Variable costs of eavesdropping a green leaf volatile on two plant species in a common garden experiment

Grace E. Freundlich and Christopher J. Frost

Department of Biology, University of Louisville, Louisville, KY, USA

Running Title: Fitness costs of plant exposure to volatile cues

Author for Correspondence:
Christopher J. Frost
139 Life Sciences Building
University of Louisville
Louisville, KY 40292
502-852-6771
chris.frost@louisville.edu

Manuscript Data:
Abstract: 208
Main Text (excluding Abstract): 3762
Introduction: 802
Materials and Methods: 1008
Results: 484
Discussion: 1463

Tables:0
Figures: 5
Number of References: 89
Supplemental Tables: 5
Supplemental Figures: 3

Abstract

1. Herbivore-induced plant volatiles (HIPVs) from one plant may cue eavesdropping neighboring plants to prepare, or ‘prime’, defenses prior to experiencing herbivory. HIPV-mediated defense priming is now a well-documented, inducible phenomenon that should—like any inducible phenomenon—incur fitness costs. Yet, ecological costs associated with exposure to volatile cues alone are not clear.
2. For the first time under field conditions, we tested the hypothesis that exposure to a single HIPV would decrease herbivore damage at the cost of reduced plant growth and reproduction. In a common garden experiment, we exposed lima bean (*Phaseolus lunatus*) and pepper (*Capsicum annuum*) plants to a persistent, low-dose (~10ng/hour) of the green leaf volatile *cis*-3-hexenyl acetate (*z*3HAC), which is a HIPV that alone can prime plant defense.
3. *z*3HAC-treated pepper plants were shorter, had less aboveground and belowground biomass, and produced fewer flowers and fruits relative to controls. In contrast, *z*3HAC-treated lima bean plants were taller and produced more leaves and flowers than did controls. Additionally, we observed species-specific differences in herbivore damage: herbivory was reduced only in *z3*HAC-exposed lima bean plants but not pepper plants.
4. Synthesis: Differential responses to an identical priming cue in a shared common field have important implications for ecological costs and benefits of volatile-mediated inter-plant communication.

Keywords: *cis*-*3*-hexenyl acetate**,** lima bean, pepper, plant fitness, plant volatiles, priming

Introduction

Production and utilization of airborne chemical cues is prevalent within the plant kingdom. Plants depend on airborne chemical signaling for pollination (Muhlemann et al., 2014), indirect defense (Mumm and Dicke, 2010), protection from pathogens (Farag et al., 2013), and herbivore resistance (Frost et al., 2008b). Volatile communication is also pivotal for plant-plant signaling, and selection for such signaling depends on honest cues that reliably confer ecologically relevant information. For example, herbivory is a fundamental ecological interaction that impacts plant fitness, and many plants increase the production and emission of volatile compounds in response to herbivore damage (Turlings et al., 1995). Such herbivore-induced plant volatiles (HIPVs) are potentially reliable cues around which plant-plant eavesdropping could be evolutionarily adaptive. Undamaged plants (or parts of the same plant (Frost et al., 2007, Heil and Silva Bueno, 2007)) eavesdropping on HIPVs from a plant experiencing herbivory may directly trigger stress responses (Heil and Karban, 2010, Pearse et al., 2013, Arimura et al., 2002), or alternatively prime responses for future potential herbivory (Frost and Hunter, 2008)).

