

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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1 **Abstract**

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

20

21 **Keywords**

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

23 Introduction

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

44 Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is a
45 sap-feeding insect on hemlock (*Tsuga*) species native to eastern Asia and the Pacific Northwest

46 region of North America (hereafter Pacific Northwest), where multiple distinct lineages of HWA
47 have coevolved with different species of hemlock trees found across its range (Havill et al.
48 2016). In the Pacific Northwest, HWA is associated with western hemlock, *Tsuga heterophylla*
49 [Rafinesque] Sargent (Pinaceae: Pinales), a species commonly found across this region (McClure
50 1992). However, while western hemlock is a dominant understory tree in this region, HWA
51 typically occurs at low densities (McClure 1992). In contrast, HWA in eastern North America is
52 a non-native pest that was introduced from Japan (Havill et al. 2016). This Japanese lineage of
53 HWA can reach high densities in its introduced range where it is responsible for the widespread
54 mortality of eastern hemlock, *T. canadensis* [L.] Carriere, and Carolina hemlock, *T. caroliniana*
55 Engelmann, across much of eastern North America (Havill et al. 2014). Various studies suggest
56 that HWA populations in eastern North America are primarily regulated by bottom-up factors in
57 the form of competition for space on hemlock twigs and HWA-induced reduction of new
58 hemlock shoots that the insect depends on for the next generation of HWA populations (McClure
59 1991; Elkinton et al. 2011; Sussky and Elkinton 2014). There are no known parasitoids of HWA
60 (Cheah et al. 2004) and natural enemies native to eastern North America play an insignificant
61 role in regulating HWA densities (McClure 1987; Montgomery and Lyon 1996; Wallace and
62 Hain 2000).

63 Given the economic and ecological importance of hemlock trees, the USDA Forest
64 Service has devoted significant funding to the importation, mass rearing, and release in eastern
65 North America of various insect predators of the Japanese HWA lineage. Some of these
66 predators, such as *Laricobius nigrinus* Fender (Coleoptera: Derodontidae) from the Pacific
67 Northwest, have established self-sustaining populations and are spreading from many release
68 locations (Mausel et al. 2010; Foley et al. 2019; Jubb et al. 2021). There has been very limited

69 success with these introductions in terms of reducing HWA populations despite high predation
70 rates (see Crandall et al. 2020; Jubb et al. 2020), suggesting that bottom-up forces might also be
71 important in determining HWA abundance in its native and introduced ranges.

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

79 **Methods**

80 **HWA life cycle and feeding**

81 HWA feeds by settling at the base of a hemlock needle and inserting their long thin
82 mouthparts, called stylets, into the twig to feed on ray parenchyma cells (Young et al. 1995).
83 HWA are parthenogenic and produce two generations per year, the overwintering sistens
84 generation and the spring progrediens generation (McClure 1987). The sistens generation hatch
85 in the summer, settle on freshly produced new growth, aestivate until early fall, feed through the
86 winter, and oviposit from late winter through early spring (McClure 1987; Sussky and Elkinton
87 2015). In spring, the progrediens eggs hatch and the progrediens first instar nymphs, commonly
88 referred to as “crawlers”, settle and feed on the same growth as the parent sistentes (plural for
89 sistens), until early to mid-summer when they lay sistens eggs (McClure 1991). The progrediens
90 generation can either become adult progredientes or winged sexuparae (McClure 1991; Sussky

91 and Elkinton 2014), which, in the native range, seek out tigertail spruce, *Picea torano* (K. Kock)
92 Koehne, to carry out a sexual generation (Havill et al. 2006). Sexuparae produced in eastern
93 North America fail to reproduce because no suitable native spruce host exists; sexuparae have
94 not been previously observed in the Pacific Northwest (Zilahi-Balogh et al. 2003).

