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Research

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Plant-Insect Interactions

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

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Abstract

Hemlock woolly adelgid is an invasive piercing-sucking insect in eastern North America, which upon infestation of its main host, eastern hemlock ('hemlock'), improves attraction and performance of folivorous insects on hemlock. This increased performance may be mediated by hemlock woolly adelgid feeding causing antagonism between the the jasmonic acid and other hormone pathways. In a common garden experiments using hemlock woolly adelgid infestation and induction with methyl jasmonate (MeJA) and measures of secondary metabolite contents and defense-associated enzyme activities, we explored the impact of hemlock woolly adelgid feeding on the local and systemic induction of jasmonic acid (JA)-elicited defenses. We found that in local tissue hemlock woolly adelgid or MeJA exposure resulted in unique induced phenotypes, whereas the combined treatment resulted in an induced phenotype that was a mixture of the two individual treatments. We also found that if the plant was infested with hemlock woolly adelgid, the systemic response of the plant was dominated by hemlock woolly adelgid, regardless of whether MeJA was applied. Interestingly, in the absence of hemlock woolly adelgid, hemlock plants had a very weak systemic response to MeJA. We conclude that hemlock woolly adelgid infestation prevents systemic induction of JA-elicited defenses. Taken together, compromised local JA-elicited defenses combined with weak systemic induction could be major contributors to increased folivore performance on hemlock woolly adelgid-infested hemlock.

Key words: induced defenses, systemic induction, invasive forest pest, herbivory, conifers

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

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

Hemlock woolly adelgid (*Adelges tsugae* Annand) is a sessile, stylet-feeding insect that is invasive to eastern North America. It has caused mass mortality of eastern hemlock ('hemlock'; *Tsuga canadensis* L.) within its invaded range. Chronic hemlock woolly adelgid infestation causes a 'hypersensitive-like' response in hemlock that is characterized by the accumulation of SA, hydrogen peroxide (H_2O_2), and proline and increases in methyl salicylate (MeSA) emissions (Radville et al. 2011, Gómez et al. 2012, Pezet et al. 2013, Pezet and Elkinton 2014, Schaeffer et al. 2018, Rigsby et al. 2019). The nature of this response led to the hypothesis that hemlock woolly adelgid infestation would increase host quality for JA-eliciting herbivores by decreasing the induction of JA-linked plant defenses. Consistent with this scenario, Wilson et al. (2016) reported increased performance of hemlock looper, *Lambdina fiscellaria* Guenée (Lepidoptera: Geometridae), on hemlock woolly adelgid-infested hemlock, and Kinahan et al. (2019) found increased gypsy moth, *Lymantria dispar* L. (Lepidoptera: Erebidae), larval preference for and performance on hemlock woolly adelgid-infested hemlocks in both field and laboratory settings.

Although the latter two studies are consistent with the hypothesis that hemlock woolly adelgid-mediated increases in SA disrupt JA-based plant defense, this linkage has not been experimentally confirmed. Although changes in the inducibility of JA-elicited defenses may be involved, SA- and JA-elicited defense responses are remarkably similar in hemlock (Rigsby et al. 2019). In an experiment that used hemlock woolly adelgid and gypsy moth larvae to directly induce SA- and JA-elicited responses, Rigsby et al. (unpublished data) found that both hemlock woolly adelgid and gypsy moth increased foliar SA levels; simultaneous herbivory by both insects had an additive effect. Gypsy moth herbivory resulted in accumulation of JA and JA-Ile, the active form of JA, whereas hemlock woolly adelgid inhibited the ability of gypsy moths to elicit JA accumulation (Rigsby et al. unpublished data). These findings support the hypothesis that hemlock woolly adelgid infestation prevents hemlock from accumulating JA phytohormones in response to JA-eliciting herbivores. Intriguingly, however, hemlock woolly adelgid infestation also increased accumulation of several bioactive gibberellins (GAs), hormones known to play a critical role in plant growth (i.e., stem elongation and leaf expansion; Davière and Achard 2013). This hemlock woolly adelgid-elicited GA accumulation is notable because GAs are also known to antagonize JA signaling (de Lucas et al. 2008). This result suggests that JA accumulation and the elicitation of JA-linked defenses could be compromised by one or both of these mechanisms.