HIPV-mediated priming appears to be a common phenomenon. HIPVs have demonstrated priming ability in corn (Farag et al., 2005, Erb et al., 2015), tomato (Farag and Paré, 2002), poplar (Frost et al., 2008b, Frost et al., 2007), blueberry (Rodriguez-Saona et al., 2009) and lima bean (Sarai Giron-Calva et al., 2012, Arimura et al., 2008). HIPVs can be diverse and taxa-specific (Arimura et al., 2009, Copolovici et al., 2011), but are often comprised of monoterpenes, sesquiterpenes, benzenoids and green leaf volatiles GLVs (Boggia et al., 2015, Engelberth et al., 2004). In contrast to volatile terpenes and benzenoids (Paschold et al., 2006, Arimura et al., 2009), GLVs are immediately released into the airspace whenever leaves are mechanically damaged (Matsui et al., 2012) serving as early indicators of wounding. GLV exposure alters gene expression profiles related to specialized metabolite production and accumulated secondary metabolite precursors in preparation for inducing resistance (Pastor et al., 2014). For example, the GLV *cis*-3-hexenyl acetate (*z*3HAC) induces transcriptional changes in poplar (Frost et al., 2008b) and maize (Engelberth et al., 2004) that prime oxylipin signaling and induced resistance. Among the GLVs, *z*3HAC may represent a reliable cue because it is typically released from herbivore-damaged leaves and not by mechanically damaged leaves in a variety of species (Matsui et al., 2012), including tomato (Farag and Paré, 2002), maize (Engelberth et al., 2004), Arabidopsis (D'Auria et al., 2007), and poplar (Frost et al., 2008b, McCormick et al., 2014).

Fitness costs incurred by plants exposed to HIPV cues are largely unknown. Ecological theory posits that induced resistance by plants against herbivores is a cost-savings strategy to restrict the deployment of costly specialized defensive metabolites until necessary (Accamando and Cronin, 2012, Cipollini et al., 2003). However, inducible resistance generates a period of vulnerability between the time of attack and the upregulation of resistance (Cipollini and Heil, 2010). Defense priming via sensory perception of early reliable cues may overcome such a vulnerability by allowing a plant to anticipate a probable attack. Therefore, priming is an inducible phenomenon and theory predicts that responding to cues alone should incur fitness costs that select against maintaining a “primed state” unless reliable cues are detected (Douma et al., 2017, Frost et al., 2008a). In other words, perception of a cue resulting in defense priming may induce physiological changes that affect fitness that are less costly than induced resistance itself. Non-volatile priming agents β-amino butyric acid (BABA) (van Hulten et al., 2006) and snail mucus (Orrock, 2018) both support this prediction. Similarly, fitness costs associated with volatile perception alone that initiate priming should be less severe than costs of induced resistance to actual herbivory. Yet there is currently limited experimental evidence of such costs with respect to anti-herbivore volatile cues. For example, whereas bacterial-derived volatiles 3-pentanol and 2-butanone decrease reproductive output in field-grown *Cucumis sativa* (Song and Ryu, 2013), wild tobacco (*Nicotiana attenuata*) exposed to airspace of experimentally clipped sagebrush produce more seeds (i.e., higher presumptive fitness) relative to control plants (Karban and Maron, 2002). These results suggest that ecological costs of exposure to volatile cues may be context dependent, but comparative cost/benefit tradeoffs for perception of HIPVs alone among sympatric field-grown plants is currently lacking.

Here, we report a common garden field experiment with lima bean (*Phaseolus lunatus*) and chili pepper (*Capsicum annum*) testing the hypothesis that field plants subject to a persistent dose of an ostensibly reliable volatile cue incur consistent fitness costs reflected in reduced growth and reproduction. We treated individuals of both species to repeated low-dose applications of *z3*HAC and measured their growth, reproduction, and herbivore damage throughout the growing season. Based on the theory of defense priming (Frost et al., 2008a), we predicted that exposure to z3HAC—regardless of plant species identity—would reduce growth and reproductive output, while also reducing natural herbivory.

