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## Impact of Hemlock Woolly Adelgid (Hemiptera: Adelgidae) Infestation on the Jasmonic Acid-Elicited Defenses of *Tsuga canadensis* (Pinales: Pinaceae)

Chad M. Rigsby

Ian G. Kinahan

Amelia May

Amy Kostka

Nick Houseman

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**Authors**

Chad M. Rigsby, Ian G. Kinahan, Amelia May, Amy Kostka, Nick Houseman, Suzanne K. Savage, Elizabeth R. Whitney, and Evan L. Preisser

1 **Impact of hemlock woolly adelgid (*Adelges tsugae*) (Hemiptera: Adelgidae) infestation on**  
2 **the jasmonic acid-elicited defenses of eastern hemlock (*Tsuga canadensis*)**

3

4 Chad M. Rigsby<sup>1,2,3</sup>, Ian G. Kinahan<sup>1</sup>, Amelia May<sup>1</sup>, Amy Kostka<sup>1</sup>, Nick Houseman<sup>1</sup>,  
5 Suzanne K. Savage<sup>1</sup>, Elizabeth R. Whitney<sup>1</sup>, and Evan L. Preisser<sup>1</sup>

6

7 <sup>1</sup>Department of Biological Sciences, The University of Rhode Island, Kingston, RI, USA

8 <sup>2</sup>Author for correspondence: crigsby@bartlettlab.com

9 <sup>3</sup>Current Address: Bartlett Tree Research Laboratories, The Morton Arboretum, Lisle, IL, USA

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## 20 **Abstract**

21 Hemlock woolly adelgid ('HWA') is an invasive piercing-sucking insect in eastern North  
22 America, which upon infestation of its main host, eastern hemlock ('hemlock'), improves  
23 attraction and performance of folivorous insects on hemlock. This increased performance may be  
24 mediated by HWA feeding causing antagonism between the the jasmonic acid and other  
25 hormone pathways. In a common garden experiments using HWA infestation and induction with  
26 methyl jasmonate (MeJA) and measures of secondary metabolite contents and defense-associated  
27 enzyme activities, we explored the impact of HWA feeding on the local and systemic induction  
28 of JA-elicited defenses. We found that in local tissue HWA or MeJA exposure resulted in unique  
29 induced phenotypes, while the combined treatment resulted in an induced phenotype that was a  
30 mixture of the two individual treatments. We also found that if the plant was infested with HWA,  
31 the systemic response of the plant was dominated by HWA, regardless of whether MeJA was  
32 applied or not. Interestingly, in the absence of HWA, hemlock plants had a very weak systemic  
33 response to MeJA. We conclude that HWA infestation prevents systemic induction of JA-elicited  
34 defenses. Taken together, compromised local JA-elicited defenses combined with weak systemic  
35 induction could be major contributors to increased folivore performance on HWA-infested  
36 hemlock.

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38 **Key Words:** induced defenses, systemic induction, invasive forest pest, herbivory, conifers

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## 40 **Introduction**

41           Plants growing under the resource-limited conditions typical of natural systems must  
42 choose how to allocate scarce resources to functions such as growth, reproduction, and defense.  
43 The induction of chemical and physical defenses in response to herbivore or pathogen attack is  
44 hypothesized to be an energetically advantageous solution to such dilemmas (Baldwin 1998;  
45 Gómez et al. 2007). When attacked by mobile herbivores that can respond to local defense by  
46 seeking out undefended plant biomass, plants can respond *via* systemic responses that stimulate  
47 defense induction in both damaged and undamaged tissues (Kant et al. 2015). Because they incur  
48 energetic costs in tissue that has not yet been – and might not be – attacked, systemic defenses  
49 are often considered a bet-hedging strategy: the cost of systemic induction is roughly half the  
50 cost borne by non-systemically-induced plants that are attacked (Reynolds et al. 2019).

51           Systemic induction can be influenced by vascular architecture and connectivity, plant size  
52 and age, and volatile production (Orians 2005; Kant et al. 2015). Several endogenous compounds  
53 that act as systemic signals include phytohormones, peptides, and volatile compounds (Kant et  
54 al. 2015). Jasmonates appear particularly important for systemic defense induction (Heil and Ton  
55 2008). Precursors to jasmonic acid (JA) conjugates, such as JA itself, are produced at the site of  
56 herbivore attack and transported through the phloem to undamaged tissues (Fürstenberg-Hägg et  
57 al. 2013). JA-elicited systemic defense expression requires both JA biosynthesis at the site of  
58 damage and JA perception in distant undamaged tissues (Heil and Walters 2009). A substantial  
59 set of literature has demonstrated the antagonistic relationship between salicylic acid (SA) and  
60 JA where the SA-induced monomerization of NONEXPRESSOR OF PATHOGENESIS-  
61 RELATED GENES1 (NPR1) suppresses JA biosynthesis and inhibits JA-responsive genes  
62 (Beckers and Spoel 2006). This antagonistic relationship suggests that the expression of JA-

63 elicited systemic defense in distal plant tissues would be compromised if locally-produced SA  
64 interfered with JA biosynthesis at the attack site.

