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Feeding by Hessian Fly (*Mayetiola destructor* [Say]) Larvae on Wheat Increases Levels of Fatty Acids and Indole-3-Acetic Acid but not Hormones Involved in Plant-Defense Signaling

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Abstract Gall-inducing insects exert a unique level of control over the physiology of their host plants. This control can extend to host–plant defenses so that some, if not most, gall-inducing species appear to avoid or modify host plant defenses to effect production of their gall. Included among gall insects is Hessian fly (*Mayetiola destructor* [Say], Diptera: Cecidomyiidae), a damaging pest of wheat (*Triticum aestivum* L.) and an emerging model system for studying plant–insect interactions. We studied the dynamics of some defense-related phytohormones and associated fatty acids during feeding of first instar Hessian fly larvae on a susceptible variety of wheat. We found that Hessian fly larvae significantly elevated in their host plants' levels of linolenic and linoleic acids, fatty acids that may be nutritionally beneficial. Hessian fly larvae also elevated levels of indole-3-acetic acid (IAA), a phytohormone hypothesized to be involved in gall formation, but not the defense-related hormones jasmonic (JA) and salicylic acids. Moreover, we detected in Hessian fly-infested plants a significant negative relationship between IAA and JA that was not present in control plants. Our results suggest that Hessian fly larvae may induce nutritionally beneficial changes while concomitantly altering phytohormone levels, possibly to facilitate plant-defense avoidance.

Keywords Auxin · Herbivory-induced responses · Jasmonic acid · Octadecanoid pathway · *Triticum aestivum* L.

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Introduction

Feeding by insect herbivores usually triggers a suite of defensive responses from host plants, including biosynthesis of plant secondary metabolites and defense-related proteins such as proteinase inhibitors and polyphenol oxidases (Karban and Baldwin 1997; Walling 2000). These inducible defenses may have evolved in response to variability in threats from herbivores in space and time because they are not as energetically demanding as constitutively expressed defenses (Karban and Baldwin 1997; van Hulten and others 2006). Inherent in inducible defenses is a signaling mechanism that can initiate appropriate defense responses following damage. Various hormones such as jasmonic (JA), salicylic (SA), and abscisic (ABA) acids participate in this signaling role for plants and trigger downstream defensive cascades that upregulate defensive genes (Davies 2004).

In recent years, defense-related plant hormones (particularly JA and SA) and their dynamics following herbivory have received substantial research attention and, as a result, a clearer understanding of their role in plants is developing (Howe and Jander 2008; Koo and Howe 2009). One tactic for understanding plant defenses and associated roles of phytohormones is to study plant–insect interactions where typical defensive cascades do not appear to occur (Sardesai and others 2005; Liu and others 2007; Tooker and De Moraes 2007, 2009; Tooker and others 2008). Studying such apparently aberrant systems in a comparative framework provides insight into coevolutionary arms races and how some herbivores cope with plant defenses.

One such group of herbivores that appears capable of avoiding typical plant defensive responses is gall-inducing insects. These species usually force their host plants to produce tumor-like growths that provide food and shelter at

the plants' expense. Gall inducers have unique influences over host-plant physiology, including distribution of plant secondary metabolites (Allison and Schultz 2005; Hartley 1998; Nyman and Julkunen-Tiitto 2000; Tooker and De Moraes 2007, 2008, 2009; Tooker and Hanks 2004; Tooker and others 2002, 2008). Some gall insects appear to avoid inducing production of phytohormones typically triggered by more generalized feeders, suggesting that the feeding strategy of at least some gall insects may not be recognized by the host plant species or possibly that the gall inducer is able to suppress typical signaling events (Sardesai and others 2005; Liu and others 2007; Tooker and De Moraes 2007, 2009; Tooker and others 2008).

