Title: Risky roots and careful herbivores: Sustained herbivory by a root-feeding herbivore attenuates indirect plant defences

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Abstract

1. Above-ground plant tissues produce characteristic blends of volatile compounds in response to insect herbivory. These herbivore-induced plant volatiles (HIPVs) function in plant defence and mediate foraging decisions by herbivores and their natural enemies. The ecological roles of HIPVs as foraging cues for different trophic levels highlight an important conflict for herbivores that need to locate suitable host plants while avoiding competition and predation.

2. Plant roots also emit HIPVs following herbivory, but our understanding of root-produced volatiles and their ecological functions in soil environments remains limited. Moreover, recent studies have documented the effects of temporal dynamics of plant volatile production on ecological interactions, but little is known about how root HIPVs change throughout herbivory or the resulting ecological implications from such changes.

3. In this study, we examined the roles of HIPVs from roots of cucumber plants Cucumis sativus as foraging cues for a specialist herbivore, striped cucumber beetle Acalymma vittatum and its natural enemies, entomopathogenic nematodes (EPNs). We predicted HIPVs from A. vittatum-damaged roots would attract EPNs, while repelling conspecific larvae that avoid competition, induced plant defences and increased risk of predation by EPNs. To capture the temporal dynamics of root HIPVs, we determined how HIPV-mediated interactions change over time with sustained herbivory.

4. Initially (after 24 hr), A. vittatum herbivory on C. sativus, or mechanical wounding, induced greater production of root volatiles. These root HIPVs recruited EPNs and repelled foraging A. vittatum larvae, although larval performance was not affected by prior damage. Sustained (7 days) herbivory by larvae reduced HIPVs to levels indistinguishable from undamaged control roots while mechanically damaged roots continued to produce higher levels of volatiles. Attenuation of HIPVs impaired indirect defence responses of C. sativus by reducing recruitment of EPNs and deterrence of A. vittatum larvae.
INTRODUCTION

Chemical information plays key roles in ecological interactions across trophic levels, as organisms forage for food while attempting to avoid competition and natural enemies (Mescher & De Moraes, 2015; Raguso et al., 2015). Insect herbivores frequently rely on olfactory cues from plants to find and evaluate hosts for feeding or oviposition, as plant-produced volatiles provide ecologically relevant information about plant identity, nutritional content and defence status (Bruce & Pickett, 2011; De Moraes, Mescher, & Tumlinson, 2001; Grunseich, Thompson, Aguirre, & Helms, 2019). It is well known that herbivore feeding triggers the production of distinct herbivore-induced plant volatiles (HIPVs), which can directly repel herbivores (Bernasconi, Turlings, Ambrosetti, Bassetti, & Dorn, 1998; De Moraes et al., 2001; Ray et al., 2019) or indirectly protect plants by attracting natural enemies that kill herbivores (Aartsma, Bianchi, van der Werf, Poelman, & Dicke, 2017; Allmann & Baldwin, 2010; De Moraes, Lewis, Pare, Alborn, & Tumlinson, 1998; Kessler & Heil, 2011; Turlings, Tumlinson, & Lewis, 1990). Herbivore avoidance of HIPVs is theorized to be a mechanism to enhance their survival or fitness by evading competition, induced plant defences and increased risk of predation by natural enemies (Bernasconi et al., 1998; De Moraes et al., 2001; Kariyat et al., 2013). Here, we test this prediction in a below-ground tritrophic system, to investigate the roles of volatiles from plant roots in guiding foraging decisions by herbivores and their natural enemies.

