c) eastern hemlock 2017-2018, and d) western hemlock 2018. Black bars: "Bag" treatment; Grey bars: "No Bag" treatment. Significant differences in density between treatments are indicated in Fig 1 by * for $P < 0.05$ and ** for $P < 0.01$. Statistical significance refers to results from our GLMM models. Density figures were split by experimental round (2015-2016 and 2017-2018) and tree species (eastern hemlock and western hemlock).

Fig. 2 Mean (± SE) a) abundance of *Adelges tsugae* (HWA) (0-3; 0 = No HWA ovisacs, 1 = 1-10 HWA ovisacs, 2 = 11-100 HWA ovisacs, and 3 = 101+ HWA ovisacs) and b) percentage of branches infested with HWA in three geographic regions of Washington (Seattle, Olympic Peninsula, and Cascades) and New England in summer of 2020. Different letters above bars indicate significant differences at $\alpha < 0.05$.

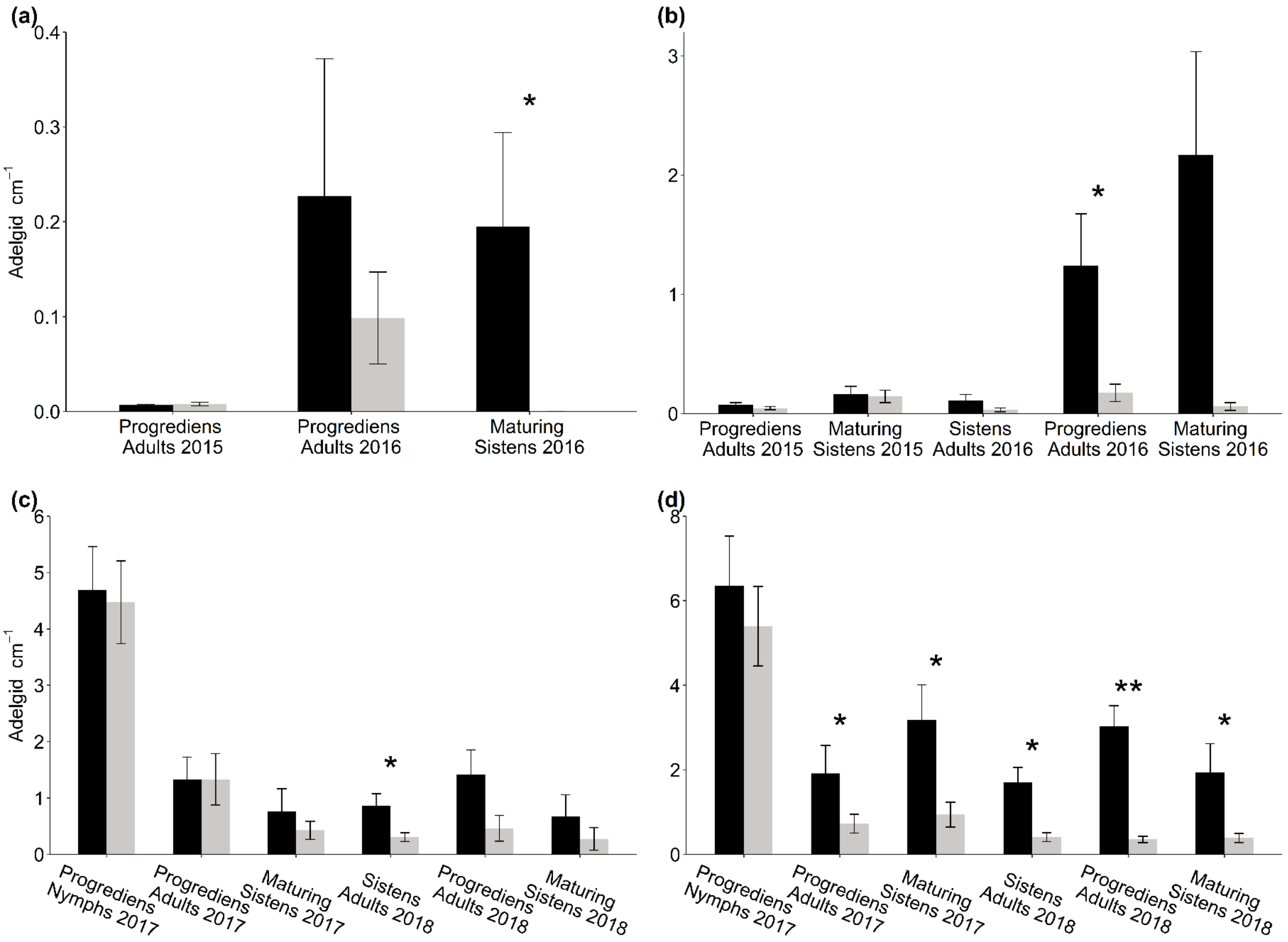
(a)
0.0
0.1
0.2
0.3
0.4
Adelgid cm⁻¹
Progrediens Adults 2015
Progrediens Adults 2016
Maturing Sistens 2016
*
(b)
0
1
2
3
Progrediens Adults 2015
Maturing Sistens 2015
Sistens Adults 2016
Progrediens Adults 2016
Maturing Sistens 2016
*
(c)
0
1
2
3
4
5
6
Adelgid cm⁻¹
Progrediens Nymphs 2017
Progrediens Adults 2017
Maturing Sistens 2017
Sistens Adults 2018
Progrediens Adults 2018
Maturing Sistens 2018
*
(d)
0
2
4
6
8
Progrediens Nymphs 2017
Progrediens Adults 2017
Maturing Sistens 2017
Sistens Adults 2018
Progrediens Adults 2018
Maturing Sistens 2018
*
*
*
**
*