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

Materials and Methods

Hemlock Common Garden, Treatments, and Sampling

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

Twelve trees from each of the two treatments (hemlock woolly adelgid-infested, uninfested controls) were selected so that each treatment was represented by at least three trees in each of four spatial blocks; trees from the fifth spatial block were excluded because this block was much shadier than the other four. A single branch was selected on each tree; all sampled branches were of similar length and diameter (ANOVA; $P > 0.05$ for all) and the branches from hemlock woolly adelgid-infested trees had moderate, but roughly equivalent hemlock woolly adelgid densities (0.5–1 hemlock woolly adelgid cm^{-1} stem). Each branch was marked by placing flagging placed at its base (Fig. 1). Twice weekly for a 2-wk period (28 August–7 September 2017), an elicitor solution containing 10 mM MeJA in a carrier solution of 0.1% (v:v) Tween-20 (MeJA treatment) or carrier solution only (control treatment) was carefully applied using a fine-tipped paint brush so that MeJA solution did not run off, to the first lateral stem proximal to the terminal stem, near the flagging. All treated branches were harvested on 11 September, placed in aluminum foil, and stored at −80°C. In order to understand how hemlock woolly adelgid affects systemic defense signaling, we harvested a stem immediately proximal (denoted as 'Systemic' stem) to the treated stem (denoted as 'Local' stem; Fig. 1). This resulted in four treatment combinations (hemlock woolly adelgid +/− and MeJA +/−; $n = 6$ biological replicates per treatment combination; 24 total), with two location categories per branch: 'Local' and 'Systemic' stems (48 total samples; Fig. 1). Lastly, in order to eliminate additional sources of variation, only foliage produced in the current growing season (i.e., newly produced foliage) was used in this study, foliage that was produced prior to the season of our experiment was not used in this study (Fig. 1).

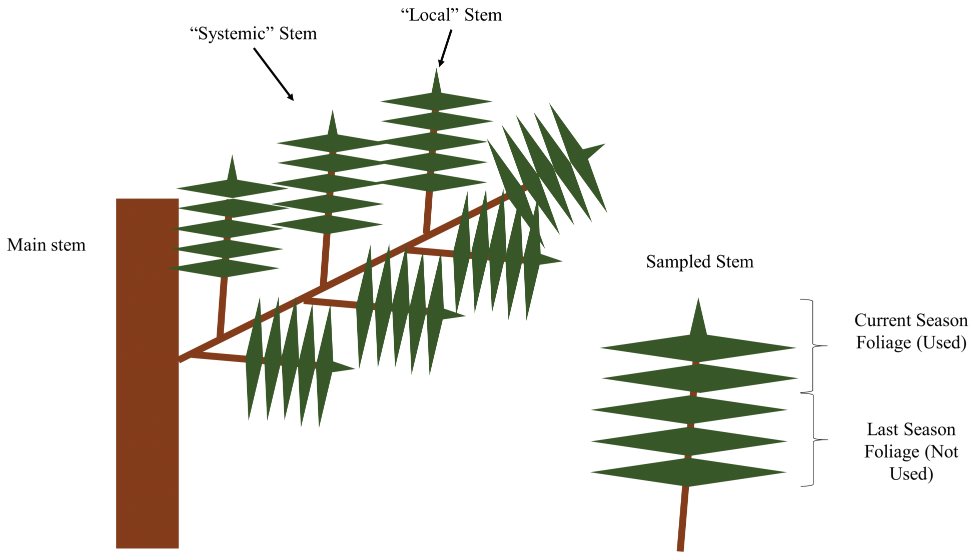

Fig. 1. Positioning of 'Local' and 'Systemic' stems used in experiment 2. Local stems directly received either 10 mM MeJA in 0.1% (v:v) Tween-20 or control solution (0.1% Tween-20) and Systemic stems received no treatment.

Chemical and Physiological Analyses

Crude levels of chemical defenses were quantified as described in Rigsby et al. (2019); any deviations from these protocols are detailed below. Briefly, needles were ground into a powder in liquid nitrogen using a mortar and pestle and 100 mg were placed in a 2-ml microtube. Tissue was twice-extracted in 0.5 ml HPLC-grade methanol. Following centrifugation at 16,000 *g* (10 min, 4°C), the supernatants were combined. Methanol-soluble terpene content was quantified immediately using chloroform and H_2SO_4 (Rigsby et al. 2019) with linalool as the standard. Soluble phenolic content was quantified via the Folin–Ciocalteu method using chlorogenic acid as standard; proanthocyanidin content was quantified using the acidified butanol method (Rigsby et al. 2019). Chlorogenic acid was used as a standard for the quantification of soluble phenolics because prior research found that chlorogenic acid dominates the soluble phenolic profile of hemlock foliage (Rigsby et al. unpublished data). The cell wall-bound phenolic (CW-bound phenolic) and lignin contents were determined as per Rigsby et al. (2019) using gallic acid and spruce lignin, respectively, as the standard. Hydrogen peroxide (H_2O_2) was quantified according to the KI method (Junglee et al. 2014, Rigsby et al. 2019).