The majority of research on chemically mediated plant–insect interactions has focused on above-ground systems; however, there is growing recognition that volatiles from plant roots facilitate a diversity of ecological interactions below-ground (Johnson & Gregory, 2006; Johnson & Nielsen, 2012; Rasmann, Hiltpold, & Ali, 2012; van Dam, Weinhold, & Garbeva, 2016; Wenke, Kai, & Plechulla, 2010). These include plant allelopathy (Huang, Gfeller, & Erb, 2019), growth promotion (Gfeller et al., 2019) and protection against pathogen infection (Lackus, Lackner, Gershenzon, Unsicker, & Köllner, 2018). Similar to above-ground plant tissues, roots also modify their production of volatiles in response to stressors, such as pathogen infection (Schulz-Bohm et al., 2018) or herbivory (Abraham, Giacomuzzi, & Angeli, 2015; Pierre et al., 2011). Although our understanding of herbivore-induced root volatiles and their roles in below-ground interactions is limited, increasing evidence suggests that HIPVs from plant roots can influence foraging by herbivores (Robert, Duployer, et al., 2012) and their natural enemies (Ali, Alborn, & Stelinski, 2010; Rasmann et al., 2005; Tonelli et al., 2016). One of the best-studied examples is the production of HIPVs from maize roots following herbivory by western corn rootworm larvae Diabrotica virgifera virgifera (Köllner et al., 2008; Rasmann et al., 2005; Robert, Erb, Duployer, et al., 2012; Robert, Veyrat, et al., 2012). Maize root HIPVs attract D. virgifera larvae, which experience enhanced performance on plants with conspecific herbivory (Robert, Erb, Duployer, et al., 2012; Robert, Veyrat, et al., 2012) and recruit entomopathogenic nematodes (EPNs), which are natural enemies of root-feeding insects (Hiltpold, Erb, Robert, & Turlings, 2011; Rasmann et al., 2005). This suggests that root herbivores can face critical trade-offs when using root HIPVs as foraging cues while also avoiding natural enemies. Our knowledge of these trade-offs in below-ground systems and across different plant, herbivore and natural enemy communities, however, remains limited, and additional research is needed to elucidate the ecological and evolutionary outcomes.

As emphasized in the above examples, plant volatile production is a dynamic process where volatile blends are modified in response to environmental changes and blend compositions fluctuate over time. Diurnal rhythms of constitutive and induced volatile production are well documented (De Moraes et al., 2001; Naranjo-Guevara, Peñaflor, Cabezas-Guerrero, & Bento, 2017; Turlings & Erb, 2018), with more recent studies investigating the role of plant circadian clocks in regulating these changes (Arimura et al., 2008; Joo et al., 2019). Emitted blends of HIPVs also change throughout the duration of herbivory. For example, some compounds are emitted rapidly following initiation of damage while production of other compounds may be delayed by several hours (Erb et al., 2015; Joo et al., 2018; Ponzio, Gols, Pieterse, & Dicke, 2013). Some studies have observed stronger production of HIPVs with increased herbivore damage (De Boer, Hordijk, Posthumus, & Dicke, 2008; Dicke, Van Loon, & Soler, 2009; Maeda & Takabayashi, 2001) while others have reported suppression of HIPVs with continuous feeding (Alba, Glas, Schimmel, & Kant, 2011; Desurmont et al., 2014; Takai et al., 2018). The temporal dynamics of plant volatile production...
influence the outcomes of ecological interactions, including the timing and magnitude of natural enemy or pollinator recruitment (Balao, Herrera, Talavera, & Dötterl, 2011; Joo et al., 2018; Kant, Ament, Sabelis, Haring, & Schuurink, 2004). A few studies have investigated production of root volatiles at multiple timepoints (Crespo et al., 2012; Danner et al., 2015; Deasy, Shepherd, Alexander, Birch, & Evans, 2016; van Dam, Samudrala, Harren, & Cristescu, 2012); however, it is currently not understood how root HIPVs change during sustained herbivory, and temporal variation in root HIPVs has not been correlated with ecological interactions.

The goal of this study was to investigate how below-ground insect herbivores use olfactory cues from plant roots to navigate the conflict of locating suitable host plants while avoiding predation. Here, we examined the role of HIPVs from roots of cucumber plants Cucumis sativus in mediating foraging decisions by larvae of the specialist cucumber beetle Acalymma vittatum and their EPN natural enemies. We predicted that cucumber roots emit HIPVs in response to herbivory from A. vittatum larvae and that these HIPVs function as an indirect defence by recruiting larvae-killing EPNs (Ali et al., 2010; Ali, Albom, & Stelinski, 2011; Ellers-Kirk, Fleischer, Snyder, & Lynch, 2000; Rasmann et al., 2005). Due to the role of HIPVs in plant defence, we hypothesized that A. vittatum larvae avoid cues from conspecific-damaged roots to avoid induced plant defences that could reduce larval performance or survival. To characterize the temporal dynamics of these interactions, we quantified changes in root HIPVs following short-term (24 hr) and sustained (7 days) herbivory and we investigated the influence of these changes on A. vittatum and EPN behaviour. We predicted that cucumber root HIPVs change over time with sustained herbivory by A. vittatum larvae, influencing the attraction of both herbivores and natural enemies. By linking herbivore and natural enemy responses to root HIPVs, we shed light on how the challenges of foraging while avoiding competition and predation, guide herbivore behaviour. Through examining these interactions over time, we can determine the ecological consequences and significance of the temporal dynamics of induced plant defences.