65 Hemlock woolly adelgid (HWA; *Adelges tsugae* Annand) is a sessile, stylet-feeding  
66 insect that is invasive to eastern North America. It has caused mass mortality of eastern hemlock  
67 ('hemlock'; *T. canadensis* L.) (Pinales: Pinaceae) within its invaded range. Chronic HWA  
68 infestation causes a 'hypersensitive-like' response in hemlock that is characterized by the  
69 accumulation of SA, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and proline and increases in methyl salicylate  
70 (MeSA) emissions (Radville et al. 2011; Gómez et al. 2012; Pezet et al. 2013; Pezet and Elkinton  
71 2014 Schaeffer et al. 2018; Rigsby et al. 2019). The nature of this response led to the hypothesis  
72 that HWA infestation would increase host quality for JA-eliciting herbivores by decreasing the  
73 induction of JA-linked plant defenses. Consistent with this scenario, Wilson et al. (2016)  
74 reported increased performance of hemlock looper (*Lambdina fiscellaria*) on HWA-infested  
75 hemlock, and Kinahan et al. (2020) found increased gypsy moth (*Lymantria dispar*) larval  
76 preference for and performance on HWA-infested hemlocks in both field and laboratory settings.

77 Although the latter two studies are consistent with the hypothesis that HWA-mediated  
78 increases in SA disrupt JA-based plant defense, this linkage has not been experimentally  
79 confirmed. While changes in the inducibility of JA-elicited defenses may be involved, SA- and  
80 JA-elicited defense responses are remarkably similar in hemlock (Rigsby et al. 2019). In an  
81 experiment that used HWA and gypsy moth larvae to directly induce SA- and JA-elicited  
82 responses, Rigsby et al. (*in review*) found that both HWA and gypsy moth increased foliar SA  
83 levels; simultaneous herbivory by both insects had an additive effect. Gypsy moth herbivory  
84 resulted in accumulation of JA and JA-Ile, the active form of JA, while HWA inhibited the  
85 ability of gypsy moths to elicit JA accumulation (Rigsby et al. *in review*). These findings support

86 the hypothesis that HWA infestation prevents hemlock from accumulating JA phytohormones in  
87 response to JA-eliciting herbivores. Intriguingly, however, HWA infestation also increased  
88 accumulation of several bioactive gibberellins (GAs), hormones known to play a critical role in  
89 plant growth (i.e., stem elongation and leaf expansion; Davière and Achard 2013). This HWA-  
90 elicited GA accumulation is notable because GAs are also known to antagonize JA signaling (de  
91 Lucas et al. 2008). This result suggests that JA accumulation and the elicitation of JA-linked  
92 defenses could be compromised by one or both of these mechanisms.

93         Previous research addressing herbivore-herbivore interactions in the HWA/hemlock  
94 system has focused on local plant defense induction (i.e., changes occurring at the site of plant  
95 damage); the impacts of HWA on systemic defense induction have not been addressed. We  
96 present the results of work assessing the potential for HWA-induced suppression of JA-elicited  
97 systemic defense induction. Using a common garden planting that contained both HWA-infested  
98 and HWA-free hemlock saplings, we induced stems with methyl jasmonate (MeJA), a  
99 methylated form of JA whose topical application induces JA-elicited responses in hemlock  
100 (Rigsby et al. 2019). We evaluated induction responses by quantifying chemical and  
101 physiological defensive responses (e.g., total soluble phenolics, peroxidase activity, etc) in  
102 foliage on stems directly sprayed with MeJA and needles not directly sprayed, but on the same  
103 branch. We hypothesized that (1) HWA infestation would attenuate local MeJA-elicited defense  
104 responses, in accordance with Rigsby et al. (2019), but would completely shut down MeJA-  
105 elicited systemic responses. Conversely, we predicted that (2) both local and systemic responses  
106 would be uninhibited in HWA-free plants.

## 107 **Materials and Methods**

108 *Hemlock common garden, treatments, and sampling.* In early spring 2014, 350 herbivore-  
109 free hemlock saplings (0.5-0.7 m tall) that were grown from seed collected in Pennsylvania and  
110 had not been treated with insecticides were purchased from Vans Pines Nursery (West Olive,  
111 MI). The 320 healthiest of these trees were planted in five 64-tree blocks (eight rows and  
112 columns with trees spaced 1-1.5 m apart) into the understory of a mixed hardwood stand at the  
113 Kingston Wildlife Research Station (South Kingstown, RI) in April 2014. As part of ongoing  
114 experiments in our laboratory, a subset of trees within each block were randomly selected for  
115 artificial infestation with HWA, performed every year at approximately mid-spring (timed with  
116 crawler emergence). Briefly, we cut HWA-infested stems from naturally growing hemlocks  
117 located less than one km from our experimental site, inspected this foliage for the presence of  
118 only HWA, and secured this cut foliage to each hemlock within this treatment using wire to  
119 secure this cut foliage to each hemlock (see Butin et al. 2007 for detailed methods). Trees in the  
120 control treatment were sham-inoculated with herbivore-free foliage to control for inoculation-  
121 related disturbance. The uninfested status of each control tree was confirmed *via* careful visual  
122 inspection of each tree prior to the removal of any foliage. Trees were protected from herbivory  
123 and treatment cross-contamination with chicken-wire cages covered in mesh bags (Agribon-15,  
124 Johnny's Selected Seeds, Waterville, ME, USA; 90% light transmission).