For enzyme activity assays, 200-mg needle powder was extracted on ice in five volumes of extraction buffer (50 mM $NaPO_4$, pH 6.8, 10% PVPP, 5% Amberlite XAD4 resin, and 1 mM EDTA) and the 10,000 *g* supernatant was used as the source of enzymes. Chitinase (CHI) and lipoxygenase (LOX) activities were quantified according to Rigsby et al. (2016) using chitin azure (OD_{575} mg^{-1}) and linoleic acid (μmoles min^{-1} mg^{-1}), respectively, as substrates. Peroxidase (POX) activity was quantified according to Rigsby et al. (2018) using guaiacol and H_2O_2 as substrates (μmoles min^{-1} mg^{-1}). Phenylalanine ammonia lyase (PAL) activity was quantified by monitoring the conversion of L-phenylalanine to *trans*-cinnamic *acid (*Chen et al. 2006; nmoles h^{-1} mg^{-1}). To express enzyme activities per unit protein, the protein content of extracts was determined using the Bradford (1976) method with bovine serum albumin as standard. During preliminary experiments, we attempted to detect polyphenol oxidase activity using multiple substrates, as well as trypsin inhibitor activity, but were unable to do so.

Statistical Analyses

The effect of hemlock woolly adelgid, MeJA, branch position (i.e., systemic induction), and their interactions on relative metabolite levels and enzyme activities was assessed using an ANOVA with stem position nested within tree identity. An ANCOVA was initially used with block as a covariate; because block was never significant, we proceeded with ANOVAs. We were interested in detecting 1) within-treatment differences in foliar position (i.e., 'Local' vs. 'Systemic' within a single treatment combination) and 2) between-treatment differences for a given foliar position (i.e., 'hemlock woolly adelgid–/MeJA–' vs 'hemlock woolly adelgid+/MeJA–' vs 'hemlock woolly adelgid–/MeJA+' vs 'hemlock woolly adelgid+/MeJA+' within a single sampling position). For posthoc comparisons of within-treatment differences between sampling positions, we used *t*-tests to directly compare Local and Systemic foliage. For posthoc comparisons of treatment combinations within a sampling position, we first performed *t*-tests comparing all combinations of interest, then the resulting *P*-values were adjusted via the Benjamini–Hochberg procedure (Benjamini and Hochberg 1995). For example, if comparing all four treatment combinations of 'Local' foliage, the six calculated *P*-values were included in the Benjamini–Hochberg correction. Because different sampling positions from different treatments were not of interest (e.g., 'Local' foliage from 'hemlock woolly adelgid+/MeJA–' vs 'Systemic' foliage from 'hemlock woolly adelgid–/MeJA–'), these comparisons were not made. These posthoc procedures were only used if significant interactions between treatments/sampling locations were detected. All statistical analyses were performed in R (R Development Core Team 2020).

Results

Secondary Metabolites

For both Local and Systemic foliage, CW-bound phenolics, lignin, and H_2O_2 all had increased tissue levels as a result of hemlock woolly adelgid infestation relative to uninfested controls (Fig. 2A–C, respectively). The application of MeJA had no effect on CW-bound phenolic or H_2O_2 contents in either Local or Systemic foliage (Fig. 2A and C, respectively), but did cause lignin to accumulate in Local foliage in the absence of hemlock woolly adelgid. However, this lignin accumulation was attenuated in the presence of hemlock woolly adelgid in Local foliage (Fig. 2B). Foliage position (i.e., 'Local' vs 'Systemic' foliage) had a significant effect on lignin and H_2O_2 contents. In the absence of hemlock woolly adelgid, MeJA application (hemlock woolly adelgid–/MeJA+) significantly increased lignin content in Local foliage but not in adjacent Systemic foliage (Fig. 2B). Additionally, in the presence of hemlock woolly adelgid and when MeJA was applied (hemlock woolly adelgid+/MeJA+), H_2O_2 content was significantly greater in Systemic than in Local foliage (Fig. 2C). There were no elicitor treatment or sampling location effects for soluble phenolics (71.70 ± 1.05 mg g^{-1} DW), proanthocyanidins (33.54 ± 1.32 OD550 g^{-1} DW), or methanol-soluble terpenes (14.05 ± 0.15 mg g^{-1} DW).