Included among gall-inducing species is Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), the most damaging pest of wheat worldwide and a gall-midge species that has become a model herbivore species for understanding plant–insect interactions. In the United States, Hessian fly causes 5–10% yield loss per year and economic damages in the millions of dollars despite the development of wheat lines resistant to Hessian fly feeding (Buntin 1999; Smiley and others 2004). Hessian fly does not induce a three-dimensional covering gall like most gallers, but it is considered a gall inducer because of similarities between the nutritive tissue at their feeding sites and those inside macroscopic galls (Harris and others 2003, 2006). Feeding by Hessian fly larvae is not well understood, but larvae feed near meristematic regions of host plants, injecting saliva and feeding upon predigested plant solutes (Stuart and Hatchett 1987; Harris and others 2003, 2006). Larvae appear to cause little mechanical damage to host plants, with their specialized mouthparts making only two small holes (Hatchett and others 1990; Harris and others 2006). Nevertheless, host plants become permanently and irreversibly stunted after 4 days of feeding by a single first-instar larva (Byers and Gallun 1972). Even if larvae are removed, evidence of stunting becomes apparent 10 days after feeding began, suggesting that larvae inject substances or effectors into host plant tissues that drastically alter plant physiology (Byers and Gallun 1972; Stuart and Hatchett 1987). Feeding by Hessian fly larvae on susceptible varieties of wheat does not trigger typical defensive responses (Sardesai and others 2005; Liu and others 2007; Tooker and De Moraes 2007). This lack of induction may be due in part to the minimal physical damage caused by larval feeding, but larval salivary components may also play a role (Stuart and Hatchett 1987). Larvae inject into their host plants various substances, resulting in a state of enhanced nutrition and reduced exposure to plant defenses that has been termed "induced susceptibility" (Liu and others 2007).

Gall-inducing species have been hypothesized to influence host-plant physiology by manipulating levels of phytohormones (Raman and others 2005), but for Hessian fly very little work appears to have addressed biochemical changes induced by feeding larvae (Harris and others 2003). In this article we investigate phytohormone dynamics of susceptible wheat during attack by first-instar Hessian fly larvae. We conducted a 7-day time-course experiment to understand how levels of phytohormones, and some of their fatty-acid precursors, change in response to Hessian fly feeding. We also include an evaluation of the response of wheat to the polyphagous caterpillar species *Heliothis virescens*, which elicits strong defensive responses from wheat (Tooker and De Moraes 2007). This experiment was included as a positive control to confirm the defensive response of wheat and determine how levels of compounds change when wheat is attacked by a generalist herbivore. Moreover, damage by this lepidopteran species provides a comparative framework in which we can interpret phytohormone changes induced by Hessian fly.

Materials and Methods

Plants and Insects

Common wheat (*Triticum aestivum* L. cultivar Centennial) was grown from seeds in a climate-controlled growth chamber (16:8 L:D; 22:20°C Day:Night; 65% RH) that was insect-free except for a small population of fungus gnats (Sciaridae). Two seeds were planted in the middle of square-shaped pots (10 × 10 cm, 9 cm tall) in a peat-based, general-purpose potting soil (Pro-Mix BX, Premier Horticulture Inc., Quakertown, PA) and watered as needed. Plants were exposed to treatments (see below) when they reached the three-leaf stage (Zadoks scale 12; Zadoks and others 1974).

Hessian flies were obtained from a laboratory colony that was established in autumn 2005 with the help of N. Bosque-Pérez (University of Idaho, Moscow, ID). Individuals used to start the colony were derived from one established in 1998 comprising Hessian fly biotypes GP, E, F, and G (Cervantes and others 2002). The colony was kept in a climate-controlled growth chamber (conditions as above) and maintained on a cultivar of common wheat (Centennial) that is susceptible to feeding by larval Hessian flies. Under these conditions, eggs hatch 3 days following oviposition and larvae begin to feed by the next day, with the first instar lasting 5 days. First-instar larvae inject salivary secretions to effect extraoral digestion, but the second instars appear to only feed because their salivary glands begin to degenerate (Stuart and Hatchett 1987); therefore, our time course spans the first instar, which induces stunting, and the beginning of the second instar, which benefits

from actions of the first. The complete life cycle (eggs to adults) takes about 28 days.

To infest plants with Hessian fly larvae, individual pots (one or two plants per pot) were covered with inverted clear plastic cups (16 oz.; Solo Cup Company, Urbana, IL) modified with a 2-cm-diameter hole (filled with a foam stopper) to allow introduction and recovery of flies and three fine mesh 'windows' (~2.5 × 2.5 cm) to allow airflow. One or two mated females (one per plant) were introduced to each cup and allowed to oviposit for 30 min. Flies were observed to confirm that they exhibited oviposition behavior. Control plants were covered with cups for 30 min but not exposed to flies. To balance the large number of plants needed for our extended time course and limited growth chamber space, plants were infested over the course of a few months in batches of 14–28 pots.