### 2 | MATERIALS AND METHODS

#### 2.1 | Plants, insects and nematodes

Cucumber plants C. sativus cv. Max Pack were grown from seed (Johnny's Selected Seeds) and used in experiments after 3–4 weeks of growth. Plants were grown in individual pots in topsoil mix (Hyponex Corporation) with 3 g Osmocote® fertilizer (15-9-12 N-P-K; Scotts) and were kept in an insect-free, climate-controlled growth room with supplemental lighting (16 hr light: 8 hr dark; 22°C; 29°C; 56% RH, Fluence). Striped cucumber beetles A. vittatum were maintained in a laboratory colony on cultivated squash Cucurbita pepo cv. Raven that was periodically supplemented with wild-caught adults. EPNs (Heterorhabditis bacteriophora) used in this study are commercially available generalists used for biological control of A. vittatum (Ellers-Kirk et al., 2000). EPNs were cultured in last-instar wax moth larvae Galleria mellonella at 27°C. Infective juveniles were harvested in White traps (White, 1927) and used within 24 hr of emergence.

#### 2.2 | Collection and analysis of root volatiles

To determine how herbivory by A. vittatum larvae affects production of olfactory cues from cucumber roots, we used dynamic headspace sampling to characterize the volatile profiles emitted by damaged and control roots after 24 hr and 7 days (Ali et al., 2010). Prior to collections, seedlings were transplanted into individual glass pots (5 cm diameter) containing clean sand (10% water W/V) and allowed to acclimate for 24 hr. One group of plants (n = 11) each received 5 second-instar A. vittatum larvae for 24 hr, one group (n = 9) received mechanical wounding (roots pierced with a metal spatula once every 8 hr for 24 hr), and another group was kept as undamaged controls (n = 11). Collections were repeated with plants damaged by 5 second-instar A. vittatum larvae for 7 days (n = 11), plants with 7 days mechanical wounding (n = 5) and 7 days controls (n = 11). Volatiles were also collected from chambers containing only clean sand as negative controls. Vacuum pumps were used to gently pull air over roots (100 ml/min) and through an adsorbent filter trap containing 60 mg of HaySep® Q (Hayes Separations, Inc.) for 8 hr (14:00–22:00). Compounds were eluted from filter traps using 150 μl dichloromethane. A 5 μl aliquot of standard solution containing nonyl acetate (80 ng/μl) was added to each sample. Roots were harvested, washed and dried, and root dry mass was recorded. After each collection, larvae were recovered and confirmed to be feeding.

Volatiles were analysed using an Agilent 7890B gas chromatograph and 5977B mass spectrometer with a splitless injector held at 250°C and helium as the carrier gas. After sample injection (1 μl), the column (HP-5MS 30 m x 0.250 mm-ID, 0.25 μm film thickness, Agilent Technologies) was held at 40°C for 5 min before the temperature was increased at 20°C/min to 250°C. Compounds were ionized by electron impact ionization at 70 eV and mass spectra were acquired by scanning from 40 to 300 m/z at 5.30 scans/s. Tentative identification of target compounds was achieved by comparison with mass spectral libraries (NIST17, Adams2 [Allured Publishing Corporation] and a University of Göteborg library), and structure assignments were confirmed where possible by comparison of mass spectra and retention times with authentic standards (Helms et al., 2019). Compounds were quantified relative to standard concentrations and calculated as ng/g dried root mass.