125 Twelve trees from each of the two treatments (HWA-infested, uninfested controls) were  
126 selected so that each treatment was represented by at least three trees in each of four spatial  
127 blocks; trees from the fifth spatial block was excluded because this block was much shadier than  
128 the other four. A single branch was selected on each tree; all sampled branches were of similar  
129 length and diameter (ANOVA;  $P > 0.05$  for all) and the branches from HWA-infested trees had  
130 moderate, but roughly equivalent HWA densities (0.5-1 HWA cm<sup>-1</sup> stem). Each branch was



131 marked by placing flagging placed at its base (Fig. 1). Twice weekly for a two-week period (28  
132 Aug - 7 Sept 2017), an elicitor solution containing 10 mM MeJA in a carrier solution of 0.1%  
133 (v:v) Tween 20 (MeJA treatment) or carrier solution only (control treatment) was carefully  
134 applied using a fine-tipped paint brush, so that MeJA solution did not run off, to the first lateral  
135 stem proximal to the terminal stem, near the flagging. All treated branches were harvested on 11  
136 Sept, placed in aluminum foil, and stored at -80°C. In order to understand how HWA impacts  
137 systemic defense signaling, we harvested a stem immediately proximal (denoted as “Systemic”  
138 stem) to the treated stem (denoted as “Local” stem) (Fig. 1). This resulted in four treatment  
139 combinations (HWA +/- and MeJA +/-; n = 6 biological replicates per treatment combination; 24  
140 total), with two location categories per branch: “Local” and “Systemic” stems (48 total samples;  
141 Fig. 1). Lastly, in order to eliminate additional sources of variation, only foliage produced in the  
142 current growing season (i.e., newly produced foliage) was used in this study, foliage that was  
143 produced prior to the season of our experiment was not used in this study (Fig. 1).

144 *Chemical and Physiological Analyses.* Crude levels of chemical defenses were quantified  
145 as described in Rigsby et al. (2019); any deviations from these protocols are detailed below.  
146 Briefly, needles were ground into a powder in liquid nitrogen using a mortar and pestle and 100  
147 mg were placed in a 2 ml microtube. Tissue was twice-extracted in 0.5 ml HPLC-grade  
148 methanol. Following centrifugation at 16,000 g (10 min, 4°C), the supernatants were combined.  
149 Methanol-soluble terpene content was quantified immediately using chloroform and H<sub>2</sub>SO<sub>4</sub>  
150 (Rigsby et al. 2019) with linalool as the standard. Soluble phenolic content was quantified *via* the  
151 Folin-Ciocalteu method using chlorogenic acid as standard; proanthocyanidin content was  
152 quantified using the acidified butanol method (Rigsby et al. 2019). Chlorogenic acid was used as  
153 a standard for the quantification of soluble phenolics because prior research found that

154 chlorogenic acid dominates the soluble phenolic profile of hemlock foliage (Rigsby et al. 2020).  
155 The cell wall-bound phenolic (CW-bound phenolic) and lignin contents were determined as per  
156 Rigsby et al. (2019) using gallic acid and spruce lignin, respectively, as the standard. Hydrogen  
157 peroxide (H<sub>2</sub>O<sub>2</sub>) was quantified according to the KI method (Junglee et al. 2014; Rigsby et al.  
158 2019).

159 For enzyme activity assays, 200 mg needle powder was extracted on ice in five volumes  
160 of extraction buffer (50 mM NaPO<sub>4</sub>, pH 6.8, 10% PVPP, 5% Amberlite XAD4 resin, and 1 mM  
161 EDTA) and the 10,000 g supernatant was used as the source of enzymes. Chitinase (CHI) and  
162 lipoxygenase (LOX) activities were quantified according to Rigsby et al. (2016) using chitin  
163 azure (OD<sub>575</sub> mg<sup>-1</sup>) and linoleic acid (μmoles min<sup>-1</sup> mg<sup>-1</sup>), respectively, as substrates. Peroxidase  
164 (POX) activity was quantified according to Rigsby et al. (2018) using guaiacol and H<sub>2</sub>O<sub>2</sub> as  
165 substrates (μmoles min<sup>-1</sup> mg<sup>-1</sup>). Phenylalanine ammonia lyase (PAL) activity was quantified by  
166 monitoring the conversion of L-phenylalanine to *trans*-cinnamic acid (Chen et al. 2006; nmoles  
167 hr<sup>-1</sup> mg<sup>-1</sup>). To express enzyme activities per unit protein, the protein content of extracts was  
168 determined using the Bradford (1976) method with bovine serum albumin as standard. During  
169 preliminary experiments, we attempted to detect polyphenol oxidase activity using multiple  
170 substrates, as well as trypsin inhibitor activity, but were unable to do so.

171 *Statistical analyses.* The effect of HWA, MeJA, branch position (i.e., systemic  
172 induction), and their interactions on relative metabolite levels and enzyme activities was assessed  
173 using an ANOVA with stem position nested within tree identity. An ANCOVA was initially  
174 used with block as a covariate; because block was never significant, we proceeded with  
175 ANOVAs. We were interested in detecting (1) within-treatment differences in foliar position  
176 (i.e., 'Local' vs. 'Systemic' within a single treatment combination) and (2) between-treatment

177 differences for a given foliar position (i.e., ‘HWA-/MeJA-’ vs. ‘HWA+/MeJA-’ vs. ‘HWA-  
178 /MeJA+’ vs. ‘HWA+/MeJA+’ within a single sampling position). For post-hoc comparisons of  
179 within-treatment differences between sampling positions, we used *t*-tests to directly compare  
180 Local and Systemic foliage. For post-hoc comparisons of treatment combinations within a  
181 sampling position, we first performed *t*-tests comparing all combinations of interest, then the  
182 resulting *P*-values were adjusted *via* the Benjamini-Hochberg procedure (Benjamini and  
183 Hochberg 1995). For example, if comparing all four treatment combinations of ‘Local’ foliage,  
184 the six calculated *P*-values were included in the Benjamini-Hochberg correction. Because  
185 different sampling positions from different treatments were not of interest (e.g., ‘Local’ foliage  
186 from ‘HWA+/MeJA-’ vs. ‘Systemic’ foliage from ‘HWA-/MeJA-’), these comparisons were not  
187 made. These post-hoc procedures was only used if significant interactions between  
188 treatments/sampling locations were detected. All statistical analyses were performed in R (R  
189 Development Core Team 2020).