Materials and Methods

Study Site and Plants

A common garden experiment was established on a 54m^2 plot within Blackacre Conservancy's community garden in Louisville, Kentucky (38°11'33.8"N 85°31'28.3"W; Supplemental Fig. 1). The field site was enclosed in a mesh fence to exclude mammalian herbivores. *Phaseolus* *lunatus,* Fabaceae, variety Fordham Hook 242 ('lima bean') and the *Capsicum annuum*, Solanaceae, variety Cayenne pepper, Joe Red Long ('pepper') were chosen as phylogenetically distinct model plants with previously established defense profiles (Ballhorn et al., 2008, Zachariah et al., 2010). Lima bean is an annual (semelparous) species, while pepper is a perennial (iteroparous) species in its native range (USDA, NRCS, 2018). Seeds were purchased from the Louisville Seed Company (Louisville, KY, USA), and germinated in Metromix 510© in May 2016 in the Biology Department's greenhouse. After reaching ~20cm in height, 132 lima bean plants were transplanted to the field May 30, 2016, and 98 pepper plants were transplanted to the field June 28, 2016. Within the field site, plants were planted in alternating rows of twos of lima bean and pepper. Previous studies with sagebrush (Karban et al., 2006) and lima bean (Heil and Adame-Alvarez, 2010) indicate that volatile cues are effective over relatively short distances of less than 100cm. Therefore, all plants in our experiment were spaced one meter apart from one another in all directions to reduce the risk of interplant communication and cue crossover.

Volatile exposure

Plants were acclimated to the field for one week after planting before volatile treatments began. To simulate a naturally occurring low dose (Engelberth et al., 2007, Shiojiri et al., 2012), plants were exposed to lanolin infused paste equivalent to 10ng/hr of *z*3HAC. This concentration is 25% of that which previously primed poplar (Frost et al., 2008b) and maize (Engelberth et al., 2004). A treatment vial contained 50mg of a 30ng/µl *z*3HAC/lanolin, while a control vial contained 50mg of lanolin. Each glass vial had a 9mm aperture and was maintained at -80□C until use. Each week, both the *z*3HAC-infused lanolin vials and lanolin-only controls were placed at the bottom of their respective plants. Each vial was inverted and supported with a wire stand and each vial was wrapped in aluminum foil to reduce photo degradation (von Merey et al., 2011) (Supplemental Fig. 2). Plants were randomly assigned to either *z*3HAC treatment (lima bean n=63; pepper n=35) or lanolin control (lima bean n=72; pepper n=43). The unit of replication was an individual plant and each plant received its own vial. Random assignment of treatments was made using blocks of 4 adjacent plants; block was included as a random factor in statistical models, and was not a significant factor in any of the models.

Growth, biomass, and reproduction measurements

We measured height and total leaf counts routinely on the experimental plants. Leaves were only counted if they were wider than 2cm across for both species while height measurements for both species were recorded from the base of the plant to the uppermost branching point. For lima bean, height was determined by measuring the longest runner within the bush, while pepper plants were measured from the base of the main stalk to the highest branching point. Along with height, the total number of leaves per plant was measured throughout the field season. A complete biomass harvest was conducted on pepper for leaves, roots, and stems at the end of the field season. All leaves and fruits were separated into paper bags before individual plants were extracted from of the ground. After removal, roots and stems were separated, roots were washed with water to remove dirt, and placed into separate paper bags. All materials were dried at 60°C for 24 hours and then weighed. A biomass harvest for lima bean was not performed because an *Epliachna varivestis* (Mexican Bean Beetle) outbreak late in the season removed much of the leaf tissue before we could determine reliable biomass measurements.

We measured total flower and fruit production as proxies for reproductive fitness. Flowers were recorded if they were true flowers with fully mature pistils and stamen. If a flower was not fully mature, it was recorded as a flower bud. Fruits were recorded as soon as fruit development was observed with either initial pod or exocarp development. Throughout the field season, fruit and flower counts per plant were recorded along with the number of mature and immature fruits.

From the fruits harvested from the final biomass harvest, ~10 randomly selected, mature fruits from each pepper plant were chosen for seed count analysis (188 fruits from z3HAC-treated plants and 210 fruits from controls). Dried fruits were dissected with a scalpel and all seeds were isolated and counted.