#### 2.3 | Larval preference assays

We conducted dual-choice experiments using below-ground olfactometers to assess the effect of olfactory cues from cucumber roots on larval foraging behaviour (Robert, Erb, Duployer, et al., 2012). One day after collecting root volatiles (24 hr or 7 days), the same plants were used for larval preference experiments (48 hr and 8 days). An
initial experiment was conducted to verify that A. vittatum larvae prefer olfactory cues from cucumber roots over sand \((n = 11)\). A second experiment was conducted to determine whether larvae differentiate between volatiles from undamaged cucumber roots and HIPVs from roots damaged by conspecifics for 48 hr \((n = 26)\). Finally, a third assay was conducted to determine whether larvae prefer volatile cues from undamaged roots or cues from roots damaged by conspecifics for 8 days \((n = 20)\). Olfactometers were assembled 30 min prior to experiments and covered to exclude light. Pots were connected with a central glass arm (13 cm) and wire mesh barriers were used to prevent larval movement into pots and larval contact with roots. Five second-instar larvae were added to each centre arm, recovered after 20 min and their positions were recorded. Treatment orientations were randomized to account for potential directional bias. Larvae were recovered from herbivory treatments to confirm active feeding.

2.4 | EPN preference assays

To determine whether EPNs use cucumber root HIPVs while foraging for insect hosts, dual-choice experiments were performed using below-ground olfactometers. Cucumber seedlings were transplanted into glass pots in 1:1 sand: topsoil mix (10% water W/V) and allowed to acclimate for 24 hr. Plants were each damaged by 5 second-instar A. vittatum larvae \((n = 12)\) for 24 hr or 7 days, or were kept as undamaged controls \((n = 12)\). Separate pairwise comparisons were conducted to determine EPN preference for (a) volatile cues from undamaged roots versus HIPVs from roots damaged by larvae for 24 hr and (b) volatile cues from undamaged roots versus cues from roots damaged by larvae for 7 days. Olfactometers were assembled with a central arm (36 cm) 1 hr prior to experiments and covered to exclude light and olfactometer orientation was randomized among trials. Wire 400 mesh screen (MSC Industrial Supply) barriers prevented EPNs from moving into pots. EPN infective juveniles (2,500) were added to the centre of each arm and their positions were recorded after 48 hr (Willett, Alborn, Duncan, & Stelinski, 2015). EPNs were extracted from sand using an adapted Baermann funnel method (MacMillan, Blok, Young, Crawford, & Wilson, 2006). After experiments, beetle larvae from damage treatments were recovered and confirmed to be feeding.

2.5 | Larval performance assays

We conducted larval performance experiments to quantify the influence of prior conspecific herbivory on A. vittatum larvae (Robert, Erb, Hibbard, et al., 2012). Cucumber seedlings were transplanted into sand and allowed to acclimate for 24 hr. In the first experiment, plants were damaged by 5 second-instar A. vittatum larvae for 24 hr \((n = 23)\) or kept as undamaged controls \((n = 23)\). After 24 hr damage, larvae were removed, and all plants were transplanted into new, individual pots. A second cohort of second-instar A. vittatum larvae were weighed and individual larvae were placed on each plant. Larvae were allowed to feed for 24 hr, then were removed and reweighed. Following these methods, a second bioassay was conducted to compare larval performance on plants damaged for 7 days \((n = 12)\) and undamaged plants \((n = 12)\).

2.6 | Root consumption assay

To determine whether herbivory by A. vittatum larvae affects the availability of resources for conspecifics (resource competition), we quantified the influence of herbivory on root loss/growth and plant mortality. Roots of 3-week-old cucumber seedlings were washed, and initial root mass was recorded. Seedlings were transplanted into individual pots with a 1:1 sand:topsoil mixture. One group of plants each received 5 second-instar A. vittatum larvae \((n = 7)\) while a second group was kept as undamaged controls \((n = 7)\). Larvae fed for 9 days, then were recovered and seedling mortality and root mass were recorded (Harrington, Mexal, & Fisher, 1994).

