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## Facilitation between invasive herbivores: hemlock woolly adelgid increases gypsy moth preference for and performance on eastern hemlock

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**Abstract.**

1. Interactions between invertebrate herbivores with different feeding modes are common on long-lived woody plants. In cases where one herbivore facilitates the success of another, the consequences for their shared host plant may be severe. Eastern hemlock (*Tsuga canadensis*), a canopy-dominant conifer native to the eastern U.S., is currently threatened with extirpation by the invasive stylet-feeding hemlock woolly adelgid (*Adelges tsugae*). The effect of adelgid on invasive hemlock-feeding folivores remains unknown.

2. We evaluated the impact of feeding by hemlock woolly adelgid on gypsy moth (*Lymantria dispar*) larval preference for, and performance on, eastern hemlock. To assess preference, we surveyed 245 field-grown hemlocks for gypsy moth herbivory damage and conducted laboratory paired-choice bioassays. To assess performance, gypsy moth larvae were reared to pupation on adelgid-infested or uninfested hemlock foliage and pupal weight, proportional weight gain, and larval period were analyzed.

3. Adelgid-infested hemlocks experienced more gypsy moth herbivory than uninfested control trees, and laboratory tests confirmed that gypsy moth larvae preferentially feed on adelgid-infested hemlock foliage. Gypsy moth larvae reared to pupation on adelgid-infested foliage gained more weight than larvae reared on uninfested control foliage.

4. Our results suggest that the synergistic effect of adelgid and gypsy moth poses an additional threat to eastern hemlock that may increase extirpation risk and ecological impact throughout most of its range.

**Key words.** Facilitation, herbivores, *Adelges tsugae*, *Lymantria dispar*, invasional meltdown

## 38 **Introduction**

39           Many interactions between co-occurring insect herbivores are mediated by their impact  
40 on the shared host plant (Kaplan & Denno, 2007). Feeding by one insect may cause alterations in  
41 plant quality, such as the induction of toxic secondary metabolites or changes to various leaf  
42 structural traits, which can affect simultaneously- or sequentially-feeding competitors (Nykänen  
43 & Koricheva, 2004). Although many such changes negatively impact the other species, they can  
44 also be facilitative (Kaplan & Denno, 2007; Ohgushi, 2008). Sap feeding by the aphid  
45 *Brevicoryne brassicae*, for example, improves the performance of folivorous *Pieris brassicae*  
46 larvae by attenuating chemical defense induction in *Brassica oleracea* (Li *et al.*, 2014).

47           Understanding herbivore-herbivore interactions is especially important in cases where  
48 one or both herbivores can substantially affect plant growth and fitness. One such species is  
49 hemlock woolly adelgid (*Adelges tsugae*; ‘adelgid’ hereafter), a destructive pest that has caused  
50 widespread mortality and decline of an ecologically significant conifer, eastern hemlock (*Tsuga*  
51 *canadensis*; ‘hemlock’ hereafter), in eastern U.S. forests. Adelgid feeds by inserting its stylet  
52 bundle into the xylem ray parenchyma cells at the base of a hemlock needle (Shields *et al.*,  
53 1995). This feeding reduces the production of new foliage (Gonda-King *et al.*, 2014; McClure,  
54 1991), alters wood morphology (Domec *et al.*, 2013; Gonda-King *et al.*, 2012), and substantially  
55 impacts plant physiology. Adelgid-infested hemlocks have elevated tissue levels of salicylic acid  
56 (SA) and emissions of its methylated form, methyl salicylate (Pezet *et al.*, 2013; Pezet &  
57 Elkinton, 2014). SA is a phytohormone that plays a critical role in plant response to abiotic  
58 stresses and biotrophic pathogens; it has also been shown to accumulate following stylet-feeding  
59 insect infestations (Walling, 2000). SA accumulation and subsequent monomerization of NPR1,  
60 a transcriptional regulator that promotes the expression of SA-responsive genes, can interfere

61 with the biosynthesis of jasmonic acid (JA)/ethylene-dependent defenses that help protect against  
62 leaf-chewing herbivores (Walling, 2008; Zarate *et al.*, 2007). Adelgid feeding has also been  
63 shown to increase nitrogen (Gonda-King *et al.*, 2014) and total amino acid content (Gomez *et al.*,  
64 2012) in hemlock needles. Because nitrogen is critical to insect growth (Awmack & Leather,  
65 2002; Kerslake *et al.*, 1998), such adelgid-mediated increases may enhance host plant quality for  
66 folivorous herbivores.

