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 the 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).

HWA is parthenogenic and produces 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. They feed by inserting their stylets into the base of the hemlock needle and feed on xylem ray parenchyma cells (Young et al. 1995). They 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 sistens parents, until early to mid-summer when they lay sistens eggs (McClore 1991). The progrediens generation can either become progrediens adults or winged sexuparvae (McClore 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). Sexuparvae produced in eastern North America fail to reproduce because no suitable native spruce host exists; sexuparvae have not been previously reported in the Pacific Norwest (Zilahi-Balogh et al. 2003).

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 *Laricocobius 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. We also estimated HWA abundance in three regions of Washington to test the validity of anecdotal observations of ubiquitous low levels of HWA in the Pacific Northwest and compared HWA abundance to the invaded range in New England.

**Methods**

**Collection of HWA inoculum and deployment on experimental branches**

Two year-long trials of inoculations and observations of HWA-infested hemlock branches were carried out in the Washington Park Arboretum in Seattle, Washington, USA in 2015 and 2017. Trial 1 began in March 2015 and natural infestations of HWA were found in Green Lake Park, Seattle, WA (47.671072, −122.344422). Trial 2 began in March 2017 and natural infestations of HWA were found in the Olympic Peninsula in the town of Sequim, WA, USA (48.078056, −123.101389). For both trials, HWA-infested 30 cm branchlets were chosen that had no or few signs of predation and 20–200 ovisacs. Branches which had few to no ovisacs disturbed were assumed to have few to no *La. nigrinus* larvae active on the branch. At the time of branch collection in March, ovisac disturbance was visible on branches which had active populations of *La. nigrinus* larvae so those were not collected for use as inoculum (sistens ovisacs containing progrediens eggs). Branchlets were clipped, the
number of sistens 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. At the time of inoculum deployment, approximately one third of progresidiens eggs inside sistens ovisacs were deep red in color and eyespots were visible on the developing progresidiens indicating that these eggs were close to hatch, with a small number of progresidiens beginning to hatch.

We deployed inoculum onto experimental branches on 23 March 2015 in Trial 1 and 27 March 2017 in Trial 2. 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 100–1400 ovisacs per bundle. Twenty hemlock trees (10 eastern and 10 western) were identified and two pairs of uninfested branches per tree with ample new growth were labeled to be used as experimental branches. Branches within each pair were given equivalent densities of sistens 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 × 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 progresidiens nymphs. In April 2015 (Trial 1) and 2017 (Trial 2), coinciding with our observations of first instar progresidiens nymphs settled on sample branches as well as few to no unhatched progresidiens eggs inside the inoculum, 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 prevent predators from reaching the HWA on that branch. In June 2015 of Trial 1, densities of HWA on sample branches were very low; < 0.1 adelgid per centimeter. Bags were kept on all artificially infested branches, despite low HWA densities, 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. Natural HWA infestations were only found on western hemlock. Before bagging the naturally infested branches, branches were shaken about 20 times to reduce or remove predators that may have been on the branch. In total, nine naturally infested western hemlock branch pairs were added to the progresidiens adults 2015 sample period in addition to the ten artificially infested western hemlock branches. The following two sample periods, maturing sistens 2015 and adult sistens 2016 consisted of only the naturally infested western hemlock branches since HWA densities on the inoculated branches no longer had detectable levels of HWA. However, by the final two sampling periods, progresidiens adults 2016 and maturing sistens 2016, originally artificially infested branch pairs on both eastern and western hemlock were sampled again due to a resurgence of the HWA populations within the bags.

Density data collection

For Trial 1 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 HWA on branchlets were observed with a dissecting microscope. In June 2015, the density of progresidiens was estimated on both inoculated and naturally infested branches. Adelgid densities on naturally infested branches were sampled in November 2015 (maturing sistens) and March 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, progresidiens densities were counted on the naturally infested branches. In November 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 progresidiens generation as their ovisacs were still present on the tree and were clearly distinguishable from the smaller fresh ovisacs of the maturing sistens generation.

Trial 2 of this experiment was initiated in March 2017, using the same inoculation method as Trial 1. From mid-June through mid-July 2017, sample branches were checked weekly to monitor the progress of the progresidiens 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. At each sampling point branch pairs which no longer had live HWA were removed from the study as they no longer provided any data on predation levels between treatments.

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 progresidiens 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, progresidiens densities were recorded.
In both trials, branches were excluded from the study if they lacked HWA infestations offering no HWA to compare between treatments.