2.7 | EPN infection assays

To confirm that EPNs locate and kill A. vittatum larvae, we quantified larval infection rates using two-choice infection assays (Zhang et al., 2019). Seedlings were transplanted into below-ground olfactometers as previously described. One damaged plant (with 5 second-instar A. vittatum larvae) and one control plant were paired \((n = 8)\). After 24 hr of herbivory, 2,500 EPNs were added to the central chamber of each olfactometer and allowed to move within the arena and into pots for 72 hr. Larvae were then recovered from the pots and monitored for EPN infection and mortality.

2.8 | Statistical analyses

Statistical analyses were performed using the software program R (R Version 3.6.1, R Core Team, 2019). Root volatile data were analysed by conducting non-metric multidimensional scaling ordinations in the package vegan to visualize blend differences (Okansen et al., 2013). Permutational multivariate analysis of variance (PERMANOVA) was conducted to quantify differences in volatile blends at different timepoints (McCormick, Gershenzon, & Unsicker, 2014). Random forest analysis was used to identify compounds with the greatest contribution to variation among treatments (Ranganathan & Borges, 2010; Ray et al., 2019). One-way ANOVAs and Dunn’s Tests were used to compare the individual compounds and total volatile production. Preference data were analysed using GLM with quasi-likelihood functions to compensate for over-dispersion (Robert, Erb, Duployer, et al., 2012). Larval performance and root biomass data were analysed using one-way ANOVA comparisons.
3 | RESULTS

3.1 | Herbivory from A. vittatum larvae initially induces, but ultimately attenuates volatile production in cucumber roots

Herbivory by larvae or mechanical wounding for 24 hr induced distinct volatile blends from cucumber roots compared to control plants (Figure 1A; PERMANOVA $F_{2,28} = 6.35, R^2 = 0.31, p < 0.001$). In contrast, the root HIPV blend after 7 days of sustained herbivory was not different from that of undamaged roots while volatile production from mechanically damaged roots remained higher (Figure 1B; PERMANOVA $F_{2,24} = 5.29, R^2 = 0.31, p < 0.001$). Undamaged cucumber roots emitted relatively small quantities of volatiles and herbivory or mechanical damage (24 hr) induced higher total volatile production (Figure 1C). Herbivory and wounding at 24 hr comparatively increased the abundance of several compounds that were already emitted in undamaged controls but did not induce production of any new compounds from cucumber roots. Random forest analysis revealed that three monoterpenes (Camphene, Sabinene and $\alpha$-Pinene) contributed most to the variation among treatments (Figure S1) and their abundances were higher in damaged roots compared to controls (Figure 1C). After 7 days, compound abundances remained higher for mechanically wounded, but not herbivore-damaged roots (Figure 1D).

3.2 | Acalymma vittatum larvae initially avoid plants with conspecific herbivory

Acalymma vittatum larvae showed a strong preference for cucumber root volatiles compared to sand (Figure 2; GLM $T_{1,10} = -7.02, p < 0.001$). Larvae also preferred volatiles from undamaged roots compared to sand.
3.4 | Prior conspecific herbivory does not affect the performance of *A. vittatum* larvae

No differences in larval performance were observed on damaged or control roots at either 24 hr or 7 days. Per cent mass gain of larvae was not different when feeding on control plants or plants damaged for 24 hr (Figure 4; ANOVA, $F_{1,44} = 0.03, p = 0.87$) or 7 days (Figure 4; ANOVA, $F_{1,22} = 0.72, p = 0.42$).

3.5 | Herbivory by *A. vittatum* larvae reduces root biomass and increases plant mortality

After 9 days of continuous herbivory, cucumber root biomass was reduced compared to controls (Figure 5; ANOVA, $F_{1,7} = 13.05, p < 0.001$). We also observed 42% mortality among damaged plants and no mortality for control plants.

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**FIGURE 2** *Acalymma vittatum* attraction to cucumber roots was modulated by conspecific herbivory. Larvae preferred volatiles from undamaged roots compared to sand or roots with 48 hr conspecific herbivory. After 8 days of herbivory, larvae did not discriminate between damaged and undamaged root volatiles (*$p \leq 0.05$). Means ± SE are presented.

**FIGURE 3** (A) Entomopathogenic nematodes (EPNs) preferred cucumber root volatiles after 24 hr of herbivory. (B) After 7 days herbivory, no difference in attraction was observed (*$p \leq 0.05$). Means ± SE are presented.