Herbivory

Since previous work has shown that z3HAC enhances induced defense against both pathogens and herbivores through defense priming (Frost et al., 2008b, Ameye et al., 2015), we monitored herbivory throughout the season. Leaf chewing damage was assessed for both pepper and lima bean as percent leaf area removed (LAR) using a visual estimation technique (Frost and Hunter, 2004, Frost and Hunter, 2008) with the following damage categories: 0%, 0-5%, 5-15%, 15-30%, 30-50%, 50-70%, 70-90%, and >90%. For each damage assessment, every leaf on a plant was categorized into one of the damage categories, and an overall percent damage was determined as a weighted average of all leaves. Plants were also routinely monitored for the presence of naturally occurring chewing and piercing/sucking herbivores.   In particular, we noted a natural occurrence of the black bean aphid (*Aphis faba*), and recorded its presence/absence on lima bean plants in the field.

Statistical analyses

All statistical analyses were performed in R (version 3.4.2) with the lme4 and multcomp packages. Growth data, such as plant height, leaf area removed, and flower counts, were analyzed using repeated measures ANOVA with the aov function with a Gaussian distribution. For repeated measures analyses, we treated date as a within-subjects effect and treatment as a between-subjects effect for all analyses. Differences between treatments at each individual time point, as well as all biomass data, were analyzed using one-way ANOVA (glmer function) followed by a Tukey's post hoc comparison.

Results

Treatment with *z*3HAC differentially affected the growth of lima bean and pepper plants. On average, *z*3HAC-treated lima bean grew 11% taller compared to control plants throughout the field season (Fig.1a; $F_{2,927}=9.688$, $P=0.002$) and produced 17% more leaves overall than did controls (Fig.1b; $F_{1,571}=4.339$, $P=0.038$). In contrast, *z*3HAC-treated pepper plants were 12% shorter relative to controls (Fig.1c; $F_{1,157}=0.005$, $P=0.942$) and produced 23% fewer leaves over the field season (Fig.1d; $F_{1,237}=21.58$, $P<0.001$). Consistent with height and leaf counts, *z*3HAC treatment reduced overall biomass of pepper plants by 24% on average (Fig.2). We destructively harvested all pepper plant biomass at the end of the season. *z*3HAC-treated pepper plants had lower leaf, stem, and root dry biomass by 21%, 31%, 29%, respectively (Fig.2a-c) ($Z=3.379$, $P<0.001$; $Z=-2.035$, $P=0.042$; $Z=-2.379$, $P=0.017$). Despite these *z*3HAC-mediated effects on biomass exposure, the aboveground-to-belowground biomass ratio was similar regardless of treatment (Fig.2d; Z=0.31, *P*=0.757). That is, pepper plants treated with *z*3HAC were smaller relative to control plants.

*z*3HAC treatment also differentially affected reproductive output between the two species, and lowered fruit output in pepper. Flower production was 30% higher in lima bean plants exposed to *z*3HAC (Fig.3a; $F_{1,576}$=15.044, *P*<0.001), while *z*3HAC-treated peppers produced 37% fewer flowers relative to control plants at the end of the field season (Fig.3b; $F_{1,43}$=14.48, *P*<0.001). *z*3HAC-treated pepper plants also produced 23% fewer fruits overall relative to controls (Fig.4a; Z=-2.035, *P*=0.042), and the fruits that were produced by *z*3HAC-treated plants had lower wet and dry masses (Fig.4b-c; Z=-2.88, *P*=0.004; Z=-2.439, *P*=0.015), and 10% lower total seed counts (Fig.4d; Z=3.524, *P*<0.001) and total seed masses (Fig.4e; Z=3.334, *P*<0.001), relative to controls. Even though total fruit and seed production was reduced by *z*3HAC treatment, the ratio of seed mass to fruit mass was similar between *z*3HAC-treated and control plants (Fig.4f; Z=0.588, *P*=0.807). Moreover, the estimated mass of an individual seed was similar between *z*3HAC-treated plants and controls in pepper (Supplemental Fig.3). There was no apparent difference in lima bean pod production (Supplemental Fig.3). However, an unexpected field-wide premature pod drop that was independent of treatment prevented us from determining lima bean seed production with confidence.