67         Recent work in the hemlock system suggests that adelgid-induced phytochemical changes  
68 may influence interactions between hemlock and other herbivores (Rigsby *et al.*, 2019; Schaeffer  
69 *et al.*, 2018; Wilson *et al.*, 2018). Larvae of the native hemlock looper (*Lambdina fiscellaria*) had  
70 higher survival and enhanced larval development when reared on adelgid-infested versus  
71 uninfested hemlock foliage (Wilson *et al.*, 2016). This work led us to explore whether similar  
72 interactions might be occurring between the adelgid and more commonly-occurring folivores.  
73 We focused our attention on gypsy moth (*Lymantria dispar*), an invasive folivore that has  
74 devastated eastern U.S. forests. Since its introduction in 1890, periodic gypsy moth outbreaks  
75 have defoliated millions of acres and altered forest structure and composition (Gandhi & Herms,  
76 2010; Lovett *et al.*, 2006). Gypsy moth can feed on eastern hemlock (Lovett *et al.*, 2006) and  
77 although it and hemlock woolly adelgid co-occur in their introduced range, their interactions  
78 have not been considered.

79         We report the results of work assessing the impact of adelgid infestation on gypsy moth-  
80 hemlock interactions. We surveyed hemlocks planted into a deciduous forest understory for  
81 gypsy moth herbivory and conducted two laboratory experiments to measure gypsy moth  
82 preference for, and performance on, adelgid-infested hemlock foliage. Because the adelgid  
83 inhibits hemlock anti-folivore defense pathways and increases the nutritional value of its needles,

84 we hypothesized that gypsy moth larvae would both prefer (consume more of) and do better  
85 (pupate at higher weights) on adelgid-infested foliage. The ‘invasional meltdown hypothesis’  
86 suggests that much of the damage caused by introduced species may result from positive  
87 interactions between invaders that can facilitate their establishment and increase their ecological  
88 impact (Simberloff & Von Holle, 1999). Our findings illustrate the potential for such facilitation  
89 between two invasive herbivores and highlight the threat this may pose to their shared host and  
90 its associated ecosystem.

## 91 **Materials and Methods**

92 *Field preference survey:* Our field preference survey took advantage of a 2016 gypsy  
93 moth outbreak to assess their impacts on field-grown eastern hemlock. The trees in this survey  
94 were planted in 2014 for use in an unrelated experiment. Briefly, 1-1.2 m-tall hemlock saplings  
95 were purchased from Van Pines Nursery (West Olive, MI) in spring 2014, planted, and grown  
96 for two years in the understory of a mixed hardwood stand at the Kingston Wildlife Research  
97 Station (South Kingstown, RI). Hemlocks were planted in five 64-tree blocks, with each tree  
98 spaced 1-1.5 m apart. Trees were protected from herbivory and cross-contamination of  
99 treatments with chicken-wire cages covered by mesh bags (Agribon-15, Johnny’s Selected  
100 Seeds, Waterville, ME, USA; 90% light transmission. Sixteen trees in each block were randomly  
101 assigned one of the following two treatments: infestation with adelgid or another invasive  
102 herbivore (*Fiorinia externa*; elongate hemlock scale, ‘scale’ hereafter). The remaining 32 trees in  
103 each block were maintained as controls. Trees in the adelgid and scale infestation treatments  
104 were inoculated in the spring of 2014, 2015, 2016, and 2017 with infested foliage collected from  
105 nearby adelgid-infested and scale-infested hemlocks, respectively; trees in the control treatment  
106 had herbivore-free hemlock foliage placed on them to control for disturbance.

107           In spring 2016, a gypsy moth outbreak occurred at our field site. Late-instar gypsy moth  
108 larvae were regularly seen roaming on the ground, where they could crawl under the mesh bags  
109 enclosing our trees. Over a short (2-3 week) time period, we observed that many of our trees  
110 received substantial damage from gypsy moth larvae. In late June 2016, 69 trees in the adelgid-  
111 infested treatment group, 69 trees in the scale-infested treatment group, and 107 trees in the  
112 control treatment group were assessed for gypsy moth herbivory damage, for a total of 245 trees.  
113 All branches emerging from the main stem of each tree were surveyed, and each tree was given a  
114 combined damage score of 0-3 (0=0-25% foliage loss, 1=26-50%, 2=51-75%, 3=76-100%). An  
115 annual, early spring survey confirmed that trees did not experience foliage loss prior to the gypsy  
116 moth outbreak. During the survey, gypsy moth larvae were confirmed to be the only folivores  
117 present on trees.