Defensive Enzyme Activities

In both Local and Systemic foliage, hemlock woolly adelgid infestation increased POX activity (Fig. 3A), whereas the application of MeJA increased LOX and PAL activities in Local foliage, only (Fig. 3B and C, respectively). Interestingly, infestation by hemlock woolly adelgid had no effect on the MeJA-elicited increase in LOX activity in Local foliage (i.e., the increase in LOX activity caused by MeJA application was not attenuated by the presence of hemlock woolly adelgid in Local foliage). However, this was the case for PAL activity, as hemlock woolly adelgid infestation severely inhibited the MeJA-elicited increase in PAL activity in Local foliage (Fig. 3C). Foliage position (i.e., 'Local' vs 'Systemic' foliage) had a

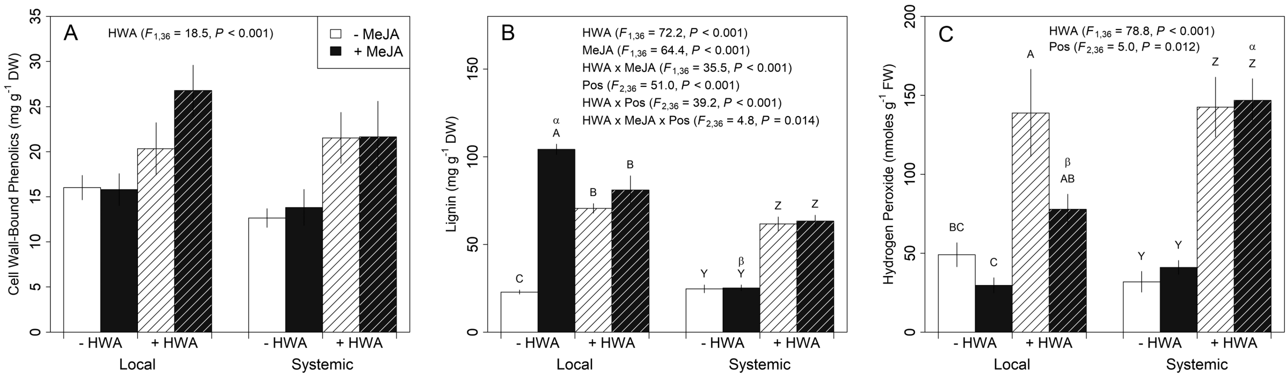

Fig. 2. Mean (± SEM) cell wall-bound phenolics (A), lignin (B), and hydrogen peroxide (C) contents of Local (left set of four bars) and Systemic (right set of four bars) foliage infested with hemlock woolly adelgid (+hemlock woolly adelgid, hatched right two bars) or not (–hemlock woolly adelgid, unhatched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

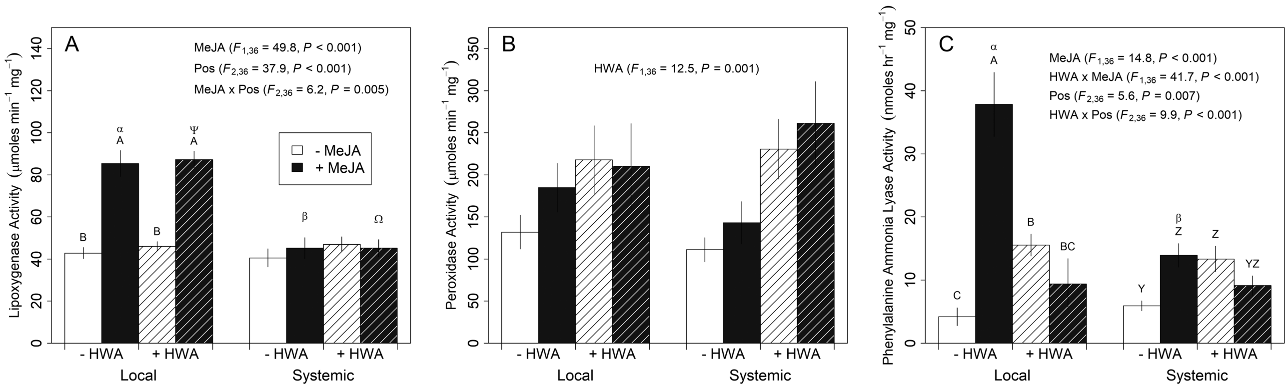

Fig. 3. Mean (± SEM) lipoxygenase (A), peroxidase (B), and phenylalanine ammonia lyase (C) activities of Local (left set of four bars) and Systemic (right set of four bars) foliage infested with hemlock woolly adelgid (+hemlock woolly adelgid, hatched right two bars) or not (–hemlock woolly adelgid,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

significant effect on both LOX and PAL activities. As with lignin content, the increase in LOX and PAL activities that were found in Local foliage in the absence of hemlock woolly adelgid and with MeJA application (hemlock woolly adelgid−/MeJA+) did not occur in Systemic foliage (Fig. 3B and C). This was also the case for LOX activity in the presence of hemlock woolly adelgid and with MeJA application (hemlock woolly adelgid+/MeJA+), where MeJA application resulted in increased activity in Local but not in Systemic foliage (Fig. 3A). There were no elicitor treatment or sampling location effects for CHI activity (0.31 ± 0.01 OD575 mg^{-1}).