Plants were harvested at 24-h intervals following infestation and time points were assigned randomly. We collected independent samples (that is, samples were not pooled) from each of five Hessian fly-infested and five control plants for each time point: 0 (oviposition), 24, 48, 72, 96, 120, 144, 168, 192, 216, and 240 (end of first instar) h. Individual control plants were always harvested at the same time as Hessian fly-infested plants of the same time point. If there were two plants in a pot, only one was harvested for tissue. Upon harvest, plants were quickly inspected for larvae, which averaged 15.4 ± 2.4 (SE) per plant. We harvested only the white crown tissue above the root–shoot junction (that is, the crown) where larvae feed (Fig. 1). Samples averaged 41.2 ± 2.5 (SE) mg of tissue. Samples were collected directly into FastPrep® tubes (Qbiogene, Carlsbad, CA) containing 1 g of Zirmil beads (1.1 mm; Saint-Gobain ZirPro, Le Pontet Cedex, France) and frozen at −80°C until processing (see below).

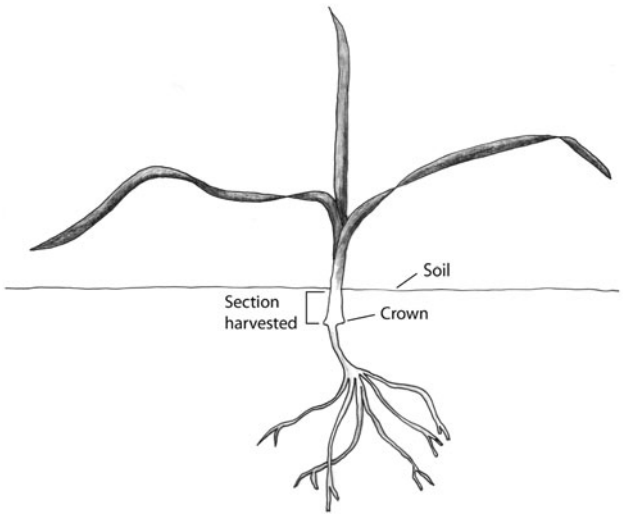

Fig. 1 Three-leaf stage of wheat showing the portion of the plant that was harvested for phytohormones analyses. This area from the soil surface to the crown is where Hessian fly larvae typically feed. Adapted from Zadoks and others (1974)

To determine the phytohormone dynamics of wheat in response to a generalist herbivore, Centennial wheat plants were also exposed to the generalist caterpillar *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae). *Heliothis virescens* does not regularly feed on wheat but will do so if starved, and so it can be used as a tool to elicit plant defensive responses (Tooker and De Moraes 2007). Moreover, the scenario established by allowing *H. virescens* to feed upon wheat should not be too different from a host plant reacting to a generalist caterpillar exploring its food options when preferred host plant species are not present. Caterpillars were reared from eggs in an incubator (16:8 L:D; 25:22°C, Day:Night; 65% RH) on an artificial casein-based diet. Third-instar caterpillars were starved for 24 h prior to being placed on wheat plants; a single caterpillar was used per pot. Caterpillars were confined to plants using tubes of clear plant tubing, which rested on the soil surface and were supported by ring stands. Tubes were topped with fine-mesh screening. For each *H. virescens*-damaged plant ($n = 5$), we collected approximately 50 mg of damaged tissue, keeping each sample separate for processing. We collected tissue of similar size and position from five healthy plants as controls.

Extraction and Quantification of Phytohormones

To extract and detect free LNA, LA, JA, SA, and IAA, we used a previously described method (Schmelz and others 2003, 2004). Briefly, we derivatized free carboxylic acids to methyl esters, which were isolated using vapor phase extraction and analyzed by GC-MS with isobutane chemical ionization using selected-ion monitoring. We quantified amounts of JA using 100 ng of dihydrojasmonic acid, SA using 100 ng of $[^2H_6]$SA, IAA using 100 ng of $[^2H_5]$IAA (CDN Isotopes, Pointe-Claire, Quebec, Canada), and LNA and LA using 100 ng of gamma-linolenic acid (Matreya LLC, Pleasant Gap, PA). Dihydrojasmonic acid was derived by subjecting methyl dihydrojasmonate (Bedoukian Research Inc., Danbury, CT) to alkaline hydrolysis. These internal standards were added to our samples prior to processing. We also processed samples without the derivatization agent to verify that the compounds recovered were not themselves present in plant or insect tissues but were derived from the carboxylic acids. To confirm the identity of methyl linolenate (meLNA), methyl linoeate (meLA), methyl jasmonate (meJA), methyl salicylate (meSA), and methyl indole-3-acetate (meIAA) in our samples, we analyzed extracts by GC-MS with electron ionization, comparing retention times and spectra with that of pure compounds. Retention times and mass spectra of meLNA, meLA, meJA, meSA, and meIAA recovered from

Hessian fly-infested and control tissue matched those of pure standards, confirming the identity of meLNA, meLA, meJA, meSA, and meIAA in our samples. Moreover, we did not recover methyl esters in the absence of the derivatization agent, verifying that the methyl esters we measured were derivatized from LNA, LA, JA, SA, and IAA and were not themselves present in samples.