118           *Laboratory preference assay:* Hemlock foliage used in the laboratory preference assay  
119 came from 0.5-0.7m hemlock saplings purchased from Vans Pines Nursery (West Olive, MI) in  
120 spring 2016. In late spring 2016, we inoculated half of the trees with adelgid-infested foliage  
121 from nearby trees; we attached adelgid-free hemlock foliage to the other trees (the control group)  
122 to control for disturbance. All trees were covered in mesh (Agribon-15, Johnny's Selected Seeds,  
123 Waterville, ME, USA; 90% light transmission) to prevent cross-contamination between  
124 treatments and grown in 1-gallon pots outside of the greenhouse complex at the University of  
125 Rhode Island (URI; Kingston, RI). Adelgid densities on each tree were assessed in late fall 2016  
126 and early spring 2017. Five secondary branches on each tree were randomly selected, and all  
127 adelgid present on the branches were counted. We used this data to ensure that both the trees and  
128 specific branches used in this experiment had similar adelgid densities (0.8-1 adelgid/cm).



129           In late spring 2017, we collected 40 gypsy moth larvae from a mixed-hardwood forest  
130 located adjacent to the URI greenhouses. We collected 4<sup>th</sup>-5<sup>th</sup> instar larvae found wandering on  
131 the ground or on tree trunks; all larvae were similarly-sized and highly active throughout the  
132 experiment. To assess gypsy moth preference for adelgid-infested hemlock, we collected 40  
133 ~10cm terminal branches: one branch from each of 20 adelgid-infested trees, and one branch  
134 from each of 20 uninfested trees. Each branch was weighed; analysis *via* two-tailed Welch's  
135 unequal variances t-test confirmed there was no significant difference in the mean branch weight  
136 experienced by larvae in each treatment group ( $t_{36} = -0.72$ ,  $P = 0.4731$ ). Following weighing, the  
137 branches were inserted into individual blocks of water-saturated floral foam (Oasis brand, Kent,  
138 OH). Two pieces of foliage (one adelgid-infested and one control) were then put in a 6L  
139 polypropylene bin (Sterilite brand, Townsend, MA). The pieces of foliage were placed at the  
140 25% and 75% marks between the left and right sides of the bin; treatment placement was  
141 alternated between left and right. After two similarly-sized gypsy moth larvae were weighed,  
142 they were both added to the center of each bin. Each bin was then covered with metal mesh held  
143 in place by a rubber band. There were a total of 20 bins in the experiment. After one day, the  
144 mesh was removed and the larvae and foliage (including any dropped needles) were weighed; the  
145 adelgid-infested and uninfested foliage were weighed separately to calculate larval consumption  
146 for each treatment.

147           *Laboratory performance assay:* Hemlock foliage used in the laboratory performance  
148 assay came from the common garden planting described in the field preference survey. In late  
149 spring 2018, 3<sup>rd</sup>-instar gypsy moth larvae were obtained from the USDA-APHIS Laboratory in  
150 Buzzards Bay, MA. These larvae originated from the New Jersey Standard Strain-APHIS  
151 substrain, a laboratory colony which has been in cultivation for >60 generations. Larvae were

152 reared on an artificial diet (Frontier Agricultural Sciences, USDA Hamden Formula) until they  
153 reached the fourth instar, at which point each larva was weighed and placed individually into one  
154 of 50 473 ml glass mason jars (Ball brand, Broomfield CO). Fourth-instar larvae were used in  
155 this experiment because younger stages have trouble consuming hemlock foliage, likely because  
156 their undeveloped mouthparts cannot penetrate lignified needles. By contrast, larvae in the fourth  
157 instar and above readily consume hemlock.