Discussion

The systemic induction of defenses is considered an important bet-hedging strategy for plants to minimize fitness costs (Reynolds et al. 2019), and systemic induction is viewed as an adaptive response against herbivores that impose chronic injury, continually increase populations on individual plants, and/or can move among plant parts (Mason et al. 2017). Like many woody plants, an abundance of folivorous insects utilize hemlock as a host resource, including a variety of leafminers, loopers, leafrollers, budworms, needleworms, tussock moths, cutworms, and others (Maier et al. 2011). Recent research has shown that hemlock woolly adelgid infestation increases the attraction to and performance of folivorous insects on hemlock (Wilson et al. 2016, Kinahan et al. 2019, Rigsby et al. 2019), and this increase in folivore performance may be facilitated by the compromising of JA-elicited defenses locally at the site of folivore attack (Rigsby et al. 2019; unpublished data). This study sought to investigate the impact of hemlock woolly adelgid infestation on the induction of systemic, JA-elicited defenses. We hypothesized that 1) the hemlock woolly adelgid-instigated attenuation of local JA induction would be accompanied by a complete lack of systemic responses, and that 2) systemic responses would occur on hemlock woolly adelgid-free plants.

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

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

Interestingly, we did not detect local or systemic accumulation of soluble phenolics, including proanthocyanidins and methanol-soluble terpenes. These classes of secondary metabolites are known to be critically important anti-herbivore defenses in conifers (Raffa et al. 2017). Previous research showed significant, positive effects of both hemlock woolly adelgid infestation and MeJA application on soluble phenolic content, including proanthocyanidins (Rigsby et al. 2019). Similar levels of CHI activity across all treatment combinations were also unanticipated, since previous research found that the activity of this enzyme was strongly enhanced by hemlock woolly adelgid infestation and MeJA application (Rigsby et al. 2019). In agreement with this previous research, we detected accumulation of CW-bound phenolics and H_2O_2, and increases in POX activity in response to hemlock woolly adelgid infestation, and a strong positive effect of MeJA application on LOX activity (Rigsby et al. 2019). One difference between these two experiments is that Rigsby et al. (2019) used potted hemlocks in full sun while this study used hemlocks planted in the understory of a mixed hardwood stand. It may be that some aspect(s) of these environmental differences had some effect on hemlock response to our treatments. In addition to normal growth, GAs are also associated with shade-avoidance and growth, and JA pathways interact directly and antagonistically through DELLA-JAZ interactions (Wasternack and Hause 2013, Davière and Achard 2016), and shaded plants are often unable to activate JA-elicited responses (Cipollini 2004). Hemlock woolly adelgid has a positive effect on a few major GAs (Rigsby et al. unpublished data), and the addition of shade may further increase gibberellin accumulation and antagonization of the JA pathway. An additive or synergistic effect between hemlock woolly adelgid infestation and shade on the inducibility of JA-elicited defenses would have major impacts on hemlock herbivore interactions, including between hemlock and hemlock woolly adelgid, itself. It has been noted by many researchers and practitioners that hemlock woolly adelgid appears to perform substantially better on its host when hemlock is shaded (Hickin and Preisser 2015).

The systemic induction of defenses is thought to be an important strategy of plants to reduce fitness costs (Kant et al. 2015, Reynolds et al. 2019), especially against herbivores that can move between plant tissues (Mason et al. 2017). Field observations and laboratory assays have shown dramatic increases in host quality and attraction to these kinds of herbivores (Wilson et al. 2016, Kinahan et al. 2019, Rigsby et al. 2019). In this study, we found that host responses to hemlock woolly adelgid infestation essentially overwhelm and prevent JA-elicited systemic defense expression, but we also detected very little JA-elicited systemic responses in hemlock in the absence of hemlock woolly adelgid. We conclude that in the absence of hemlock woolly adelgid, some JA-associated signal may be translocated and systemically perceived, as evidenced by significantly elevated PAL activity. Environmental conditions of our experiment may have played a role in this lack of response, however, hemlock often exists in the environment in dense, shaded conditions (Hadley 2000), still allowing our results to be ecologically meaningful. Future research should explore the role of shade on local and systemic SA- and JA-elicited responses in hemlock.

Acknowledgments

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