*z*3HAC exposure reduced natural herbivory in lima bean but not pepper plants. Chewing herbivory on pepper plants was low throughout the season and statistically higher in *z*3HAC-treated plants; however, this effect was driven by only the first assessment date (Fig.5a, F=$_{1,193}$=5.627, *P*=0.019). In contrast, chewing damage to lima bean leaves increased as the field season progressed, with *z*3HAC-treated plants having overall 26% less chewing damage than did control plants (Fig.5b; $F_{1,539}$=21.745, *P*<0.001). In addition to chewing herbivory, black bean aphids (*Aphis faba*) colonized 87% of the *z*3HAC-treated lima bean plants, compared with only 21% of control plants (Fig.5c; $\chi^2 = 50.11$, df=1, $P<0.001$). *A.faba* colonized early in the season and was only observed June 15-31 (Julian dates 166-181) because a heavy rainfall event reduced their population to undetectable levels. Piercing/sucking herbivores were rare for the remainder of the experiment.

Discussion

We show that a persistent, low-dose application of *z*3HAC differentially affects growth and reproduction in two plant species under identical field conditions. Based on previous work on plant defense priming and sensory perception of volatiles (Frost et al., 2007, Engelberth et al., 2004), we hypothesized *z3*HAC application would decrease growth and reproductive fitness in both plant species. The rationale for this hypothesis was a central assumption of induced resistance theory that ecological costs modulate the deployment particular defensive phenotypes until necessary (Agrawal, 1999, Baldwin, 1998, Cipollini et al., 2003, Didiano et al., 2014, Koricheva, 2002, Cipollini and Heil, 2010, Mauricio, 1998). Volatile-mediated priming, even if regulated by a different mechanism from resistance (Hilker et al., 2016), is an inducible phenomenon that theoretically should incur such fitness costs (Martinez-Medina et al., 2016). Yet, our results clearly indicate that pepper and lima bean had divergent fitness outcomes when subjected to a single GLV under identical field conditions. Whereas *z3*HAC-treated pepper plants had reduced growth (Fig. 1) and no effect on herbivore resistance (Fig. 5) relative to controls, *z3*HAC-treated lima bean plants grew more and produced more flowers (Fig. 1), and suffered less chewing herbivory (Fig. 5b) compared to controls. This result—that some plants experience fitness costs while others have minimal or even positive effects when exposed to the same HIPV—has important implications for how volatile cues may structure interspecific competition and ecological communities. That is, HIPVs alone may be sufficient to influence plant communities if their presence results in differential fitness effects among species.

What might affect the response of plants to volatile exposure? One possibility is that differences in life history traits among plant species influence plant sensory perception and the outcome of defense priming. For example, short-lived semelparous (annual) species may invest more into reproductive output when exposed to herbivory (Pilson and Decker, 2002), whereas iteroparous (perennial) species may reserve resources for growth and reproduction for times when herbivores are absent (Hughes, 2017, Miller et al., 2008). Additionally, annuals may optimize reproductive output over seed quality to increase progeny success, where perennials may do the opposite (Rasmann et al., 2012, Blue et al., 2015). It is plausible that herbivore-associated cues such as *z*3HAC may induce similar divergent fitness effects between annual and perennial species as those induced by herbivory. Previous work on the role of HIPVs in plant anti-herbivore resistance focused on priming-mediated defense with consistent results between annual and perennial species: wheat (Ameye et al., 2015, Walters et al., 2008), corn (Engelberth et al., 2007, Farag et al., 2005), lima bean (Arimura et al., 2002, Choh and Takabayashi, 2006, Heil and Silva Bueno, 2007), tomato (Acevedo et al., 2017), blueberry (Rodriguez-Saona et al., 2009), sagebrush (Karban et al., 2006), and poplar (Frost et al., 2008b) all show evidence of defense priming and enhanced resistance. In contrast, we specifically focused on indicators of plant fitness in a semelparous species (lima bean) and an iteroparous species (pepper) (USDA, NRCS, 2018) in a common garden experiment. Consistent with effects observed with direct herbivory, *z*3HAC treatment alone increased growth and flowering in lima bean, while reducing growth and reproductive output in pepper (Fig. 3 and 5). Such divergent fitness effects from exposure to a single ubiquitous herbivore-associated cue underscore the potential for functional similarity in the mechanisms by which annual and perennial plants modulate responses to herbivory and volatile indicators of herbivory.