158         Half of the jars contained foliage from adelgid-infested hemlocks, while the other half of  
159 the jars contained foliage from uninfested hemlocks, for a total of 25 replicates per treatment.  
160 The foliage in each jar consisted of a single ~17cm sprig of foliage kept upright in hydrated  
161 floral foam (Oasis brand, Kent, OH); foliage was checked every day and replaced if >50% of the  
162 needles had been consumed. The top of each jar was covered with nylon mesh and all jars were  
163 kept in a growth chamber (15:9 L:D, 24° C, 60-70% RH). Larvae were checked every two days  
164 and the position of the jars rotated within the growth chamber; the date of and weight at pupation  
165 was recorded for each individual.

166         *Statistical analysis:* All data were inspected for normality (Shapiro-Wilk test) and  
167 homoscedasticity (Bartlett's test) prior to analysis; data were log-transformed where necessary to  
168 meet assumptions. Damage scores were tabulated by treatment group and analyzed *via* Pearson's  
169 chi-squared test. Data from the laboratory preference assay were analyzed *via* two-tailed Welch's  
170 unequal variances t-test. Percent weight gain, pupal weight and larval period were analyzed  
171 separately *via* three-way analysis of covariance (ANCOVA), with foliage type and sex as the  
172 predictors, initial larval weight as a covariate, and all two-way interactions. We classified larvae  
173 as male or female because the sexes differ substantially in their time to and weight at pupation  
174 (Myers et al., 1998); this allowed us to analyze percent weight gain, pupal weight, and larval

175 period of the two sexes separately for both foliage treatment groups. Tukey's test was used to  
176 separate the mean response of the two sexes in either foliage treatment group. Figures were  
177 created using *ggplot2* (Wickham, 2016). R software v. 3.5.0 was used for all statistical analyses  
178 (R Development Core Team, 2018).

179

## 180 **Results**

181 *Field preference survey:* Adelgid-infested hemlocks experienced significantly more  
182 gypsy moth herbivory damage than scale-infested or control trees ( $X^2 = 48.96$ ,  $P < 0.0001$ ; Fig.  
183 1). Nearly 40% of adelgid-infested trees lost more than half of their foliage to gypsy moth  
184 herbivory, while fewer than 10% of scale-infested trees and 5% of control trees experienced  
185 similar levels of damage. Conversely, 84% of both control and scale-infested trees experienced  
186 minimal (0-25% foliage loss) herbivory.

187 *Laboratory preference assay:* When allowed to choose between adelgid-infested and  
188 control foliage, larvae consumed an average of 37% more adelgid-infested foliage than control  
189 foliage (0.36 g +/- 0.054 SE and 0.22 g +/- 0.034 SE, respectively;  $t_{31} = -2.17$ ,  $P = 0.0380$ ).

190 *Laboratory performance assay:* Larvae reared to pupation on adelgid-infested hemlock  
191 foliage gained more weight, and pupated at a higher weight, than larvae reared on uninfested  
192 foliage (both  $P < 0.05$ ; Fig. 2 A, B). Female larvae gained more weight, pupated at a higher  
193 weight, and took longer to pupate than male larvae (all  $P < 0.05$ ; Fig. 2 A, B, C). Initial larval  
194 weight affected larval weight gain and weight at pupation, but not larval period.

195 Female larvae reared on adelgid-infested foliage gained 256% of their initial weight,  
196 while those fed control foliage gained 120% of their initial weight ( $P < 0.001$ ; Fig. 2 A). Male

197 larvae reared on adelgid-infested and uninfested foliage gained 115% and 67% of their initial  
198 weight, respectively ( $P < 0.001$ ; Fig. 2 A).

199 Female larvae reared on adelgid-infested foliage pupated at weights 25% greater than  
200 those reared on uninfested foliage ( $F_{1,36} = 12.5$ ,  $P = 0.0011$ ; Fig. 2 B). Conversely, male larvae  
201 reared on adelgid-infested and control foliage pupated at similar weights ( $P = 0.88$ ; Fig. 2 B).

202 Larval period was not affected by treatment or initial weight (both  $P > 0.4$ ; Fig. 2 C),  
203 although female larvae reared on adelgid-infested hemlock foliage had a larval period five days  
204 longer than that of male larvae reared on uninfested foliage ( $P = 0.0249$ ; Fig. 2 C).