**Estimating HWA survival**

Survival was estimated only in Trial 2 due to lack of adequate data in Trial 1. Sample branchlets 30 cm in length were destructively sampled and brought to the laboratory for processing with a dissecting microscope under 60x–500x magnification. Only two of the generations were viable for survival estimates, the progrediens adults 2017, and the maturing sistens 2016. Survival was estimated as the proportion of live HWA out of the total number of HWA that settled on the branch sample. Both generations were sampled well after aestivation break so first instar nymphs which never produced “wool” were considered dead. Nymphs which woolled up but did not have eggs or chorion were probed to encourage movement as well as to depress the body wall and were considered dead if they did not move or if they body wall did not quickly return to its original shape. Hemolymph color was also an indicator with bright orange/red hemolymph indicating a live HWA and maroon or black hemolymph indicating the HWA was dead. For the progrediens adults 2017, eggs and chorion inside the ovisac were also an indication that the HWA was alive. It is impossible to directly measure the cause of HWA mortality and even if killed by predators, HWA remain tethered to the branch by their mouthparts without any signs of a causal mortality agent. Therefore, we did not directly measure predation. Instead, predation was assumed to be the most likely cause for any observed differences in HWA survival or density detected by our paired experimental design which compares HWA populations on branches with predators (unbagged) and without predators (bagged).

**Predator collection**

Predator samples were collected opportunistically from progrediens ovisacs while estimating density. Samples were stored in 95% ethanol and were later identified using cytochrome oxidase subunit I (CO1) DNA barcoding (Foottit et al. 2009).

**Surveying natural HWA abundance**

In the summer of 2021, HWA abundance data were collected from hemlock forests across western Washington to test the validity of anecdotal observations suggesting that HWA occur only at low density in forests across the Pacific Northwest. 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. Western hemlock trees were checked for HWA presence in three regions: 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 460 m 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, the analysis was limited to data from branches with predator exclusion bags. By looking at the bag data separately, we removed any effect of predation on HWA establishment and isolated 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. This allowed us to look at the proportion of branches with HWA by tree species at each sampling point. Analysis was limited to data from Trial 2 because the low density of HWA in Trial 1 made tests for survival infeasible.

Densities of HWA were compared between treatments by branch pairs for each generational life stage on each tree species using two generalized linear mixed models (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” data set failed to converge using the ‘glmmTMB’ function due to low sample size and the data being heavily zero-inflated. Instead, we analyzed those 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). The proportion of HWA surviving from settled first instar nymph was compared between species and
treatments for the progrediens generation in June 2017 as well as the maturing sistens generation in November 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 table containing the counts of live and dead HWA for each branch sample, and the binomial distribution was 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. These data were used to test for between-treatments 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 2 years. Data were summarized to daily average temperatures and analyzed using the ‘lm’ function (Package = “stats”, Version = 4.1.2).

HWA survey abundance data were 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).

### Results

#### Effect of tree species on HWA establishment and survival

The overall proportion of branches colonized by HWA for all generations was significantly higher on western hemlock than on eastern hemlock (Table 1). With the data split by HWA life stage, the proportion of branches colonized by the progrediens generation in June 2017 was similar but lower on eastern hemlock compared to western hemlock however, the proportion of branches that remained colonized for all other HWA life stages was significantly lower on eastern hemlock compared to western hemlock (Table 1).

#### HWA densities and survival exclusion treatment

In the first experiment (Trial 1, 2015–2016 sampling period) (Fig. 1A–B), HWA densities in both bagged and unbagged treatments were generally low (< 1 HWA per cm). HWA densities were significantly higher on bagged branches for the progrediens adults 2016 on western hemlock (Table S1D); all other sample periods were not significant (Table S1A–C and S1E). On eastern hemlock, densities of progrediens adults in June 2015 and progrediens adults in June 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 with higher HWA density in the bagged treatment (S2E; Fig. 1A). Densities of HWA were too low for collection on eastern hemlock for the maturing sistens in November 2015 and for the adult sistens in March 2016.

In the second experiment (Trial 2, 2017–2018 sampling period) (Fig. 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 significantly higher densities in the

### Table 1 Proportion of branches with HWA by tree species in Trial 2 (2017–2018).

| Generation                  | No. branches with HWA/total branches | z value | P value |
|-----------------------------|--------------------------------------|---------|---------|
|                             | Eastern hemlock | Western hemlock |         |         |
| 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 | 2.087 | 0.0369*  |
| Maturing Sistens 2018       | 0.300 | 0.600 | 2.807 | 0.0025** |

Significant effects at P < 0.05 are in bold. Significance symbols for P < 0.001 (***) , P < 0.01 (**), P < 0.05 (*)
bagged treatment at all the subsequent sampling points (5 life stages, 4 consecutive generations) (Table S1G–K). On eastern hemlock (Table S2F–K), the bagged treatment density was significantly higher for the adult sistens in March 2018, all other life stages had no significant differences between the bag treatment (Table S2I). 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 difference between treatments for the survival data was highly significant for both tree species in the progresiens generation in June 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 progresiens nymphs apart when settled together, we only analyzed these data for the progresiens in June 2017 and the maturing sistens in November 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**

Fly larvae found on progresiens 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*.