Flower and fruit production is a key component of plant fitness potential. We show that *z*3HAC treatment alone differentially affected flower production in lima bean and pepper (Fig. 3). Insect herbivory can increase or decrease floral production depending on the system and environmental conditions (Lucas-Barbosa, 2016, Agrawal et al., 1999, Pashalidou et al., 2013). Whereas increased flower production is a strategy assumed to ameliorate fitness losses in the presence of an environmental stress (Agrawal, 2000, Agrawal, 1999), decreased flower production may be related to costs of chemically mediated defense (Heath et al., 2014). Additionally, herbivory affects floral attractiveness (Halpern et al., 2010, Hoffmeister and Junker, 2016), which may ultimately influence fitness (Kessler et al., 2011). Our data indicate that a volatile cue alone is sufficient to trigger changes in floral biology, but the magnitude and direction of those changes are plant species-specific. Additionally, *z*3HAC-treated pepper produced fewer fruits (Fig. 4) but lima bean did not (Supplemental Fig. 3). The mechanisms underlying *z*3HAC-mediated effects on flower and fruit production are unknown, but may be similar to those induced by herbivory (Lucas-Barbosa, 2016).

Resource allocation between different tissues is pivotal for growth, reproduction, and defense, and can be influenced by environmental stress. For example, direct herbivory alters resource allocation between aboveground tissue and belowground tissue (Frost and Hunter, 2008, Machado et al., 2013, Eichenberg et al., 2015), as does application of the anti-herbivore phytohormone jasmonic acid (Gomez et al., 2010, Schweiger et al., 2014). Volatile cues can also affect biomass allocation. For example, barley exposed to volatiles from unwounded neighboring plants of different cultivars increases root and leaf biomass (Ninkovic, 2003), while exposure to volatiles decreases aboveground biomass in other systems (Lu et al., 2017, Cipollini, 2010). In our case, volatile treatment reduced overall aboveground and belowground biomass in pepper, but did not appear to alter overall biomass allocation patterns. In other words, *z*3HAC-treated pepper plants were smaller overall, and therefore produced fewer seeds.

Volatile cues may impact ecological communities in both expected and pleiotropic ways. HIPVs are well-established mediators of multitrophic antagonistic and mutualistic interactions (Kessler and Baldwin, 2001, Heil, 2008, Peñaflor et al., 2017), and manipulations of chemical signals and volatile blends have been used for biological control in a wide range of systems (Stenberg et al., 2015, Peñaflor and Bento, 2013). For example, HIPV-infused sticky traps in a grape (*Vitis vinifera*) orchard differentially attracted lacewings, hoverflies, and parasitoids (Lucchi et al., 2017). Exogenous GLV manipulation using "dispensers" under field conditions altered the arthropod community composition in maize (von Merey et al., 2011). In our study, *A.faba* were clearly and unexpectedly attracted to *z*3HAC-exposed plants (Fig. 5). Under glasshouse conditions, *A.faba* were repelled by *z*3HAC alone (Webster et al., 2008), which suggests that the cue that mediated attraction was not our treatment alone. It is tempting to speculate that aphid attraction combined with reduced chewing herbivory in lima bean may be reflective of *z*3HAC effects on Jasmonic Acid (JA) and Salicylic Acid (SA) signaling, which would be consistent with a JA-SA tradeoff (Huot et al., 2014, Wei et al., 2014). Ultimately, however, the utility of GLVs (or other VOCs) in field applications will depend on understanding community-level effects of the application.