205

## 206 **Discussion**

207 Here we present evidence that one destructive forest pest, hemlock woolly adelgid,  
208 facilitates the development of the invasive gypsy moth. We found that gypsy moth larvae prefer  
209 hemlock foliage infested with hemlock woolly adelgid (Fig. 1), and that feeding on this infested  
210 foliage facilitates gypsy moth larval development. Female larvae reared on adelgid-infested  
211 hemlock foliage gained more than twice as much of their initial weight (Fig. 2 A) and pupated at  
212 25% higher weights (Fig. 2 B) than larvae reared on uninfested foliage. Male larvae reared to  
213 pupation on adelgid-infested foliage also gained 48% more weight than those fed uninfested  
214 foliage (Fig. 2 A) but pupated similar weights (Fig. 2 B). Additionally, gypsy moth larvae  
215 exhibited a preference for adelgid-infested foliage over uninfested foliage, and in a natural  
216 setting, adelgid-infested hemlocks experienced substantially more gypsy moth herbivory than  
217 uninfested trees. Our results are consistent with findings from previous studies documenting a  
218 facilitative effect of hemlock woolly adelgid on other leaf-chewing herbivores.

219           The enhanced performance of gypsy moth larvae reared on adelgid-infested foliage may  
220 result from adelgid-induced changes to hemlock defenses. Adelgid infestation of hemlock  
221 increases foliar emissions of methyl salicylic acid (Pezet *et al.*, 2013; Pezet & Elkinton, 2014)  
222 and triggers salicylic acid (SA) accumulation in needles (Schaeffer *et al.*, 2018; Rigsby *et al.*,  
223 2019), activating SA-linked stress responses in hemlock. The accumulation of SA, and  
224 subsequent monomerization of NPR1, has been shown to inhibit jasmonic acid (JA) biosynthesis  
225 and JA-responsive gene expression (Zarate *et al.*, 2007). Plant defense against leaf-chewing  
226 herbivores is primarily mediated by JA (Gilbert & Liebhold, 2010; Kroes *et al.*, 2014), and  
227 blocking the induction of JA-related defenses may make the foliage of adelgid-infested hemlocks  
228 more digestible and/or easily converted into body mass by gypsy moth larvae. This interpretation  
229 is supported by work on other systems where negative cross-talk between these pathways has  
230 been shown to improve the performance of a later-arriving herbivore (reviewed in Stam *et al.*,  
231 2014).

232           The improved performance of gypsy moth may also be driven by enhanced foliar  
233 nutritional quality in adelgid-infested hemlocks. Hemlock needles on adelgid-infested stems are  
234 higher in nitrogen, suggesting that hemlock woolly adelgid may turn needles into nitrogen-rich  
235 sinks. For instance, amino acid content in adelgid-infested hemlock foliage has been measured at  
236 levels 3.3-fold greater than uninfested foliage (Gomez *et al.*, 2012). Nitrogen plays a key role in  
237 the development and fecundity of herbivorous insects (Awmack & Leather, 2002; Kerslake *et*  
238 *al.*, 1998). High concentrations of dietary nitrogen have been shown to increase gypsy moth  
239 larval survival and pupal weights (Lindroth *et al.*, 1997), and gypsy moth fecundity has been  
240 positively correlated with host plant foliar nitrogen content (Hough & Pimentel, 1978). This is  
241 consistent with prior work in this system by Wilson *et al.* (2016) that found hemlock looper

242 larvae reared on adelgid-infested foliage had higher early-instar survival and attained higher  
243 pupal weights than larvae reared on uninfested foliage.

244 Adelgid-infested hemlocks in our common garden planting experienced significantly  
245 higher rates of defoliation compared to both control (herbivore-free) and scale-infested trees  
246 (Fig. 1). Laboratory choice-assays confirmed that gypsy moth larvae preferentially feed on  
247 adelgid-infested hemlock foliage. In addition to documenting increased overall nitrogen and  
248 amino acid concentrations in adelgid-infested hemlocks, Gomez *et al.* (2012) reported substantial  
249 increases in levels of the amino acid proline. Proline can act as an indicator of plant stress  
250 (Mattson & Haack, 1987), and is an important source of stored energy for insects (Gäde &  
251 Auerswald, 2002). In this case, elevated proline content in adelgid-infested hemlocks may act as  
252 a phagostimulatory signal of vulnerability and elevated nutrient content. This pattern has been  
253 documented in other plant-insect systems, particularly for various Hemiptera, Lepidoptera,  
254 Orthoptera and mite species (Mattson & Haack, 1987).