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

96 Two year-long rounds of inoculations and observations of hemlock woolly adelgid-
97 infested hemlock branches were carried out in the Washington Park Arboretum in Seattle,
98 Washington, USA in 2015 and 2017. In March of 2015 natural infestations of HWA were found
99 in Green Lake Park, Seattle, WA (47.671072, -122.344422). In March of 2017, natural
100 infestations of HWA were found in the Olympic Peninsula in the town of Sequim, WA, USA
101 (48.078056, -123.101389). Hemlock woolly adelgid-infested branchlets were chosen that had no
102 or few signs of predation and 20 to 200 ovisacs. Branchlets were clipped, the number of ovisacs
103 counted, and branchlets randomly grouped into bundles of 1, 3, or 5 and placed in bricks of
104 water-soaked floral foam (Smithers-Oasis Kent Co. OH, USA) to prevent desiccation. Branchlets
105 were stored in bins that were kept indoors at room temperature for approximately four days until
106 deployment.

107 We deployed inoculum onto experimental branches on 23-March-2015 and 27-March-
108 2017. Inoculum branchlets deployed at Washington Park Arboretum in bundles of 1, 3, 10, and
109 20 on experimental branches to create varying densities of ovisacs with a range of 100 to 1,400
110 ovisacs per bundle. Twenty hemlock trees (10 eastern and 10 western) were identified and two
111 pairs of uninfested branches per tree were labeled to be used as experimental branches. Branches
112 within each pair were given equivalent densities of ovisacs. Inoculum densities were randomly
113 assigned to branch pairs. The pairs were then given bundles of 1, 3, 10, or 20 branchlets,

114 corresponding to their assigned inoculum density, that were then fixed onto the branches with
115 plastic cable ties. Predator exclusion bags (1-m-long by 0.5-m-wide Equinox® No-See-Um
116 mosquito netting, ~569 holes per square centimeter) were then placed over all branches and tied
117 on using strips of self-stick vinyl foam insulation (3.2 cm x 48 mm) between the branch and the
118 cable tie so that ties did not cut off nutrient and water flow. Sample branches were monitored
119 over the next few weeks to confirm that nymphs had settled by examining inoculum ovisacs with
120 a hand lens for the presence of eggs, as well as checking the sample branches visually for settled
121 nymphs. In April of 2015 and 2017, the mesh bags were removed from one branch in each pair
122 and the inoculation bundles were removed from all branches. The mesh bag remained on the
123 second branch in each pair to restrict predators from reaching the HWA on that branch. In June
124 of 2015, densities of HWA were very low; >0.1 adelgid per centimeter. Branches with some
125 HWA were kept in the study and natural infestations found in the arboretum were incorporated
126 into the study by choosing branch pairs with similar densities of HWA and bagging one of the
127 branches. Before bagging, branches were shaken about 20 times to remove any predators that
128 may have been on the branch.

129 **Density data collection**

130 For round one of this experiment (i.e., 2015-2016), densities of HWA were estimated by
131 removing 30-cm branchlets and counting HWA life stages. Length of branchlet growth and
132 counts of hemlock woolly adelgid on branchlets were observed with a dissecting microscope. In
133 June of 2015, the density of progrediens was estimated on both inoculated and naturally infested
134 branches. Adelgid densities on naturally infested branches were sampled in November of 2015
135 (maturing sistens) and March of 2016 (adult sistens). During these sampling periods, HWA
136 populations on inoculated branches were still establishing, and therefore had very low sistens

137 densities and were not sampled until November 2016. In June 2016, progrediens densities were
138 counted on the naturally infested branches. In November of 2016, the maturing sistens
139 generation was quantified on both the inoculated branches and naturally infested branches. The
140 inoculated branches were also sampled for the past progrediens generation as their ovisacs were
141 still present on the tree and were clearly distinguishable from the smaller fresh ovisacs of the
142 maturing sistens generation.

143 Round two of this experiment was initiated in March 2017, using the same inoculation
144 method as round 1. From mid-June through mid-July of 2017, sample branches were checked
145 weekly to monitor the progress of the progrediens generation to measure settlement on sample
146 branches. Of the 20 paired branches inoculated for each tree species, 17 pairs were successfully
147 inoculated for western hemlock and 13 pairs were successfully inoculated for eastern hemlock.