As a caveat, volatile identity, concentration, and duration may affect the reliability of a cue and therefore the costs associated with eavesdropping. Plants experiencing insect herbivory frequently generate species-specific blends of volatile compounds (Ameye et al., 2017, Holopainen and Gershenzon, 2010), which can influence fitness in neighboring plants (Karban, 2017, Caparrotta et al., 2018, Kessler et al., 2006). Plant-derived compounds associated with herbivory include GLVs (Lu et al., 2017, Farag et al., 2005, Frost et al., 2008b), phenylpropanoid derivatives (Erb et al., 2015), and terpenes (Arimura et al., 2012). However, individual compounds within a blend can affect plant defense and priming as much as the blend. We used *z*3HAC in this study because it is released primarily from herbivore-damaged leaves and not just wounded leaves (Engelberth et al., 2004), which differs from other wound-released GLVs (Ameye et al., 2017, Matsui et al., 2012) and ostensibly allows *z*3HAC to confer reliable ecological information. Moreover, plants detect and respond specifically to *z*3HAC (Frost et al., 2008b), and the costs associated with that response were the focus of this investigation. Additionally, concentration of a cue may influence plant resistance (Lu et al., 2017, Bissmeyer et al., 2018). For example, a repeated, low-dose exposure to a GLV blend enhances plant resistance compared to a single application (Shiojiri et al., 2012) while *z*3HAC emissions can be as high as 66 ng/cm^3 after herbivory (Boggia et al., 2015). For these reasons, we chose to use a low-dose exposure to *z*3HAC (25% of the concentration that primed poplar (Frost et al., 2008b) and maize (Engelberth et al., 2004)), and still observed divergent fitness effects between the two plant species (Figs.1, 3, and 5).

In summary, our key finding is that a persistent application of a low dose of a single volatile compound *z*3HAC, a common HIPV and GLV, in field conditions leads to divergent growth and reproductive fitness effects between two plant species. This result underscores the variable nature of HIPV-eavesdropping, and that plants with different life histories may have evolved distinct mechanisms for responding to volatile cues. Ultimately, the adaptive significance of eavesdropping for enhancing plant immunity will depend on plant life history, physiology, and other ecological factors to determine whether a plant will benefit from eavesdropping VOCs or not, and therefore what impact priming might have on plant communities.

Acknowledgments

We are grateful to Allie Peot and Abhinav Maurya for field assistance, and to A.Peot for assistance processing the plant materials in the laboratory. Comments from Heidi Appel, Ian Kaplan, and Amy Austin greatly improved the manuscript. We are grateful to A. Dale Josey and Susan Ballerstedt for permission and logistical support to work in the community gardens at Blackacre Conservancy. This work was supported by NSF-IOS grant 1656625 to CJF.

Author Contributions

CJF conceived the study. GEF and CJF designed the experiments. GEF performed the experiments. GEF and CJF analyzed the data, wrote the paper, and gave final approval for publication.

References

Accamando, A. K. & Cronin, J. T. (2012) Costs and Benefits of Jasmonic Acid Induced Responses in Soybean. *Environmental Entomology,* **41,** 551-561.

Acevedo, F. E., Peiffer, M., Tan, C. W., Stanley, B. A., Stanley, A., Wang, J., Jones, A. G., Hoover, K., Rosa, C., Luthe, D. & Felton, G. (2017) Fall Armyworm-Associated Gut Bacteria Modulate Plant Defense Responses. *Molecular Plant-Microbe Interactions,* **30,** 127-137.

Agrawal, A. A. (1999) Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. *Ecology,* **80,** 1713-1723.

Agrawal, A. A. (2000) Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *Trends in Plant Science,* **5,** 309-313.