255 The fact that adelgid feeding enhances gypsy moth preference for, and performance on,  
256 eastern hemlock, makes it likely that their co-occurrence on hemlock can additively stress and  
257 further threaten this important conifer. In southern New England, adelgid infestation has caused  
258 extensive mortality of overstory hemlocks (Eschtruth *et al.*, 2006; Orwig *et al.*, 2002; Preisser *et*  
259 *al.*, 2008), altering understory conditions that put hemlock seedlings at a competitive  
260 disadvantage (Orwig *et al.*, 2013; Orwig *et al.*, 2008). Hemlocks are adapted to cool  
261 microclimates and low light levels (Hadley, 2000), and increased light exposure due to crown  
262 thinning and mortality of mature trees inhibits recruitment of hemlock seedlings and favors  
263 establishment of black birch (*Betula lenta*) and other deciduous trees (Ingwell *et al.*, 2012; Orwig  
264 & Foster, 1998; Orwig *et al.*, 2002). Preferential feeding by gypsy moth larvae on adelgid-

265 infested overstory hemlocks may exacerbate this effect, reducing the likelihood of new hemlock  
266 recruits eventually repopulating devastated hemlock forests. The damage inflicted by gypsy  
267 moths on adelgid-infested hemlock saplings may further compromise regeneration. Over a four-  
268 year period, hemlock regeneration in adelgid-infested forests declined by 46% (Preisser *et al.*,  
269 2011). Feeding by both species may accelerate this decline, if inhibited seedling recruitment is  
270 coupled with significant mortality of juvenile hemlock saplings.

271         Enhanced performance of gypsy moth larvae on adelgid-infested hemlock may also have  
272 a cascading effect on other plant taxa that grow with hemlock in forests of the eastern U.S. Oaks  
273 (*Quercus* spp.) are a preferred host of gypsy moth (Barbosa *et al.*, 1979; Hough & Pimentel,  
274 1978), and feeding by gypsy moth larvae has caused extensive mortality and decline of overstory  
275 oaks throughout this region (Gandhi & Herms, 2010). Total basal area of overstory oaks has  
276 decreased due to gypsy moth herbivory, and mortality of white oak (*Quercus alba*), northern red  
277 oak (*Quercus rubra*), and chestnut oak (*Quercus montana*) specifically has increased by 40%  
278 (Fajvan & Wood, 1976). Gypsy moth herbivory in southern New England forests has increased  
279 oak mortality and reduced the growth of surviving canopy trees by as much as 65% (Gottschalk  
280 *et al.*, 1990). It is plausible that the enhanced growth of female gypsy moth larvae on adelgid-  
281 infested hemlock may translate to greater fecundity, which could increase gypsy moth population  
282 densities in southern New England forests. Since tree mortality increases as the intensity and  
283 frequency of gypsy moth defoliation increases (Davidson *et al.*, 1999), larger gypsy moth  
284 populations here could speed oak decline.

285         It is important to realize that ecological traits of the gypsy moth larvae used in the  
286 laboratory performance assay may not be comparable with those of wild gypsy moth larvae.  
287 Larvae used in the laboratory performance assay were part of the New Jersey Standard Strain-

288 APHIS substrain, a mass-reared colony of gypsy moth larvae that has been in cultivation for >60  
289 generations. Because this colony is intended for research, certain selective regimes and control  
290 measures have been enacted upon it to maximize the survival and fecundity of the gypsy moths.  
291 These include laboratory selection for higher survival and fecundity, and an artificial diet  
292 (Frontier Agricultural Sciences, USDA Hamden Formula), which may incidentally select for  
293 genotypes that show reduced performance on a natural diet (Grayson *et al.*, 2015). However, a  
294 comparison of development between gypsy moth larvae from the New Jersey Standard Strain-  
295 APHIS substrain, -FS substrain, and three wild populations all reared on a natural diet, found no  
296 population-level differences in male and female pupal weights (Grayson *et al.*, 2015).  
297 Additionally, our observation of substantial wild gypsy moth larval herbivory damage to adelgid-  
298 infested field-grown eastern hemlocks, as well as a confirmed wild gypsy moth larval preference  
299 for adelgid-infested hemlocks, mirror results from the laboratory performance assay and further  
300 support their ecological relevance.