Statistical Analyses

For all analyses of variance, fatty-acid and hormone levels were natural-log transformed to normalize data and stabilize variance. We made comparisons of LNA, LA, JA, SA, and IAA levels by ANOVA (PROC GLM) with Hessian fly larvae (present or absent), day of infestation, and their interaction as main effects (SAS Institute 2003). For all ANOVAs, we confirmed that there were no patterns in the dispersion of residuals that would indicate nonlinear relationships between variables and compared individual means with Tukey's HSD mean separation test (Sokal and Rohlf 1995). Although we collected samples for 10 days following oviposition, we present data only for days 1–7 following egg hatch, that is, the days larvae were feeding. The 3 days following oviposition showed no significant changes from time zero (data not shown).

Student's *t* tests (PROC TTEST) were used to compare levels of free LNA, LA, JA, SA, and IAA in *H. virescens*-damaged leaves and undamaged controls (Sokal and Rohlf 1995; SAS Institute 2003). We tested for linear relationships between LNA, LA, JA, SA, and IAA within Hessian fly-infested plants and controls using regression analysis (PROC REG, SAS Institute 2003).

Because amounts of LNA and LA and of JA and IAA were significantly correlated within some treatments, we also subjected the transformed data to a multivariate analysis of variance, using Wilks Lambda to test for significance of main effects of gall, tissue, and their interaction (MANOVA; PROC GLM, SAS Institute 2003). The multivariate approach addresses intercorrelations between dependent variables and controls the Type I error rate, which can inflate when conducting multiple tests of correlated dependent variables.

Results

After 72 h of feeding, *H. virescens* caterpillars severely damaged wheat seedlings. Their feeding strongly increased levels of the fatty acids LNA and LA to 12 and 3.5 times, respectively, above those from undamaged controls (Fig. 2a; LNA: $t = 2.58$, df $= 5$, $p = 0.049$; LA: $t = 3.33$, df $= 5$, $p = 0.021$). Similarly, levels of JA, an oxidation product derived from LNA, in *H. virescens*-damaged tissue

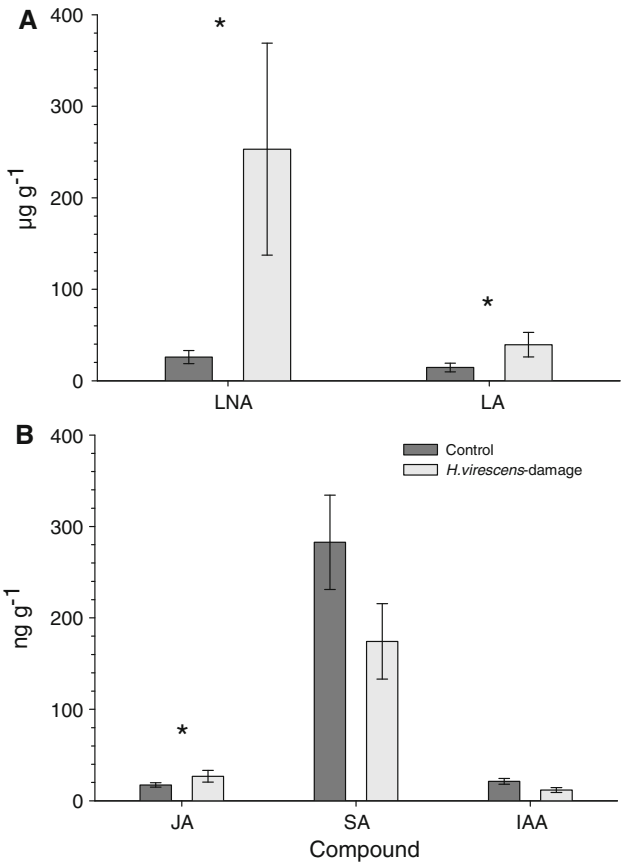

Fig. 2 Levels (mean ± SE) of **a** linolenic (LNA) and linoleic (LA) acid and **b** jasmonic (JA), salicylic (SA), and indole-3-acetic (IAA) acids in *H. virescens*-damaged and control wheat plants after 72 h of feeding. *Asterisks* indicate significant differences of acid levels between caterpillar-damaged and control plants (Student *t*-test $p < 0.05$; see text for details)