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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 trial (Trial 1: 2015–2016 and Trial 2: 2017–2018) and tree species (eastern hemlock and western hemlock).
Survey for natural HWA abundance

HWA abundance and percentage of branches with HWA were higher in New England than Washington in the summer of 2021 (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. Comparisons of HWA between Washington and New England are comparisons between the western North American lineage and Japanese lineage of HWA respectively.

Discussion

While we had trouble inoculating both hemlock species in Trial 1, possibly a result of predator compromised or poor-quality inoculum, in Trial 2, 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 bottom-up interactions between HWA and its hemlock host were responsible for suppressing HWA densities on our sample branches. Our findings instead suggest that the western North American lineage of HWA had better survival on western hemlock than eastern hemlock (Table 1). Eastern hemlock cultivar variety could have had an influence in the success of HWA on that species, however, because HWA
populations were able to reach levels similar to those in eastern North America and were able to persist on western hemlock for multiple generations, bottom-up effects did not appear to have a strong influence on HWA survival.

Our predator exclusion results provided strong support for the hypothesis that predator-caused mortality is responsible for suppressing rare and localized HWA outbreaks 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 June 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 stage (data collected in November). These data support the recent decision of the USDA Forest Service to augment the HWA biological control effort by 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). Due to the nature of our study, where summer-active predators set the initial differences in density between treatments, significantly higher densities in the bag treatment in following generations on western hemlock in Trial 2 cannot be specifically attributed to a winter-active predator, though we suspect they are having a significant impact on HWA survival and density. We believe 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. Thus far only the winter-active predator La. nigrinus has been established in substantial numbers in eastern North America and there is evidence that, by itself, La. nigrinus cannot suppress high-density HWA populations (Crandall et al. 2020).

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 collected 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 June 2017 progresdiens 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 density of the subsequent sistens generation 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, which are the same material as the bags used in the Washington, 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-treatments differences in air temperature (Fig. 1A).

The large differences in HWA abundance and percentage of HWA-infested branches between New England and Washington (Fig. 2) support what we and others (McClure 1992; Mausel 2005) have long observed: high-density HWA infestations are rarely found on western hemlock in western forests but occur in localized pockets. Higher HWA densities at the urban site (Seattle, WA) may possibly be explained by the lower densities of HWA predators in such non-forest settings. For example, the HWA predator La. nigrinus requires a duff layer for pupation, which is not available in many urban settings where hemlock trees are growing surrounded by lawns and in some cases pavement. Disjunct populations of hemlock may also lack silver fly populations which could take longer to find HWA outbreaks occurring in urban settings as opposed to a natural forest setting. However, Whitmore et al. (unpublished) suggest that occasionally higher densities of HWA predators are found in some urban areas compared to more rural areas. It may be that HWA populations on trees in both urban and rural areas can occasionally ‘escape’ predator control, reaching high densities until predators aggregate to their location and decrease their numbers. Currently there are no published data describing HWA predator abundance in urban and rural areas. A study on HWA predator abundance is necessary to definitively
assess the relative abundance and identify factors affecting the abundance of HWA predators in rural and urban settings.

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. McClure (1992), Oten (2011) and Weston and Harper (2009) found better performance of HWA on eastern hemlock compared to western hemlock. However, Jetton et al. (2008) found mixed results with higher HWA population densities on eastern hemlock but similar fecundity between eastern hemlock and western hemlock. Joseph et al. (2011) also found that there was no difference in fecundity between the two hosts and furthermore found equivalent infestation rates on unfertilized eastern hemlock and western hemlocks. Contrary to the studies in the eastern U.S., Mausel (2005) found that HWA fecundity was higher on western hemlock than on eastern hemlock in the western U.S. Similarly, Weed et al. (2016) found significantly higher densities of HWA on western hemlock than on eastern hemlock when sampling naturally occurring HWA infestations in the Washington Park Arboretum in Seattle, WA, the same location as our present study. It is worth noting that McClure (1992), Jetton et al. (2008), Oten (2011), and Weston and Harper (2009) all used the Japanese HWA lineage collected in the eastern U.S.; this lineage has no evolutionary history with T. heterophylla (Havill et al. 2016). In contrast, the western North American HWA lineage, native to the Pacific Northwest, used by Mausel (2005) and sampled by Weed et al. (2016), has a long evolutionary history with T. heterophylla. Similarly, 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. Therefore, for the success of the biological control program for HWA, it is important that HWA is studied in its native range to elucidate the role that bottom-up effects (host tree resistance) and top-down effects (natural enemies) play in HWA population dynamics.

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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**Data availability** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Code availability** The R-script used to analyze the dataset used and/or analyzed during the current study is available from the corresponding author on reasonable request.

**Declarations**

**Conflict of interest** The authors declare that they have no conflicts of interest.

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