301 Adelgid-induced hemlock mortality has severely affected ecosystem dynamics in eastern  
302 U.S. forests. Hemlock supports critical habitat for unique vertebrate and invertebrate  
303 communities (Ellison *et al.*, 2010), and dramatic shifts in understory vegetation, soil nutrient  
304 cycling and hydrological regimes may have long-lasting changes that compromise these areas  
305 (Orwig *et al.*, 2008). Future work should evaluate the extent to which adelgid and gypsy moth  
306 act synergistically to speed the decline of eastern hemlock and other canopy-dominant species,  
307 and the impact this could have on hemlock-associated ecosystems.

308

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320

### 321 **Contribution of Authors**

322 IGK, ELP, CMR, and CMO were responsible for project design. IGK, AKB, ERW, SKS, EES,  
323 and ELP were responsible for data collection and analysis. IGK, ELP, AKB, CMR, and CMO  
324 were responsible for paper writing.

325

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458 **Figure Legends**

459 **Figure 1.** Gypsy moth larval herbivory damage to eastern hemlocks in adelgid (*Adelges*  
460 *tsugae*)-infested, uninfested control, and scale (*Fiorinia externa*)-infested treatment groups.  
461 Damage was quantified on a scale of 0-3, representing % foliage loss of trees in each treatment  
462 group (0=0-25% foliage loss, 1=26-50%, 2=51-75%, 3=76-100%).

463 **Figure 2.** Percent weight gain (A), pupal weight (B), and larval period (C) of gypsy moth  
464 larvae reared on either adelgid-infested or uninfested control hemlock foliage. Bars represent  
465 means +/- 1 SE; F = female larvae, M = male larvae. Capital letters denote significant treatment-  
466 level differences ( $P < 0.05$ ).

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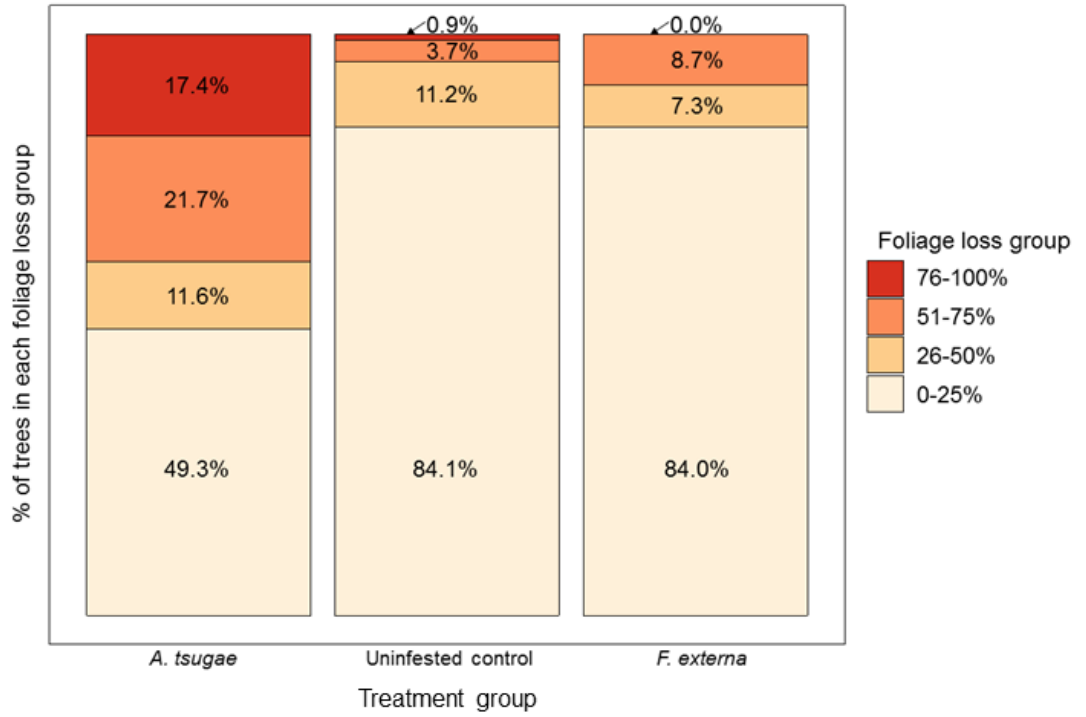
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Figure 1.



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Figure 2.