were significantly increased, being nearly double the levels in undamaged wheat seedlings (Fig. 2b; JA: $t = 4.06$, df $= 5$, $p = 0.010$). Caterpillar feeding did not alter levels of SA or IAA (Fig. 2; SA: $t = 1.10$, df $= 5$, $p = 0.32$; IAA: $t = 1.8$, df $= 5$, $p = 0.14$). Regression analysis of compounds in caterpillar-damaged wheat revealed a significant positive relationship between LNA and LA (best-fit regression line: LA $= 0.11 \times$ LNA $+ 13777$, $r^2 = 0.90$, $p < 0.0001$) and a marginally significant multivariate relationship between LNA and LA and JA (best-fit regression line: JA $= 0.00008 \times$ LNA $+ 0.0009 \times$ LA $+ 7.8$, $r^2 = 0.74$, $p = 0.069$). No other significant relationships were detected.

Similar to *H. virescens*, Hessian fly larvae also significantly affected levels of LNA and LA in wheat seedlings relative to control plants (Table 1). Linolenate was significantly influenced by the effect of treatment (that is, Hessian fly versus control), with Hessian fly larvae-infested plants having significantly more LNA. Moreover, LNA was also marginally influenced by the day effect and the

Table 1 Results of ANOVA on linolenic, linoleic, jasmonic, salicylic, and indole-3-acetic acids measured in Hessian fly-infested and control wheat plants

Compound	Overall ANOVA [$F_{13,69}$ (p)][a]	Main effects[a]		
		Treatment [$F_{1,69}$ (p)]	Day [$F_{6,69}$ (p)]	Interaction [$F_{6,69}$ (p)]
Linolenic	2.7 (0.006)	10.9 (0.002)	1.9 (0.096)	2.0 (0.075)
Linoleic	2.0 (0.04)	10.3 (0.002)	1.1 (0.40)	1.5 (0.20)
Jasmonic	0.97 (0.49)	–	–	–
Salicylic	0.78 (0.67)	–	–	–
Indole-3-acetic	2.0 (0.04)	2.73 (0.10)	3.2 (0.01)	0.67 (0.68)

[a] Statistics performed on log-transformed data

"treatment × day" interaction, which revealed that Hessian fly-infested wheat from day 7 had amounts of LNA that were greater than those measured in control plants on days 1–5 and those from Hessian fly-infested plants from days 1–3 (Table 1; Fig. 3a). Amounts of LA were also influenced by the treatment effect, with Hessian fly-infested plants having levels significantly greater than control plants, but LA levels were not significantly influenced by day or the "treatment × day" interaction (Table 1; Fig. 3b).

Although *H. virescens* significantly altered levels of JA but not SA, feeding by Hessian fly larvae did not significantly influence levels of either compound relative to control plants (Table 1; Fig. 4a, b). Hessian fly larvae did, however, alter levels of IAA (Table 1; Fig. 4c). Despite high variation, Hessian fly-infested plants contained elevated levels of IAA relative to controls at the 0.1 significance level (Table 1; Fig. 4c). The significant "day" effect revealed that regardless of Hessian fly infestation, the amount of IAA on day 5 was significantly greater than that on day 3 (Table 1; Fig. 4c). The "treatment × day" interaction term was not significant.

Multivariate analysis of Hessian fly-infested and control wheat revealed two significant associations: LNA was significantly and positively associated with LA (partial correlation coefficient [PCC] = 0.92, $p < 0.0001$), whereas IAA was significantly and negatively associated with JA (PCC = −0.38, $p = 0.001$). The multivariate analysis further showed significant effects of treatment and day, but not their interaction (Wilks Lambda for treatment: $F_{5,52} = 2.8$, $p = 0.027$; day: $F_{30,210} = 1.7$, $p = 0.014$; interaction: $F_{30,210} = 1.0$, $p = 0.41$). These results support our univariate analyses and indicate that Hessian fly larvae strongly influenced fatty-acid and phytohormone content relative to uninfested control plants and altered these levels over time.

