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

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

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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.

37

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 (H₂O₂), 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⁻¹ 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 H₂SO₄
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 (H₂O₂) 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 NaPO₄, 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 (OD₅₇₅ mg⁻¹) and linoleic acid (μmoles min⁻¹ mg⁻¹), respectively, as substrates. Peroxidase
164 (POX) activity was quantified according to Rigsby et al. (2018) using guaiacol and H₂O₂ as
165 substrates (μmoles min⁻¹ mg⁻¹). 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⁻¹ mg⁻¹). 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 H₂O₂ 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 H₂O₂ 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 H₂O₂ 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+), H₂O₂
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 H₂O₂, 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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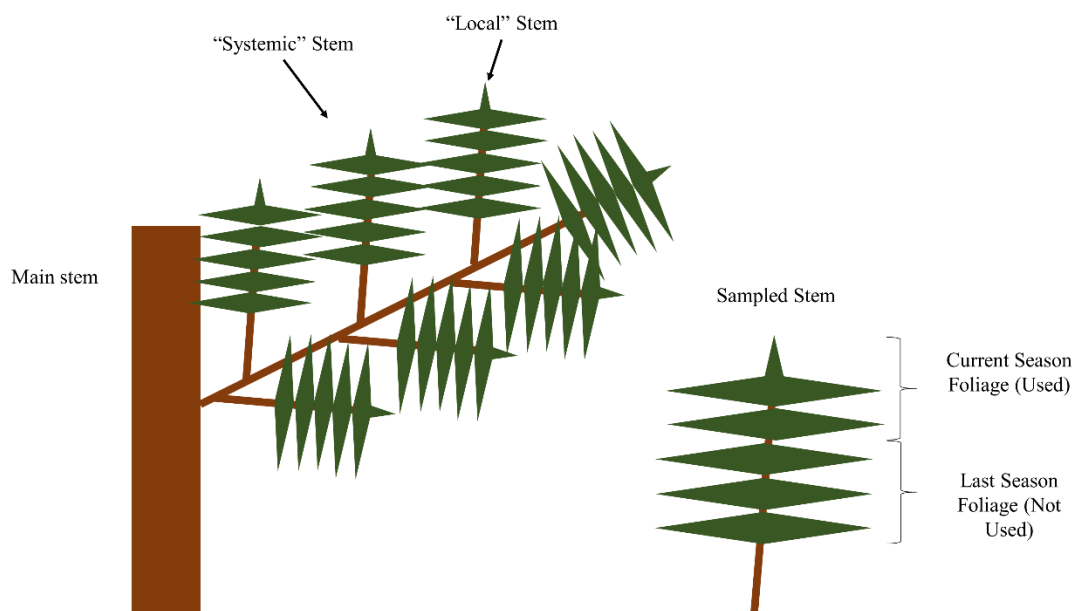
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399 **Figures**

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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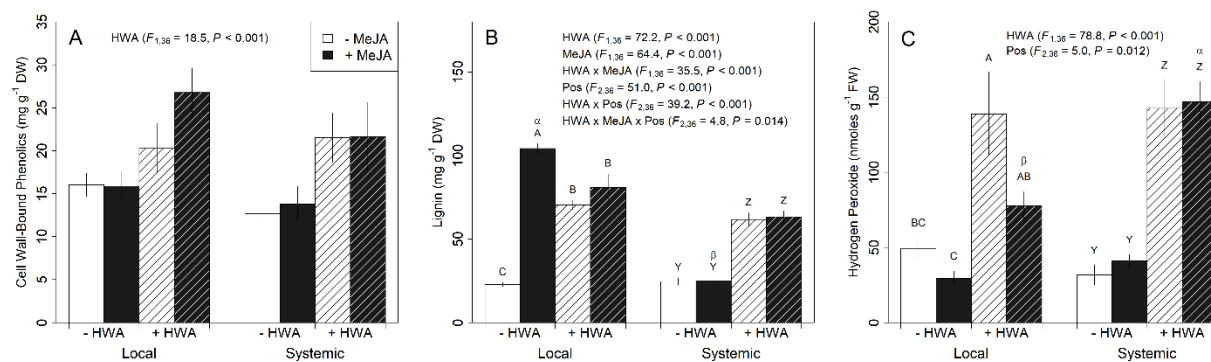
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410 **Fig. 2.** Mean (\pm SEM) cell wall-bound phenolics (A), lignin (B), and hydrogen peroxide (C)
 411 contents of Local (left set of four bars) and Systemic (right set of four bars) foliage infested with
 412 hemlock woolly adelgid (+ HWA, hatched 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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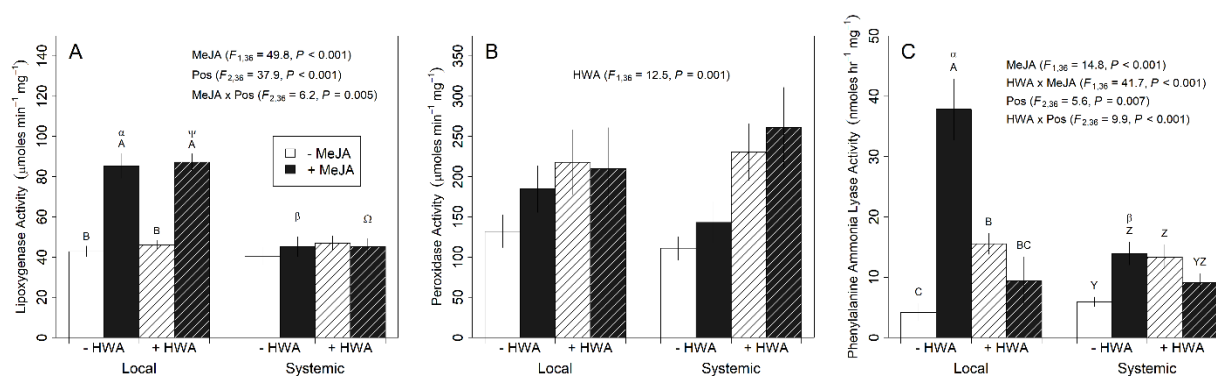


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428 **Fig. 3.** Mean (\pm SEM) lipoxygenase (A), peroxidase (B), and phenylalanine ammonia lyase (C)
 429 activities of Local (left set of four bars) and Systemic (right set of four bars) foliage infested with
 430 hemlock woolly adelgid (+ HWA, hatched 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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