Figure 1.

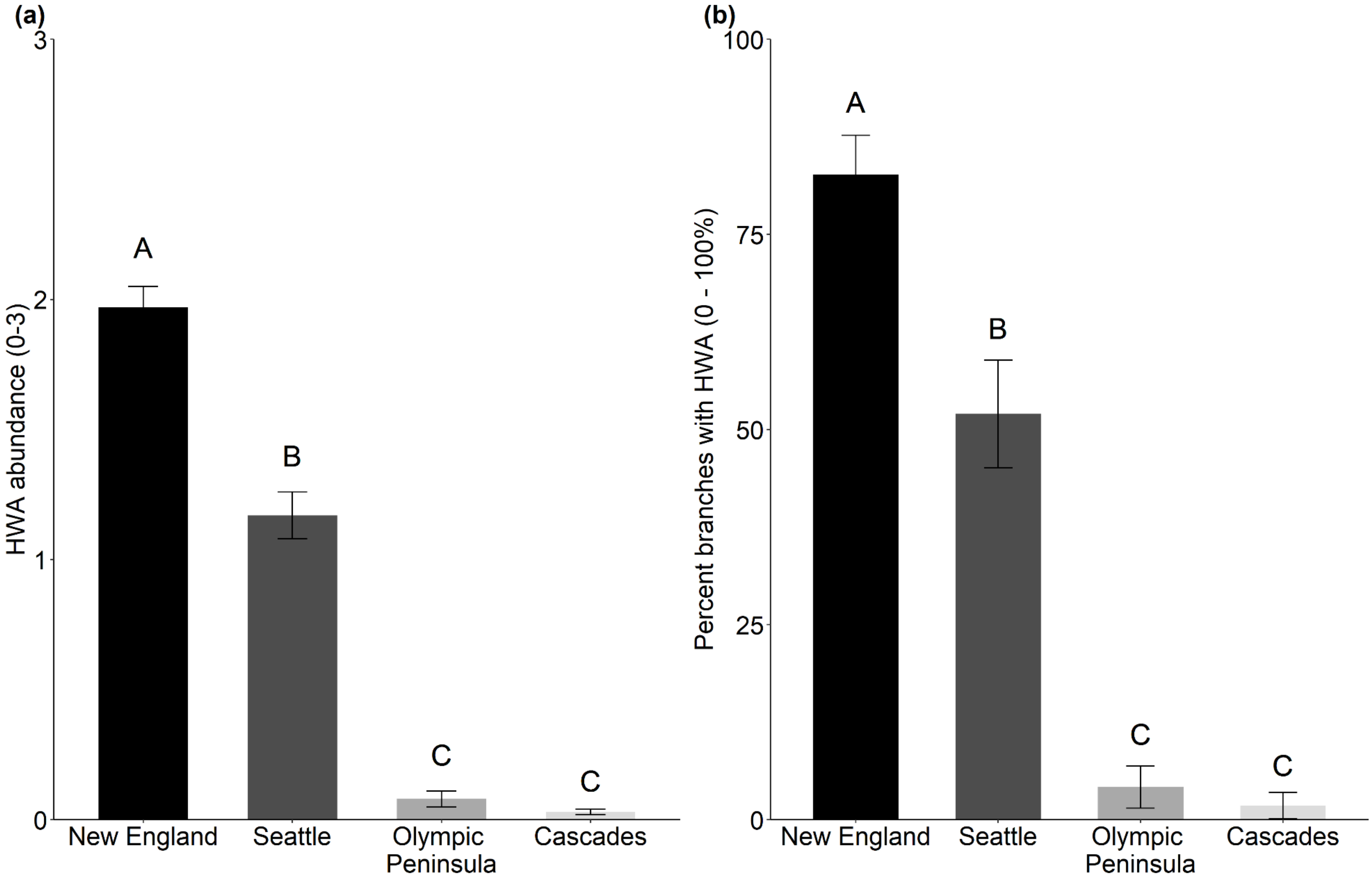
(a)
HWA abundance (0-3)
3
2
1
0
A
B
C
C
New England
Seattle
Olympic Peninsula
Cascades
(b)
Percent branches with HWA (0 - 100%)
100
75
50
25
0
A
B
C
C
New England
Seattle
Olympic Peninsula
Cascades