(over HIPVs from roots damaged by conspecifics for 48 hr (Figure 2; GLM $T_{1,51} = 2.78, p = 0.007$). However, larval preference was not different between damaged and control roots after 8 days of herbivory (Figure 2; GLM $T_{1,35} = -0.58, p = 0.56$).

3.3 | EPNs are initially attracted to herbivore-damaged roots

More EPNs were attracted to roots with 24 hr of herbivory compared to undamaged control roots (Figure 3A; GLM $T_{1,11} = 7.13, p < 0.001$). However, after 7 days of herbivory, no attraction was observed, with few EPNs choosing either treatment (Figure 3B; GLM $T_{1,11} = 0.87, p = 0.39$).

**FIGURE 4** Larval performance was not different on roots damaged by conspecifics or control roots at 24 hr or 7 days. Means ± SE are presented.

**FIGURE 5** Cucumber root mass was reduced after 9 days of herbivory (*$p \leq 0.05$). Means ± SE are presented.
3.6 | EPNs infect and kill *A. vittatum* larvae

We observed that 71% of larvae recovered from the two-choice infection assays were infected and killed by EPNs.

4 | DISCUSSION

This study demonstrates that olfactory cues from plant roots guide the foraging decisions of herbivores and their natural enemies, by helping herbivores assess their risk of competition and predation, and helping natural enemies locate prey. We found that HIPVs from cucumber roots initially (after 24 hr) function as direct and indirect plant defences by repelling *A. vittatum* larvae and attracting beneficial EPNs that kill larvae. Furthermore, our study revealed that root HIPV emissions change over the course of herbivory, which modifies their roles in below-ground multi-trophic interactions. Initial feeding by *A. vittatum* larvae (24 hr) induced a distinct blend of HIPVs, but after 7 days of sustained herbivory, root volatile production was reduced to levels indistinguishable from controls. This reduction in HIPVs attenuated the avoidance of conspecific larvae and attraction of EPNs. These findings suggest that *A. vittatum* larvae ultimately reduce or suppress production of root HIPVs, thereby disrupting plant defences and altering chemically mediated interactions below-ground.

4.1 | HIPVs as honest signals of reward and risk

Plant-produced volatile compounds mediate ecological interactions at multiple trophic levels, communicating messages of risk and reward to herbivores and their natural enemies. HIPVs are widely recognized as indirect defences through their attraction of natural enemies (Ali et al., 2010; Allmann & Baldwin, 2010; Hiltpold et al., 2011; Narango-Guevara et al., 2017; Rasmann et al., 2005). In this role, HIPVs are honest signals of reward, exposing herbivores—which are often cryptic—to foraging predators and parasitoids seeking them as food for themselves or their offspring. Just as HIPVs make prey more apparent to natural enemies, foraging herbivores can also use HIPVs to assess the possibility of encountering natural enemies (Joo et al., 2018; Shiojiri, Ozawa, & Takabayashi, 2006), although this has not been previously investigated below-ground, where volatile compounds are dominant foraging cues (Johnson & Nielsen, 2012). In our study, we found that root HIPVs initially attract EPNs and repel *A. vittatum* larvae after short-term herbivory. This is one of a growing number of studies demonstrating that the functions of below-ground HIPVs are analogous to above-ground HIPV-mediated defences (Pearse et al., 2020), and suggests that herbivores can use these cues to avoid increased predation risk across a variety of ecological contexts.

Herbivore-induced plant volatiles also directly defend plants against herbivores, reducing herbivore performance through toxic or deterrent properties (Brzozowski, Mazourek, & Agrawal, 2019; Veyrat, Robert, Turlings, & Erb, 2016). Furthermore, volatile production is often correlated or metabolically linked to other defences (Christensen et al., 2013). This includes cucurbitacins in cucumber, although the variety used in this study produces extremely low levels of cucurbitacins (Agrawal, Janssen, Bruin, Posthumus, & Sabelis, 2002). When HIPVs directly reduce herbivore performance or signal production of other defences, they communicate direct risks for foraging herbivores (Bernasconi et al., 1998; Ray et al., 2019). Although no differences in larval performance were observed in this study, we found that after 9 days of larval herbivory, all damaged plants lost root mass and 42% of these plants eventually died. Thus, HIPVs may also serve as indicators of resource competition for below-ground herbivores (De Moraes et al., 2001; Valladares, Coll-Araóz, Alderete, Vera, & Fernández, 2019; Zakir et al., 2013).