## 190 **Results**

191 *Secondary Metabolites.* For both Local and Systemic foliage, CW-bound phenolics,  
192 lignin, and H<sub>2</sub>O<sub>2</sub> all had increased tissue levels as a result of HWA infestation relative to  
193 uninfested controls (Figs. 2A, 2B, and 2C, respectively). The application of MeJA had no effect  
194 on CW-bound phenolic or H<sub>2</sub>O<sub>2</sub> contents in either Local or Systemic foliage (Figs. 2A and 2C,  
195 respectively), but did cause lignin to accumulate in Local foliage in the absence of HWA.  
196 However, this lignin accumulation was attenuated in the presence of HWA in Local foliage (Fig.  
197 2B). Foliage position (i.e., “Local” vs. “Systemic” foliage) had a significant effect on lignin and  
198 H<sub>2</sub>O<sub>2</sub> contents. In the absence of HWA, MeJA application (HWA-/MeJA+) significantly  
199 increased lignin content in Local foliage but not in adjacent Systemic foliage (Fig. 2B).

200 Additionally, in the presence of HWA and when MeJA was applied (HWA+/MeJA+), H<sub>2</sub>O<sub>2</sub>  
201 content was significantly greater in Systemic than in Local foliage (Fig. 2C). There were no  
202 elicitor treatment or sampling location effects for soluble phenolics ( $71.70 \pm 1.05 \text{ mg g}^{-1} \text{ DW}$ ),  
203 proanthocyanidins ( $33.54 \pm 1.32 \text{ OD}_{550} \text{ g}^{-1} \text{ DW}$ ), or methanol-soluble terpenes ( $14.05 \pm 0.15 \text{ mg}$   
204  $\text{g}^{-1} \text{ DW}$ ).

205 *Defensive Enzyme Activities.* In both Local and Systemic foliage, HWA infestation  
206 increased POX activity (Fig. 3A), while the application of MeJA increased LOX and PAL  
207 activities in Local foliage, only (Figs. 3B and 3C, respectively). Interestingly, infestation by  
208 HWA had no effect on the MeJA-elicited increase in LOX activity in Local foliage (i.e., the  
209 increase in LOX activity caused by MeJA application was not attenuated by the presence of  
210 HWA in Local foliage). However, this was the case for PAL activity, as HWA infestation  
211 severely inhibited the MeJA-elicited increase in PAL activity in Local foliage (Fig. 3C). Foliage  
212 position (i.e., “Local” vs. “Systemic” foliage) had a significant effect on both LOX and PAL  
213 activities. As with lignin content, the increase in LOX and PAL activities that were found in  
214 Local foliage in the absence of HWA and with MeJA application (HWA-/MeJA+), did not occur  
215 in Systemic foliage (Figs. 3B and 3C). This was also the case for LOX activity in the presence of  
216 HWA and with MeJA application (HWA+/MeJA+), where MeJA application resulted in  
217 increased activity in Local but not in Systemic foliage (Fig. 3A). There were no elicitor treatment  
218 or sampling location effects for CHI activity ( $0.31 \pm 0.01 \text{ OD}_{575} \text{ mg}^{-1}$ ).

## 219 **Discussion**

220 The systemic induction of defenses is considered an important bet-hedging strategy for  
221 plants to minimize fitness costs (Reynolds et al. 2019), and systemic induction is viewed as an  
222 adaptive response against herbivores that impose chronic injury, continually increase populations

223 on individual plants, and/or can move among plant parts (Mason et al. 2017). Like many woody  
224 plants, an abundance of folivorous insects utilize hemlock as a host resource, including a variety  
225 of leafminers, loopers, leafrollers, budworms, needleworms, tussock moths, cutworms, and  
226 others (Maier et al. 2011). Recent research has shown that HWA infestation increases the  
227 attraction to and performance of folivorous insects on hemlock (Wilson et al. 2016; Rigsby et al.  
228 2019; Kinahan et al. 2020), and this increase in folivore performance may be facilitated by the  
229 compromising of JA-elicited defenses locally at the site of folivore attack (Rigsby et al. 2019;  
230 2020). This study sought to investigate the impact of HWA infestation on the induction of  
231 systemic, JA-elicited defenses. We hypothesized that (1) the HWA-instigated attenuation of local  
232 JA induction would be accompanied by a complete lack of systemic responses, and that (2)  
233 systemic responses would occur on HWA-free plants.

234         With regards to our first hypothesis that HWA infestation would attenuate local MeJA-  
235 elicited defense responses, our data somewhat agree with this though defenses presented rather  
236 as a blend between HWA-induced and MeJA-induced responses. This was consistent with  
237 previous research that found local JA-elicited defense expression is altered by HWA infestation  
238 (Rigsby et al. 2019). The second part of the hypothesis, that this local attenuation would be  
239 accompanied by complete inhibition of systemic responses, which also appears to be supported  
240 generally as systemic defense expression was completely masked by the local response to HWA  
241 infestation. Patterns of metabolite accumulation and enzyme activities of this treatment-position  
242 combination (i.e., systemic HWA+/MeJA+) was most similar to both the local and systemic  
243 HWA+/MeJA- treatments. Even if JA-elicited host responses were not locally compromised, the  
244 lack of systemic responses to mobile herbivores would pose a serious problem for a woody plant,  
245 as mobile folivores could simply move to these undefended tissues (Mason et al. 2017).