Regression analyses of control plants pooled across days revealed significant positive relationships between LNA and LA (best-fit regression line: LNA = 0.55 × LA + 3758, $r^2 = 0.76$, $p < 0.0001$) and between LNA and IAA (best-fit regression line: LNA = 278 × IAA + 20417, $r^2 = 0.12$, $p = 0.04$), although this latter relationship is weak and does not describe much of the variation. No other significant relationships between compounds were found for control plants.

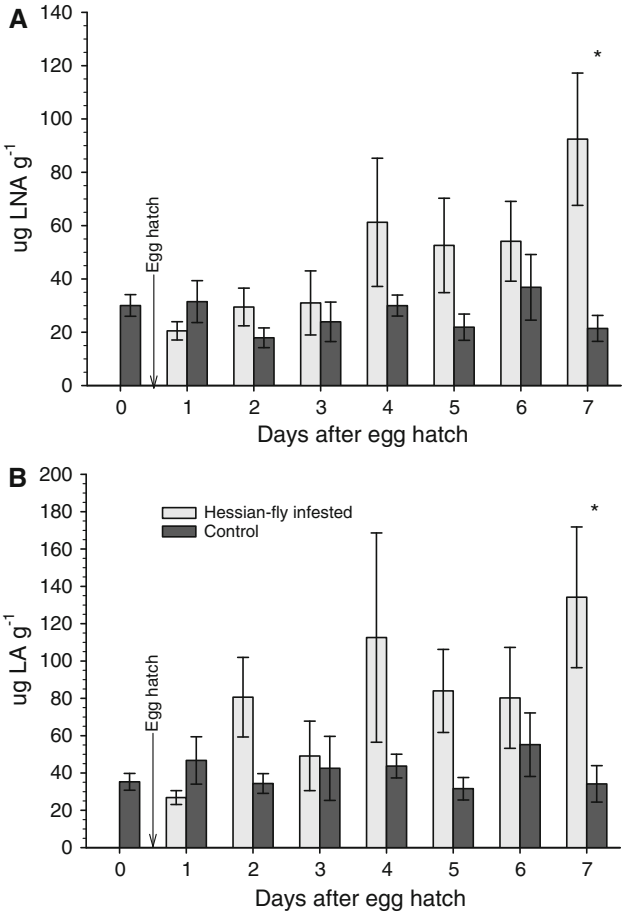

Fig. 3 Levels (mean ± SE) of **a** linolenic (LNA) and **b** linoleic (LA) acids from crowns of Hessian fly-infested and control wheat plants. Oviposition occurred on day 0 and 3 days later eggs hatched. *Asterisks* indicate significant differences between acid levels on particular days (Tukey's HSD $p < 0.05$; data are shown untransformed; see Table 1 and text for details on statistics)

Similar to controls, when Hessian fly-infested plants were pooled across days, regression analyses revealed a strong relationship between LNA and LA (best-fit regression line: LNA = 0.54 × LA + 5092, $r^2 = 0.87$, $p < 0.0001$). Unlike control plants, Hessian fly-infested plants did not yield a significant relationship between LNA and IAA ($r^2 = 0.04$, $p = 0.23$) but did reveal a significant negative relationship between JA and IAA (best-fit regression line: JA = −0.78 × IAA + 43.4, $r^2 = 0.21$, $p = 0.006$),

indicating that high levels of IAA were associated with low levels of JA. No other significant relationships between compounds were detected for Hessian fly-infested plants.

Discussion

Feeding by *H. virescens* on wheat leaves strongly increased levels of LNA, LA, and JA but did not significantly alter SA or IAA levels. Importantly, levels of LNA, LA, and JA were significantly associated with each other, supporting the notion that *H. virescens* induced an octadecanoid cascade. In other plant–herbivore systems, the octadecanoid pathway appears to be expressed constitutively and free LNA is quickly oxidized through a series of steps to form JA (Farmer and Ryan 1992). However, in this wheat system the constitutive nature of the pathway remains unclear because our samples were collected after 72 h of damage, providing ample time for production of octadecanoid-related enzymes (Tooker and De Moraes 2009). Because leaves are typically well defended, for the purposes of this study the dynamics of LNA, LA, JA, SA, and IAA following *H. virescens* damage can be considered "typical" of how wheat can react to generalized feeding in a controlled setting and deviations from this standard, as seen with Hessian fly larvae, may be notable.