Figure 2.

Table 1. Establishment of HWA by tree species in 2017-2018. Values listed are the number of branches with live HWA out of the total number of branches inoculated.

	No. Branches with HWA / Total Branches			
Generation	Eastern Hemlock	Western Hemlock	z value	P value
All Generations Combined	0.414	0.807	6.579	**<0.001*****
Progrediens Nymphs 2017	0.65	0.850	1.426	0.1540
Progrediens Adults 2017	0.650	0.850	1.426	0.1540
Sistens Nymphs 2017	0.400	0.850	2.762	**0.0058****
Maturing Sistens 2017	0.350	0.850	3.009	**0.0026****
Adult Sistens 2018	0.300	0.850	3.252	**0.0012****
Progrediens Adults 2018	0.250	0.800	3.265	**0.0011****
Maturing Sistens 2018	0.300	0.600	2.087	**0.0369***

Significant effects at $P < 0.05$ are in bold. Significance symbols for $P < 0.001$ (***), <0.01 (**), <0.05 (*)

Table 2. Mean (± SE) percent survival, number of branch pairs, and results of the Generalized Mixed Model analyses comparing survival of *Adelges tsugae* by treatment for each generation (progrediens, sistens) and host tree species (western hemlock, eastern hemlock).

	HWA Generation	Hemlock Species	Treatment	Survival (%)	n (pairs)	z value	*P*
A)	Progrediens Adults June 2017	Western	Bag	31.6 ±5.6	17	-23.97	**<0.001*****
			No Bag	12.2 ±4.6			
		Eastern	Bag	25.1 ±5.2	13	-4.17	**<0.001*****
			No Bag	19.5 ±5.0			
B)	Maturing Sistens November 2017	Western	Bag	65.7 ±6.2	14	-1.158	0.2467
			No Bag	60.4 ±10.8			
		Eastern	Bag	30.4 ±12.2	7	-2.362	**0.0182***
			No Bag	23.9 ±10.9			

Significant effects at $P < 0.05$ are in bold. Significance symbols for $P < 0.001$ (***), <0.01 (**), <0.05 (*)

Table 3. Results from multiple studies testing host susceptibility of eastern hemlock (T. canadensis) and western hemlock (T. heterophylla) to hemlock woolly adelgid by quantifying adelgid density, survival, fecundity, and proportion of host trees infested. Significant effects (*P* < 0.05) shown as "<" and ">" while non-significant effects shown as "=". HWA lineages as described by Havill et al. 2016.

	Experimental Location and HWA Lineage	HWA density, survival, and fecundity on T. canadensis vs T. heterophylla	Study
A)	Hampden, CT, USA Japanese	T. canadensis > T. heterophylla (survival and fecundity)	McClure 1992
B)	Raleigh, NC, USA Japanese	T. canadensis > T. heterophylla (progrediens density) T. canadensis = T. heterophylla (fecundity)	Jetton et al. 2008
C)	Blairsville, GA, USA Japanese	T. canadensis = T. heterophylla (total adelgid per centimeter) T. canadensis = T. heterophylla (fecundity) (Looking only at unfertilized tree results)	Joseph et al. 2011
D)	Crossnore, NC, USA Japanese	T. canadensis > T. heterophylla (36 times more likely to be infested)	Oten 2011 (Thesis Chapter 7)
E)	Katonah, NY, USA Japanese	T. canadensis > T. heterophylla (percentage of trees infested)	Weston and Harper 2009
F)	Seattle, WA, USA Western North American	T. canadensis < T. heterophylla (fecundity)	Mausel 2005

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CrandalletalData02.09.2022.xlsx
- CrandalletalSupplementaryMaterials02.09.2022.pdf