246           The apparent lack of systemic induction by MeJA+ plants was unanticipated and the  
247 opposite of our second hypothesis. Several variables could have contributed to this, such as  
248 interspecific species variation in systemic inducibility (e.g., Heil and Ploss 2006), site conditions  
249 (e.g., shade is known to inhibit JA responses; Cipollini 2004), MeJA dose (e.g., Naidoo et al.  
250 2013), and/or vascular architecture (e.g., the stems chosen for our experiment may not have been  
251 as connected as we perceived; Oriens 2005). However, the differential responses of LOX and  
252 PAL activities in the systemic tissues was particularly interesting (Figs. 3A and 3C). The activity  
253 of LOX, which should be an excellent of JA-elicitation indicator as it is directly involved in JA  
254 synthesis (Beckers and Spoel 2006) and directly (Felton et al. 1994) and indirectly (War et al.  
255 2012) involved in defense, was not increased systemically by MeJA. However, PAL activity was  
256 increased systemically with MeJA application, perhaps demonstrating that PAL activity may  
257 better indicate JA-elicitation than LOX activity. Regardless of this, systemically increased PAL  
258 activity indicates that some sort of signal likely made it to this stem and was perceived by these  
259 tissues.

260           Interestingly, we did not detect local or systemic accumulation of soluble phenolics,  
261 including proanthocyanidins, and methanol-soluble terpenes. These classes of secondary  
262 metabolites are known to be critically important anti-herbivore defenses in conifers (Raffa et al.  
263 2017). Previous research showed significant, positive effects of both HWA infestation and MeJA  
264 application on soluble phenolic content, including proanthocyanidins (Rigsby et al. 2019).  
265 Similar levels of CHI activity across all treatment combinations was also unanticipated, since  
266 previous research found that the activity of this enzyme was strongly enhanced by HWA  
267 infestation and MeJA application (Rigsby et al. 2019). In agreement with this previous research,  
268 we detected accumulation of CW-bound phenolics and H<sub>2</sub>O<sub>2</sub>, and increases in POX activity in

269 response to HWA infestation, and a strong positive effect of MeJA application on LOX activity  
270 (Rigsby et al. 2019). One difference between these two experiments is that Rigsby et al. (2019)  
271 used potted hemlocks in full sun while this study used hemlocks planted in the understory of a  
272 mixed hardwood stand. It may be that some aspect(s) of these environmental differences had  
273 some effect on hemlock response to our treatments. In addition to normal growth, GAs are also  
274 associated with shade-avoidance and growth, and JA pathways interact directly and  
275 antagonistically through DELLA-JAZ interactions (Wasternack and Hause 2013; Davière and  
276 Achard 2016), and shaded plants are often unable to activate JA-elicited responses (Cipollini  
277 2004). HWA has a positive effect on a few major GAs (Rigsby et al. *In Review*), and the addition  
278 of shade may further increase gibberellin accumulation and antagonization of the JA pathway.  
279 An additive or synergistic effect between HWA infestation and shade on the inducibility of JA-  
280 elicited defenses would have major impacts on hemlock herbivore interactions, including  
281 between hemlock and HWA, itself. It has been noted by many researchers and practitioners that  
282 HWA appears to perform substantially better on its host when hemlock is shaded (Hickin and  
283 Preisser 2015).

284         The systemic induction of defenses is thought to be an important strategy of plants to  
285 reduce fitness costs (Kant et al. 2015; Reynolds et al. 2019), especially against herbivores that  
286 can move between plant tissues (Mason et al. 2017). Field observations and laboratory assays  
287 have shown dramatic increases in host quality and attraction to these kinds of herbivores (Wilson  
288 et al. 2016; Rigsby et al. 2019; Kinahan et al. 2020). In this study, we found that host responses  
289 to HWA infestation essentially overwhelm and prevent JA-elicited systemic defense expression,  
290 but we also detected very little JA-elicited systemic responses in hemlock in the absence of  
291 HWA. We conclude that in the absence of HWA, some JA-associated signal may be translocated

292 and systemically perceived, as evidenced by significantly elevated PAL activity. Environmental  
293 conditions of our experiment may have played a role in this lack of response, however, hemlock  
294 often exists in the environment in dense, shaded conditions (Hadley 2000), still allowing our  
295 results to be ecologically meaningful. Future research should explore the role of shade on local  
296 and systemic SA- and JA-elicited responses in hemlock.

297

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312 **References**

- 313 **Baldwin, I. T. 1998.** Jasmonate-induced responses are costly but benefit plants under attack in  
314 native populations. *Proc. Natl. Acad. Sci. USA.* 95: 8113-8118.
- 315 **Beckers, G. J. M., and S. H Spoel. 2006.** Fine-tuning plant defence signaling: Salicylate versus  
316 jasmonate. *Plant. Biol.* 8: 1-10.
- 317 **Benjamini, Y., and Y. Hochberg. 1995.** Controlling the false discovery rate: a practical and  
318 powerful approach to multiple testing. *J. R. Stat. Soc. B* 57: 289-300.
- 319 **Butin, E., E. L. Preisser, and J. S. Elkinton. 2007.** Factors effecting settlement rate of the  
320 hemlock woolly adelgid, *Adelges tsugae*. *Agric. For. Entomol.* 9: 215-219.
- 321 **Chen, J-Y., P-F. Wen, W-F. Kong, Q-H. Pan, J-C. Zhan, J-M. Li, and W-D. Huang. 2006.**  
322 Effect of salicylic acid on phenylpropanoids and phenylalanine ammonia-lyase in  
323 harvested grape berries. *Postharvest Biol. Tec.* 40: 64-72.
- 324 **Cipollini, D. 2004.** Stretching the limits of plasticity: Can a plant defend against both  
325 competitors and herbivores?. *Ecology.* 85: 28-37.
- 326 **Davière, J-M., and P. Achard. 2013.** Gibberellin signaling in plants. *Development.* 140: 1147-  
327 1151.
- 328 **Davière, J-M., and P. Achard. 2016.** A pivotal role of DELLAs in regulating multiple hormone  
329 signals. *Mol. Plant.* 9: 10-20.
- 330 **de Lucas, M., J-M. Davière, M. Rodríguez-Falcón, M. Pontin, J. M. Iglesias-Pedraz, S.**  
331 **Lorrain, C. Fankhauser, M. A. Blázquez, E. Titarenko, and S. Prat. 2008.** A