Hessian fly larvae significantly elevated amounts of LNA and LA in wheat crowns on the seventh day of feeding (Table 1; Fig. 2). Feeding by Hessian fly also marginally influenced levels of IAA but did not alter JA or SA (Fig. 3). These results agree with recently published work that found that 1 and 3 days of Hessian fly feeding on a susceptible variety of wheat did not significantly alter levels of LNA, JA, or SA but increased IAA (Zhu and others 2010). In contrast, Hessian fly larvae feeding on resistant lines of wheat induce substantial increases of LNA within 3 days of feeding (Zhu and others 2010).

The increases we detected in LNA and LA in Hessian fly-infested plants were only a fraction of the increases found for *H. virescens*-damaged plants (Figs. 1, 2). This difference may be explained by the disparate feeding styles of the two herbivore species, the part of the leaf on which they feed (leaf blades versus leaf bases in the crown), and/or the defense pathways their feeding induces. Russian wheat aphid (RWA; *Diuraphis noxia*) can also increase LNA levels in wheat leaves but to a degree lower than we detected (Smith and others 2010). Hessian fly and RWA have feeding mechanisms that are more similar to each other than to the chewing mouth parts of *H. virescens*, but Hessian fly appears to more strongly alter LNA levels, perhaps reflecting a unique influence of Hessian fly (Fig. 3).

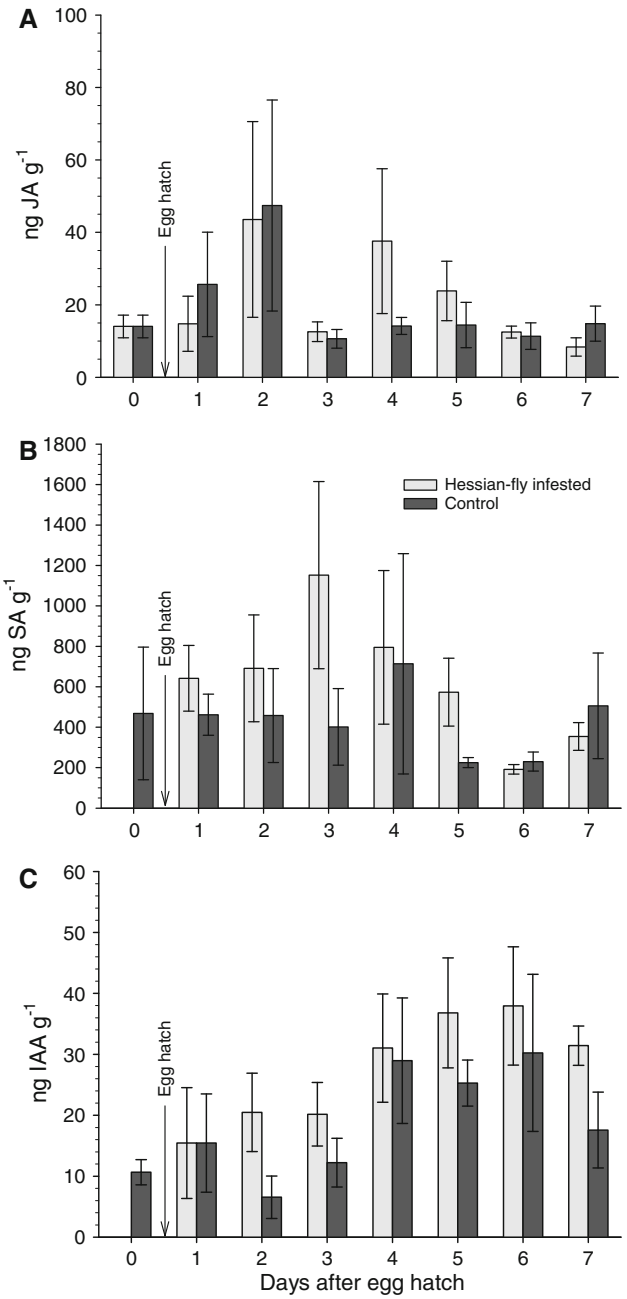

Fig. 4 Levels (mean ± SE) of **a** jasmonic (JA), **b** salicylic (SA), and **c** indole-3-acetic acids from crowns of Hessian fly-infested and control wheat plants. Oviposition occurred on day 0 and 3 days later eggs hatched. *Asterisks* indicate significant differences between acid levels on particular days (Tukey's HSD $p < 0.05$; data are shown untransformed; see Table 1 and text for details on statistics). Levels of IAA were marginally influenced by Hessian fly feeding, but the "treatment × day" interaction term was insignificant (Table 1), so no differences are noted here