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

153 **Surveying natural HWA abundance**

154 HWA densities were quantified at 36 sites in three regions in western Washington state
155 (12 sites per region) to compare HWA abundance in each region. Hemlock trees were checked
156 for HWA presence in three locations: Seattle, Washington (urban), Route 90 and Route 2 (rural),
157 and the Olympic Peninsula (rural). Sites were either forests or wooded areas in parks and were at
158 least one kilometer from other sites. In mountainous regions, we limited sites to below 1,500 ft

159 elevation to reduce the effect of elevation as a confounding factor. Where possible, 20 trees per
160 site (10 trees minimum where hemlock was less abundant) were sampled which involved
161 checking the top and bottom of one-meter-long branch and rating the HWA infestation or lack
162 thereof. We used a 0-3 scale of HWA infestation (0 = none; 1 = 1-10 ovisacs/m branch; 2 = 11-
163 100 ovisacs/m branch; 3 = 101-1000 ovisacs/m branch), adapted from the methods used in
164 Preisser et al. (2008). We also added in sites from New England to compare the densities from
165 Washington to those in the invaded range of HWA. Sites were mainly from Massachusetts, but
166 included one site from Vermont, one site from New Hampshire, and three sites from Maine. The
167 same techniques were used to collect HWA abundance as described for Washington.

168 **Data analysis**

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

175 Densities of hemlock woolly adelgid were compared between treatments by branch pairs
176 for each generational life stage on each tree species using two generalized linear mixed model
177 (GLMM). The ‘glmmTMB’ function (Package = *glmmTMB*, Version 1.1.2.3) was used for zero-
178 inflated data by specifying in the model a zero-inflation factor and using a zero-inflated Gamma
179 family of distributions. Each model had a random effect for branch pair (Bates et al. 2015). The
180 “Maturing Sistens 2016” failed to converge using the ‘glmmTMB’ function due to low sample
181 size and the data being heavily zero-inflated. Instead, we ran that data with the ‘glmer’ function,

182 using the Gamma distribution, and added a small constant (0.0001) to the response variable
183 (HWA density) to permit analysis even when HWA were absent (Zar 2010). In addition to
184 testing our data with GLMM models, we also analyzed HWA densities with the Wilcoxon signed
185 rank test since our data was not normally distributed. Adding a non-parametric test compliments
186 and supports the results from our parametric GLMM tests.

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

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

202 HWA survey abundance data was analyzed using a non-parametric Kruskal-Wallis test
203 because the data were non-normally distributed. The Kruskal-Wallis test allowed us to test for
204 differences in average abundance between each of the regions that we sampled, acting

205 analogously to a one-way ANOVA test. We also looked for differences in the percentage of
206 branches with HWA by region using a ‘glm’ model with a quasibinomial distribution.

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

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

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

217 **Results**

218 **Effect of tree species on HWA establishment and survival**

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

223 **HWA densities and survival exclusion treatment**

224 In the first experiment (Round one, 2015-2016 sampling period) (Figs. 1A-B), HWA
225 densities in both bagged and unbagged treatments were generally low (<1 HWA per cm). HWA

226 densities on bagged and unbagged western hemlock branches were only significantly different
227 for the progrediens adults 2016 (Table S1D); all other sample periods were not significant (Table
228 S1A-C and S1E). On eastern hemlock, densities of progrediens adults 2015 and progrediens
229 adults 2016 were not significantly different between the bagged and unbagged treatments (Table
230 S2A and S2D) but there was a significant difference for the maturing sistens in November 2016
231 (S2E; Fig. 1A). Densities of HWA were too low for collection on eastern hemlock for the
232 maturing sistens in 2015 and for the adult sistens in 2016.

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

243 The results of the Wilcoxon signed rank test were mostly in agreement with those from
244 our GLMM models (Table S1 and S2). Results did differ for western hemlock sistens adults
245 2016 (Table S1C) with the Wilcoxon signed rank test finding a significant difference where our
246 GLMM model did not. For eastern hemlock, the Wilcoxon test did not find the maturing sistens
247 2016 to be significantly different (Table S2E), and it did find the maturing sistens 2017 to have a

248 significant treatment effect (Table S2H). For all other generations, the Wilcoxon signed rank test
249 agreed with the results from our GLMM models.