- 332 molecular framework for light and gibberellin control of cell elongation. *Nature*. 451:  
333 480-483.
- 334 **Felton, G. W., C. B. Summers, and A. J. Mueller. 1994.** Oxidative responses in soybean  
335 foliage to herbivory by bean leaf beetle and three-cornered alfalfa hopper. *J. Chem. Ecol.*  
336 20: 639-650.
- 337 **Fürstenberg-Hägg, J., M. Zagrobelny, and S. Bak. 2013.** Plant defense against insect  
338 herbivores. *Int. J. Mol. Sci.* 14: 10242-10297.
- 339 **Gómez, S., V. Latzel, Y. M. Verhulst, and J. F. Stuefer. 2007.** Costs and benefits of induced  
340 resistance in a clonal plant network. *Oecologia*. 153: 921-930.
- 341 **Hadley, J. L. 2000.** Understory microclimate and photosynthetic response of saplings in an old-  
342 growth eastern hemlock (*Tsuga canadensis* L.) forest. *Ecoscience*. 7: 66-72.
- 343 **Heil, M., and K. Ploss. 2006.** Induced resistance enzymes in wild plants—do ‘early birds’ escape  
344 from pathogen attack?. *Naturwissenschaften*. 93: 455-460.
- 345 **Heil, M., and J. Ton. 2008.** Long-distance signalling in plant defence. *Trends Plant Sci.* 13:  
346 264-272.
- 347 **Heil, M., and D. R. Walters. 2009.** Ecological consequences of plant defense signaling, pp.  
348 667-716. In L. C. Van Loon (ed), *Advances in Botanical Research* Vol. 51, Elsevier,  
349 Amsterdam, Netherlands.
- 350 **Hickin, M., and E. L. Preisser. 2015.** Effects of light and water availability on the performance  
351 of hemlock woolly adelgid (Hemiptera: Adelgidae). *Environ. Entomol.* 44: 128-135.

- 352 **Junglee, S., L. Urban, H. Sallanon, and F. Lopez-Lauri. 2014.** Optimized assay for hydrogen  
353 peroxide determination in plant tissue using potassium iodide. *Am. J. Anal. Chem.* 5:  
354 730-736.
- 355 **Kant, M. R., W. Jonckheere, B. Knecht, F. Lemos, J. Liu, C. J. Schimmel, C. A. Villarroel,**  
356 **L. M. S. Ataide, W. Dermauw, J. J. Glas, M. Egas, A. Janssen, T. Van Leeuwen, R.**  
357 **C. Schuurink, M. W. Sabelis, and J. M. Alba. 2015.** Mechanisms and ecological  
358 consequences of plant defence induction and suppression in herbivore communities. *Ann.*  
359 *Bot.* 115: 1015-1051.
- 360 **Maier, C. T., C. R. Lemmon, J. M. Fengler, D. F. Schweitzer, R. C. Reardon. 2011.**  
361 Caterpillars on the Foliage of Conifers in the Northeastern United States (Revised).  
362 USDA FHTET-2011-07:1-153
- 363 **Mason, C.J., C. Villari, K. Keefover-Ring, S. Jagemann, J. Zhu, P. Bonello, K. F. Raffa.**  
364 **2017.** Spatial and temporal components of induced plant responses in the context of  
365 herbivore life history and impact on host. *Funct. Ecol.* 31: 2034-2050.
- 366 **Naidoo, R., L. Ferreira, D. K. Berger, A. A. Myburg, and S. Naidoo. 2013.** The identification  
367 and differential expression of *Eucalyptus grandis* pathogenesis-related genes in response  
368 to salicylic acid and methyl jasmonate. *Front. Plant Sci.* 4: 43.
- 369 **Orians, C. 2005.** Herbivores, vascular pathways, and systemic induction: Facts and artifacts. *J.*  
370 *Chem. Ecol.* 31: 2231-2242.
- 371 **Raffa, K. F., C. J. Mason, P. Bonello, S. Cook, N. Erbilgin, K. Keefover-Ring, J.G. Klutsch,**  
372 **C. Villari, and P. A. Townsend. 2017.** Defence syndromes in lodgepole–whitebark pine