The increases in fatty acids associated with Hessian fly feeding in our study and those associated with resistant lines of wheat (Zhu and others 2010) are especially intriguing because they occurred in crown tissue, which is white and not obviously photosynthetic. LNA, in particular, tends to

be strongly associated with chloroplasts and is usually less common in weakly photosynthetic or nonphotosynthetic shoot and roots (del Rio-Celestino and others 2008; Harwood and Russell 1983); therefore, Hessian fly appears to increase LNA content in tissues that are not typically rich in LNA, possibly suggesting that Hessian fly larvae may benefit from having more LNA and LA in their diet than would otherwise be present (Tooker and De Moraes 2009). Hessian fly is, of course, a maggot and neither LNA nor LA has been reported to be necessary for dipteran development (Dadd 1977; Downer 1985); however, such dietary work is based largely upon research with mosquitoes, house flies, and parasitic flies and not dipteran herbivores (Dadd 1977). Phytophagous fly species may still require LNA to complete development (Chang and Vargas 2007). Unfortunately, determining the dietary importance of LNA (or LA) for Hessian fly will be challenging given the complexity of its feeding habit (that is, its concealed feeding site and induced nutritive tissue) unless nutritionally deficient wheat mutants are developed.

Although increased levels of LNA and LA may enhance the nutrition of Hessian fly larvae, they could also pose threats to feeding larvae because both fatty acids themselves, as well as their metabolites, can induce plant defenses (Farmer and Ryan 1992). Feeding by Hessian fly larvae on susceptible plants, however, does not appear to produce a typical induction of plant defenses, suggesting that larvae somehow avoid triggering a typical defense response (Harris and others 2003; Liu and others 2007; Tooker and De Moraes 2007). We have previously hypothesized that gall insects, which have an unparalleled ability to influence host-plant physiology, should be under strong selection pressure to avoid plant defenses, including JA signaling, because they are sedentary and usually incapable accessing other host plants (Tooker and De Moraes 2007, 2008, 2009). The absence of JA induction in our current work is consistent with this hypothesis, as is lack of induction of six-carbon, green-leafy volatiles, which are additional metabolites of LNA (Tooker and De Moraes 2007). Russian wheat aphid also appears to avoid increasing JA content despite elevating LNA levels (Smith and others 2010), so it would seem that other insect species have also evolved tactics to avoid inducing typical host-plant defenses.

Although Hessian fly larvae failed to increase levels of the phytohormones JA and SA, they upregulated amounts of IAA in their host plants. This finding is consistent with other work with Hessian fly feeding on susceptible varieties of wheat (Zhu and others 2010). Indole-3-acetic acid is the main auxin in plant tissues and is well known to cause plant cells to grow and divide (Davies 2004); therefore, perhaps the higher levels of IAA that we detected could play a role in generating the nutritive cells at larval feeding sites. Alternatively, IAA has also been implicated as a negative regulator of JA, which is both a defense-signaling molecule and a powerful growth regulator (Saniewski and others 2002; von Dahl and Baldwin 2004). The significant negative relationship that we detected between IAA and JA in Hessian fly-infested plants, but not in control plants or *H. virescens*-damaged plants, is consistent with the notion that IAA levels may contribute to suppression of JA. Limiting JA production near feeding sites via IAA could benefit Hessian fly larvae in two ways: first, by helping larvae avoid triggering fatal defense responses mediated by JA and, second, by facilitating cell growth, possibly of nutritive tissues, by minimizing the presence of a growth regulator.

Indole-3-acetic acid has long been suspected to be involved in gall formation (Raman and others 2005), particularly for producing macroscopic "covering" galls (that is, the insect induces the plant to grow a swelling that covers the insect); however, finding higher levels of IAA associated with a species that does not make a covering gall is significant for trying to understand the evolution of the gall-inducing habit. Because Hessian fly feeds between leaf sheaths, it is already surrounded by tissue and might not derive much more benefit from producing a covering gall. Nevertheless, Hessian fly appears to elevate IAA, possibly to facilitate formation of nutritive tissue or to counter plant defenses. If a free-feeding common ancestor of Hessian fly and gall midges that induces covering galls similarly altered IAA levels, it would seem conceivable that selection could then increase levels of IAA to the point where producing a covering gall would be possible.

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