250 The difference between treatments for the survival data was highly significant for both
251 tree species in the progrediens generation in 2017, with higher survival in the bagged treatment
252 (eastern hemlock: $Z = -4.17$, $P = <0.001$; western hemlock: $Z = -23.97$, $P = <0.001$) (Table 2A).
253 For the maturing sistens in November 2017, we found that there was significantly higher survival
254 in bagged treatments on eastern hemlock, however, there were no significant differences in
255 survival on western hemlock (eastern hemlock: $Z = -2.362$, $P = 0.0182$; western hemlock: $Z = -$
256 1.158 , $P = 0.2467$) (Table 2B). Due to destructive sampling and difficulty in telling sistens
257 nymphs and progrediens nymphs apart when settled together, we only analyzed this data for the
258 progrediens in 2017 and the maturing sistens in 2017.

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

261 **Predator collections**

262 Predator samples, collected opportunistically from progrediens ovisacs while estimating
263 density, were identified using cytochrome oxidase subunit I (CO1) DNA barcoding. Fly larvae
264 found foraging on progrediens ovisacs included three species of chamaemyiid flies (*Leucotaraxis*
265 *piniperda* Malloch, *Le. argenticollis* Zetterstedt and one *Neoleucopis* sp.), two species of
266 cecidomyiid flies (not identified to genus), and syrphid flies (not identified to genus). We also
267 frequently observed predatory true bugs (Hemiptera) including Lygaeidae (*Kieidocerys resedae*
268 (Panzer)), Anthocoridae, Reduviidae, and green lacewing nymphs (Chrysopidae) (all three not

269 identified to genus) and much less frequently (3-4 individuals over the course of the experiment)
270 the coccinellid *Harmonia axyridis*.

271 **Survey for natural HWA abundance**

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

277 **Findings from HWA literature review**

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

282 **Discussion**

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

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

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

311 that a combination of summer-active and winter-active predators will be required to suppress
312 densities of HWA in eastern North America.

313 One alternative explanation for higher HWA densities in bagged versus unbagged
314 treatments is that bags may inhibit crawler dispersal and thus artificially inflate the bagged
315 treatment densities. This effect would not influence the data we took on HWA survival (Table
316 2A), which was measured as a proportion of the HWA nymphs settled on hemlock twigs post-
317 dispersal. Furthermore, it would not have affected the difference between treatments in HWA
318 density in the 2017 progrediens generation, because bags were placed on both bagged and
319 unbagged treatments until progrediens crawlers had finished dispersing and settling on new
320 branches. However, it might have affected the density of the subsequent sistens generation. In a
321 separate study conducted in Deerfield, MA in 2020 (unpublished data) we tested for a mesh bag
322 effect on sistens crawler dispersal. We compared the ratio of observed to expected sistens
323 nymphs between bag treatments and found that there was significant but small effect of bag ($T =$
324 -2.78 , $df = 28$, $P = 0.0096$) with a ratio of $3.14 (\pm 0.25)$ for bag vs $3.02 (\pm 0.35)$ for no bag
325 (Crandall unpublished data). These small but significant differences were expected because the
326 holes in the predator exclusion bags are larger than the size of a first instar HWA crawler and we
327 have witnessed them moving through the bag. We also addressed the possibility of a temperature
328 related bag effect by measuring temperature inside and outside of predator exclusion bags and
329 found no between-treatment differences in air temperature (Fig. A1).

330 The large differences in HWA abundance and percentage of HWA-infested branches
331 between New England and Washington (Fig. 2) confirm what we and others (McClure 1992;
332 Mausel 2005) have long observed: HWA is rarely found on western hemlock in western forests.
333 Higher HWA densities at the urban site (Seattle, WA) are likely explained by the lower densities

334 of HWA predators in such non-forest settings. When we and previous investigators (e.g., Weed
335 et al. 2016) searched for high-density HWA populations to obtain inoculum for these
336 experiments, we nearly always found them in urban areas (Seattle) or on isolated trees, such as in
337 parking lots, along the side of the road, and elsewhere. When we returned to those same trees in
338 the following years, we nearly always found low densities of HWA. It may be that HWA
339 populations on trees at such sites can occasionally ‘escape’ predator control, reaching high
340 densities until predators aggregate to their location and decrease their numbers.