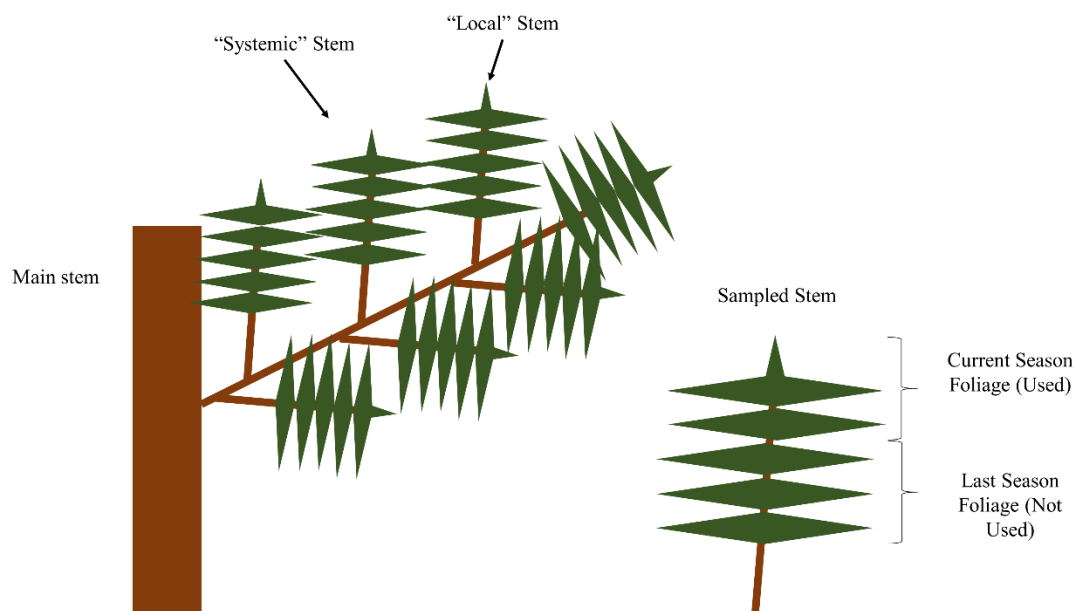
- 373 ecosystems relate to degree of historical exposure to mountain pine beetles. *Plant Cell*  
374 *Environ.* 40: 1791-1806.
- 375 **Reynolds, G. J., T. R. Gordon, and N. McRoberts. 2019.** Using game theory to understand  
376 systemic acquired resistance as a bet-hedging option for increasing fitness when disease  
377 is uncertain. *Plants.* 8: 219.
- 378 **Rigsby, C. M., D. A. Herms, P. Bonello, and D. Cipollini. 2016.** Higher defense-associated  
379 enzymes may contribute to greater resistance of Manchurian ash to emerald ash borer  
380 than a closely related and susceptible congener. *J. Chem. Ecol.* 42: 782-797.
- 381 **Rigsby, C. M., C. Villari, D. L. Peterson, D. A. Herms, P. Bonello, and D. Cipollini. 2018.**  
382 Girdling increases survival and growth of emerald ash borer larvae on Manchurian ash.  
383 *Agr. For. Entomol.* 21: 130-135.
- 384 **Rigsby, C. M., E. E. Shoemaker, M. M. Mallinger, C. M. Orians, and E. L. Preisser. 2019.**  
385 Conifer responses to a stylet-feeding invasive herbivore and induction with methyl  
386 jasmonate: Impact on the expression of induced defences and a native folivore. *Agr. For.*  
387 *Entomol.* 21: 227-234.
- 388 **Rigsby, C. M., M. J. A. Body, A. May, A. Oppong, A. Kostka, N. Houseman, S. Savage, E.**  
389 **R. Whitney, I. G. Kinahan, B. DeBoef, C. M. Orians, H. A. Appel, J. C. Schultz, and**  
390 **E. L. Preisser. *In Review.*** Impact of chronic stylet-feeder infestation on folivore-induced  
391 signaling and defenses. *Plant Cell Environ.*
- 392 **War, A. R., M. G. Paulraj, T. Ahmad, A. A. Buhroo, B. Hussain, S. Ignacimuthu, and H. C.**  
393 **Sharma. 2012.** Mechanisms of plant defense against insect herbivores. *Plant Sig. Behav.*  
394 7: 1306-1320.

395 **Wasternack, C., and B. Hause. 2013.** Jasmonates: biosynthesis, perception, signal transduction  
396 and action in plant stress response, growth and development. An update to the 2007  
397 review in *Annals of Botany*. *Ann. Bot.* 111: 1021-1058.

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399 **Figures**

400 **Fig. 1.** Positioning of “Local” and “Systemic” stems used in Experiment 2. Local stems directly  
401 received either 10 mM MeJA in 0.1% (v:v) Tween-20 or control solution (0.1% Tween-20) and  
402 Systemic stems received no treatment.