4.2 | Attenuation of indirect plant defences

One hypothesis to explain our finding of reduced root HIPVs with sustained herbivore damage is that defences are suppressed by microbes or effector molecules in *A. vittatum* oral secretions. Plant defence suppression by herbivore oral secretions has been observed in above-ground tissues for several plant and herbivore species (Chung et al., 2013; Sarmento et al., 2011; Schausberger, 2018; Takai et al., 2018). For example, effector molecules in saliva of *Helicoverpa zea* larvae were found to suppress production of defences like nicotine in tomato *Nicotiana tabacum* (Musser et al., 2002). Similarly, bacteria in oral secretions from Colorado potato beetle larvae *Leptinotarsa decemlineata* suppressed anti-herbivore defences in tomato plants *Solanum lycopersicum* (Chung et al., 2013). While there is some evidence for herbivore manipulation of root defences, suppression of below-ground indirect defences by root herbivores has not been previously documented (Robert et al., 2013). Future research is needed to identify the mechanisms underlying root HIPV reduction by *A. vittatum* larvae and to determine whether defence suppression occurs or is widespread in below-ground plant–herbivore interactions.

Alternative hypotheses to explain our finding of reduced HIPVs with sustained herbivore damage are that plants attenuate root HIPVs to reduce attraction of subsequent herbivores or that they shift defensive strategies over time. Previous studies have found that HIPVs can simultaneously attract natural enemies and subsequent herbivores (Ali et al., 2011; Dicke & Baldwin, 2010; El-Sayed, Hale, 2010), indicating a trade-off for plants between indirect defense and herbivore attraction. Here, we observed EPN attraction to root HIPVs but did not assess whether this attraction extends to other herbivore species. While reducing HIPVs, plants could also invest in alternative defence strategies following sustained herbivory. We did not, however, observe a reduction in herbivore performance as evidence of induced plant defences after 7 days. Our findings suggest that plants can balance the costs and benefits of defence and herbivory by modifying HIPVs over relatively short time-scales.
(7 days), initially increasing indirect defences, then reducing possible attraction of subsequent herbivores.

5 | CONCLUSIONS

This work highlights the critical functions of root HIPVs in mediating multitrophic interactions among plants, herbivores and natural enemies in below-ground ecosystems. Our findings indicate that plants produce HIPVs for indirect defence, as EPNs use these cues to locate prey, and herbivores use HIPVs to avoid antagonistic interactions with natural enemies and conspecifics. This work also sheds light on the temporal dynamics of below-ground chemically mediated interactions, revealing that olfactory cues and their ecological functions can shift over relevant time-scales.

ACKNOWLEDGEMENTS

We thank the Helms lab members for assistance with maintaining plants and colonies for experiments. The authors also thank the A.W.E.S.O.M.E. faculty writing group in the College of Agriculture and Life Sciences at Texas A&M University. Group financial support was provided by NSF ADVANCE Institutional Transformation Award 1008385 and the college. Research was supported by funding from Texas A&M University and USDA NIFA Award No. 2017-67012-31498. Any opinions, findings and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the USDA. We also thank the editors and anonymous reviewers for their helpful feedback.

AUTHORS’ CONTRIBUTIONS

J.M.G. and A.M.H. conceived the ideas and designed the methodology; J.M.G., M.N.T., Z.G. and A.A.H. collected the data; J.M.G. and A.M.H. analysed the data. All authors contributed to writing the manuscript.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.0rxwdbrx6 (Grunseich et al., 2020).

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**How to cite this article:** Grunseich JM, Thompson MN, Hay AA, et al. Risky roots and careful herbivores: Sustained herbivory by a root-feeding herbivore attenuates indirect plant defences. *Funct Ecol*. 2020;34:1779–1789. https://doi.org/10.1111/1365-2435.13627