341 Previous artificial hemlock inoculation studies have reported conflicting results regarding
342 the relative performance (i.e., ability to settle, survive and reproduce) of HWA in the eastern
343 U.S. on eastern hemlock versus western hemlock (Table 3). It is worth noting that the studies
344 from Table 3A-E used the Japanese HWA lineage collected in the eastern U.S.; this strain has no
345 evolutionary history with *T. heterophylla* (Havill et al. 2016). In contrast, the western HWA
346 lineage used by Mausel (2005) (Table 3F) has a long evolutionary history with *T. heterophylla*.
347 Chinese hemlock, *Tsuga chinensis* (Franch.) E. Pritz, is widely regarded as being completely
348 resistant to HWA (McClure 1992; Bentz et al. 2002; Del Tredici and Kitajima 2004; Hoover et
349 al. 2009; Weston and Harper 2009; Joseph et al. 2011; Lappanen et al. 2019) in studies involving
350 infestation with the Japanese HWA lineage in the eastern U.S. However, Havill and Montgomery
351 (2008) described finding *T. chinensis* in its native range infested with HWA and even having
352 “dense populations”, likely the result of coevolution between *T. chinensis* and its associated
353 lineage of HWA possessing the ability to establish. These conflicting results along with ours
354 suggest that resistance of any *Tsuga* species to HWA likely depends upon whether the lineage of
355 HWA being tested has co-evolved with that species.

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

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373 **Declarations**

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377 **Ethics approval:** Not applicable.

378 **Consent to participate:** Not applicable.

379 **Consent for publication:** Not applicable.

380 **Availability of data and materials:** The datasets used and/or analyzed during the current study
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382 **Code availability:** The R-script used to analyze the dataset used and/or analyzed during the
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384 **Author contributions:** JE obtained funding and provided overall management of the project. JE
385 and JL formulated the study design. JL and RC collected the data. RC conducted statistical
386 analyses and wrote the manuscript. JE and JL edited drafts of the manuscript