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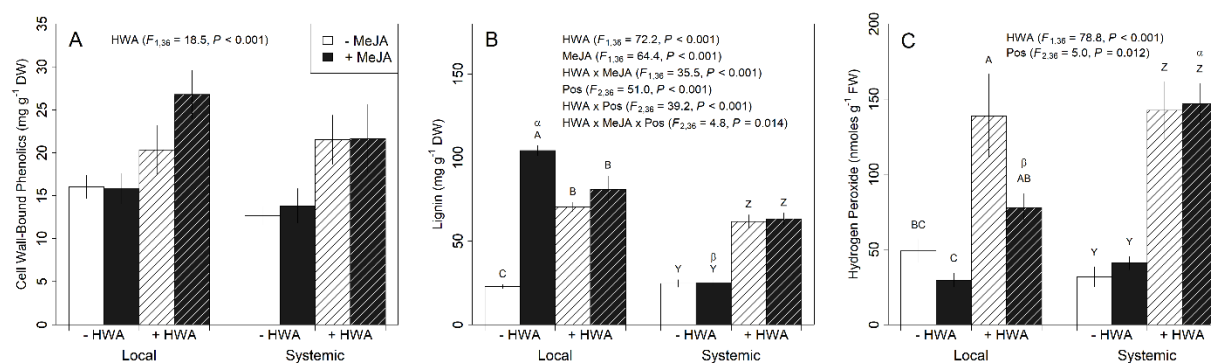
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410 **Fig. 2.** Mean ( $\pm$  SEM) cell wall-bound phenolics (A), lignin (B), and hydrogen peroxide (C)  
 411 contents of Local (left set of four bars) and Systemic (right set of four bars) foliage infested with  
 412 hemlock woolly adelgid (+ HWA, hatched right two bars) or not (- HWA, unhatched left two  
 413 bars) and/or treated with methyl jasmonate (+ MeJA, black bars) or not (- MeJA, white bars).  
 414 Significant treatment and interaction effects are listed for each response. Different uppercase  
 415 letters indicate significant differences within foliage position and different lowercase Greek  
 416 letters indicate significant differences between foliar positions within a treatment combination.  
 417 For cell wall-bound phenolics (A), MeJA ( $F_{1,36} = 1.0$ ;  $P = 0.317$ ), HWA x MeJA ( $F_{1,36} = 0.6$ ;  $P =$   
 418  $0.453$ ), stem position ( $F_{2,36} = 1.1$ ;  $P = 0.336$ ), HWA x stem position ( $F_{2,36} = 0.0$ ;  $P = 0.971$ ),  
 419 MeJA x stem position ( $F_{2,36} = 0.2$ ;  $P = 0.836$ ), and HWA x MeJA x stem position ( $F_{2,36} = 0.3$ ;  $P =$   
 420  $0.726$ ) were all not significant predictors. For lignin (B), only MeJA x stem position ( $F_{2,36} =$   
 421  $0.8$ ;  $P = 0.455$ ) was not a significant predictor. For hydrogen peroxide (C), MeJA ( $F_{1,36} = 2.8$ ;  $P =$   
 422  $0.103$ ), HWA x MeJA ( $F_{1,36} = 1.4$ ;  $P = 0.252$ ), HWA x stem position ( $F_{2,36} = 1.7$ ;  $P = 0.193$ ),  
 423 MeJA x stem position ( $F_{2,36} = 1.0$ ;  $P = 0.377$ ), and HWA x MeJA x stem position ( $F_{2,36} = 0.2$ ;  $P =$   
 424  $0.805$ ) were all not significant predictors.

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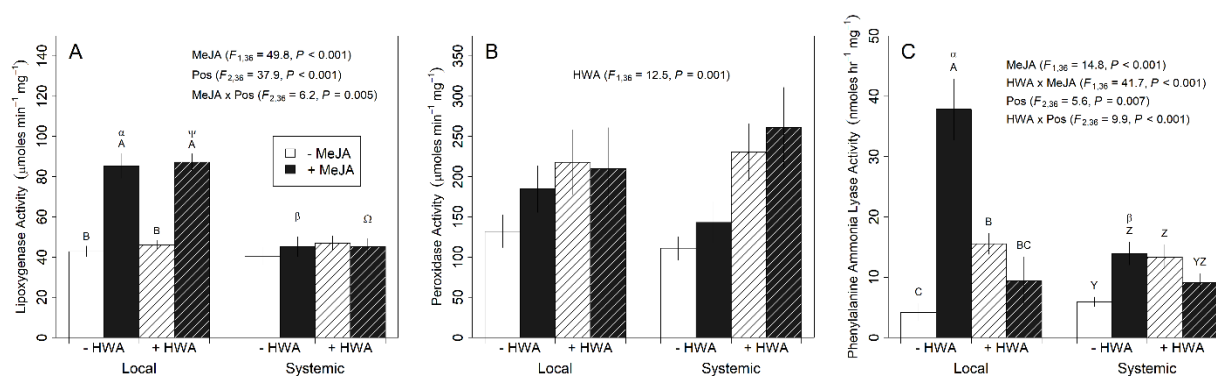


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428 **Fig. 3.** Mean ( $\pm$  SEM) lipoxygenase (A), peroxidase (B), and phenylalanine ammonia lyase (C)  
 429 activities of Local (left set of four bars) and Systemic (right set of four bars) foliage infested with  
 430 hemlock woolly adelgid (+ HWA, hatched right two bars) or not (- HWA, unhatched left two  
 431 bars) and/or treated with methyl jasmonate (+ MeJA, black bars) or not (- MeJA, white bars).  
 432 Significant treatment and interaction effects are listed for each response. Different uppercase  
 433 letters indicate significant differences within foliage position and different lowercase Greek  
 434 letters indicate significant differences between foliar positions within a treatment combination.  
 435 For lipoxygenase activity (A), HWA ( $F_{1,36} = 0.9$ ;  $P = 0.357$ ), HWA x MeJA ( $F_{1,36} = 0.4$ ;  $P =$   
 436  $0.526$ ), HWA x stem position ( $F_{2,36} = 1.5$ ;  $P = 0.227$ ), and HWA x MeJA x stem position ( $F_{2,36} =$   
 437  $0.1$ ;  $P = 0.915$ ) were all not significant predictors. For peroxidase activity (B), MeJA ( $F_{1,36} = 1.2$ ;  
 438  $P = 0.282$ ), HWA x MeJA ( $F_{1,36} = 0.4$ ;  $P = 0.535$ ), stem position ( $F_{2,36} = 0.2$ ;  $P = 0.785$ ), HWA  
 439 x stem position ( $F_{2,36} = 1.1$ ;  $P = 0.334$ ), MeJA x stem position ( $F_{2,36} = 0.8$ ;  $P = 0.473$ ), and HWA  
 440 x MeJA x stem position ( $F_{2,36} = 1.4$ ;  $P = 0.253$ ) were all not significant predictors. For  
 441 phenylalanine ammonia lyase activity (C), HWA ( $F_{1,36} = 3.1$ ;  $P = 0.088$ ), MeJA x stem position  
 442 ( $F_{2,36} = 0.0$ ;  $P = 0.966$ ), and HWA x MeJA x stem position ( $F_{2,36} = 1.9$ ;  $P = 0.162$ ) were all not  
 443 significant predictors.

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