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521 **Figure Captions**

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

527

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

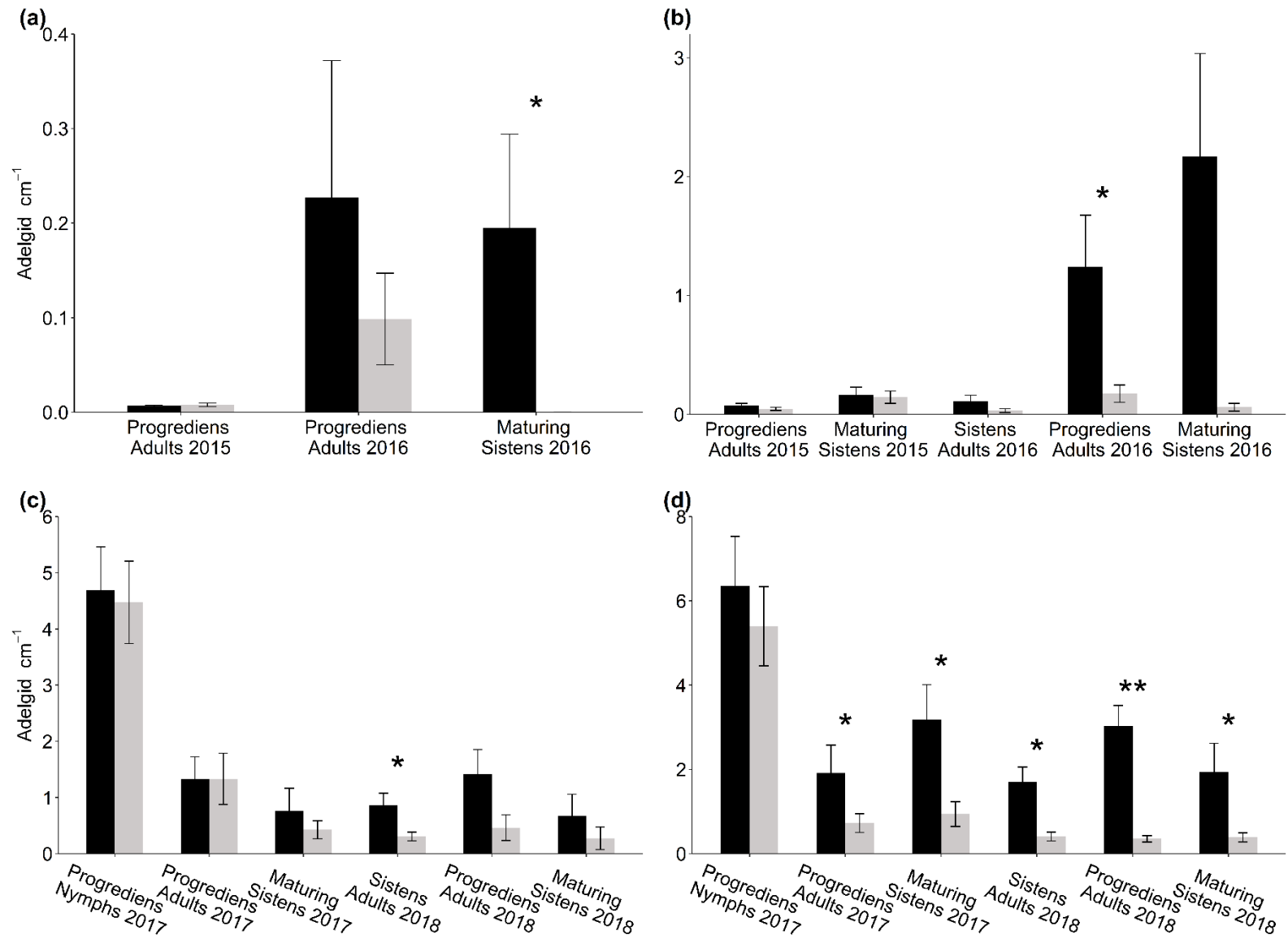


Figure 1.

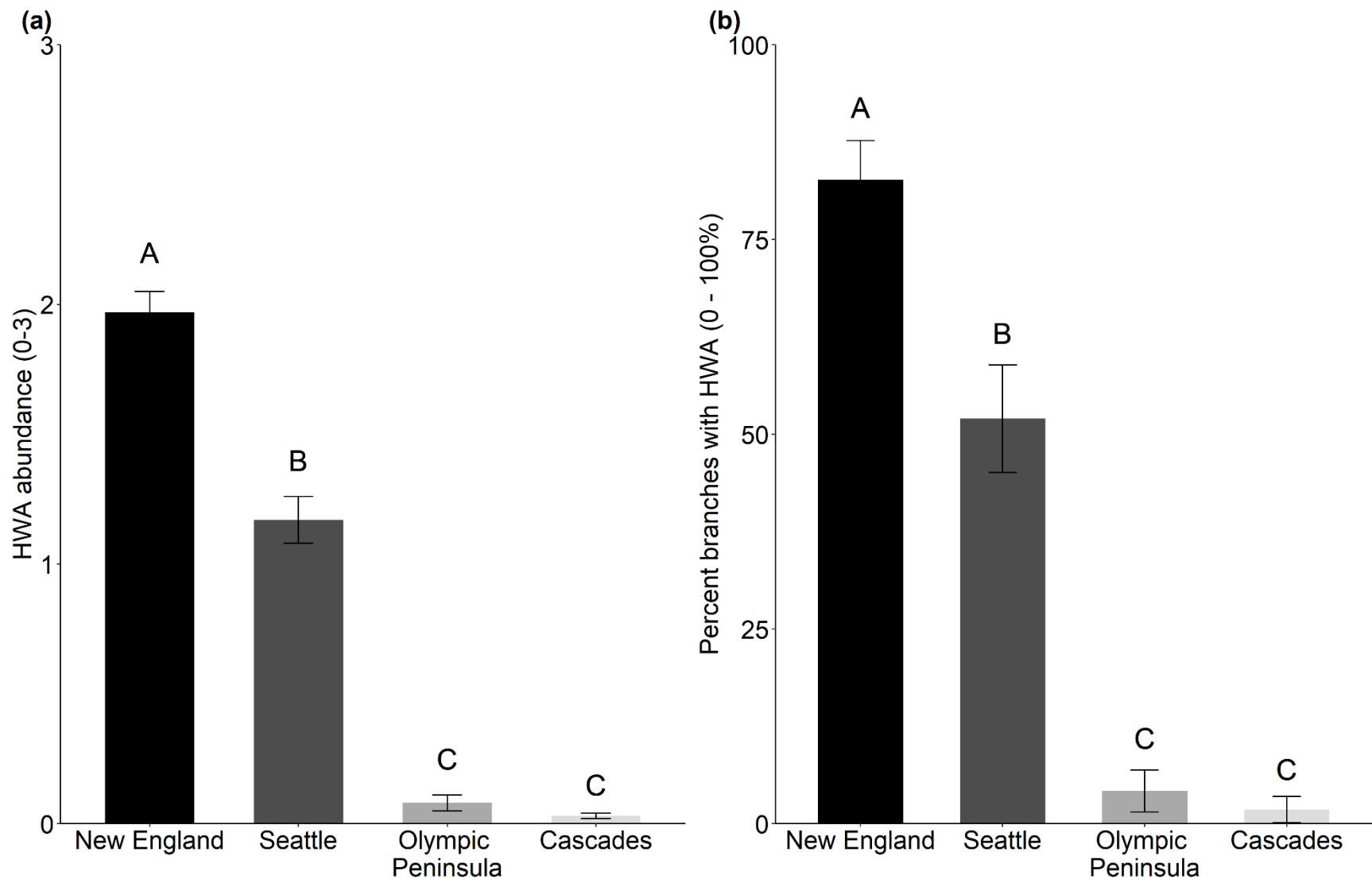


Figure 2.

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.

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	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 (\pm 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 (progre diens, sistens) and host tree species (western hemlock, eastern hemlock).

	HWA Generation	Hemlock Species	Treatment	Survival (%)	n (pairs)	z value	<i>P</i>
A)	Progre diens Adults June 2017	Western	Bag	31.6 \pm 5.6	17	-23.97	<0.001***
			No Bag	12.2 \pm 4.6			
		Eastern	Bag	25.1 \pm 5.2	13	-4.17	<0.001***
			No Bag	19.5 \pm 5.0			
B)	Maturing Sistens November 2017	Western	Bag	65.7 \pm 6.2	14	-1.158	0.2467
			No Bag	60.4 \pm 10.8			
		Eastern	Bag	30.4 \pm 12.2	7	-2.362	0.0182*
			No Bag	23.9 \pm 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 <i>T. canadensis</i> vs <i>T. heterophylla</i>	Study
A) Hampden, CT, USA Japanese	<i>T. canadensis</i> > <i>T. heterophylla</i> (survival and fecundity)	McClure 1992
B) Raleigh, NC, USA Japanese	<i>T. canadensis</i> > <i>T. heterophylla</i> (progreiens density) <i>T. canadensis</i> = <i>T. heterophylla</i> (fecundity)	Jetton et al. 2008
C) Blairsville, GA, USA Japanese	<i>T. canadensis</i> = <i>T. heterophylla</i> (total adelgid per centimeter) <i>T. canadensis</i> = <i>T. heterophylla</i> (fecundity) (Looking only at unfertilized tree results)	Joseph et al. 2011
D) Crossnore, NC, USA Japanese	<i>T. canadensis</i> > <i>T. heterophylla</i> (36 times more likely to be infested)	Oten 2011 (Thesis Chapter 7)
E) Katonah, NY, USA Japanese	<i>T. canadensis</i> > <i>T. heterophylla</i> (percentage of trees infested)	Weston and Harper 2009
F) Seattle, WA, USA Western North American	<i>T. canadensis</i> < <i>T. heterophylla</i> (fecundity)	Mausel 2005

Supplementary Files

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