Agrawal, A. A., Strauss, S. Y. & Stout, M. J. (1999) Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution,* **53,** 1093-1104.

Ameye, M., Allmann, S., Verwaeren, J., Smagghe, G., Haesaert, G., Schuurink, R. C. & Audenaert, K. (2017) Green leaf volatile production by plants: a meta-analysis. *New Phytologist***,** 1-18.

Ameye, M., Audenaert, K., De Zutter, N., Steppe, K., Van Meulebroek, L., Vanhaecke, L., De Vleesschauwer, D., Haesaert, G. & Smagghe, G. (2015) Priming of Wheat with the Green Leaf Volatile Z-3-Hexenyl Acetate Enhances Defense against *Fusarium graminearum* But Boosts Deoxynivalenol Production. *Plant Physiology,* **167,** 1671-1684.

Arimura, G., Matsui, K. & Takabayashi, J. (2009) Chemical and Molecular Ecology of Herbivore-Induced Plant Volatiles: Proximate Factors and Their Ultimate Functions. *Plant and Cell Physiology,* **50,** 911-923.

Arimura, G., Muroi, A. & Nishihara, M. (2012) Plant-plant-plant communications, mediated by (E)-beta-ocimene emitted from transgenic tobacco plants, prime indirect defense responses of lima beans. *Journal of Plant Interactions,* **7,** 193-196.

Arimura, G., Ozawa, R., Nishioka, T., Boland, W., Koch, T., Kuhnemann, F. & Takabayashi, J. (2002) Herbivore-induced volatiles induce the emission of ethylene in neighboring lima bean plants. *Plant Journal,* **29,** 87-98.

Arimura, G. I., Kopke, S., Kunert, M., Volpe, V., David, A., Brand, P., Dabrowska, P., Maffei, M. E. & Boland, W. (2008) Effects of feeding *Spodoptera littoralis* on lima bean leaves: IV. Diurnal and nocturnal damage differentially initiate plant volatile emission. *Plant Physiology,* **146,** 965-973.

Baldwin, I. T. (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences of the United States of America,* **95,** 8113-8118.

Ballhorn, D. J., Schiwy, S., Jensen, M. & Heil, M. (2008) Quantitative Variability of Direct Chemical Defense in Primary and Secondary Leaves of Lima Bean (*Phaseolus lunatus*) and Consequences for a Natural Herbivore. *Journal of Chemical Ecology,* **34,** 1298-1301.

Bissmeyer, S., Freundlich, G. & Frost, C. (2018) The influence of dose of a plant-derived volatile cue on *Arabidopsis thaliana* resistance against

insect herbivores. *Kentucky Journal of Undergraduate Research,* **2,** 84-95.

Blue, E., Kay, J., Younginger, B. S. & Ballhorn, D. J. (2015) Differential effects of type and quantity of leaf damage on growth, reproduction and defence of lima bean (*Phaseolus lunatus* L.). *Plant Biology,* **17,** 712-719.

Boggia, L., Sgorbini, B., Bertea, C. M., Cagliero, C., Bicchi, C., Maffei, M. E. & Rubiolo, P. (2015) Direct Contact-Sorptive Tape Extraction coupled with Gas Chromatography-Mass Spectrometry to reveal volatile topographical dynamics of lima bean (*Phaseolus lunatus* L.) upon herbivory by *Spodoptera littoralis* Boisd. *BMC Plant Biology,* **15,** 1-13.

Caparrotta, S., Boni, S., Taiti, C., Palm, E., Mancuso, S. & Pandolfi, C. (2018) Induction of priming by salt stress in neighboring plants. *Environmental and Experimental Botany,* **147,** 261-270.

Choh, Y. & Takabayashi, J. (2006) Herbivore-induced extrafloral nectar production in lima bean plants enhanced by previous exposure to volatiles from infested conspecifics. *Journal of Chemical Ecology,* **32,** 2073-2077.

Cipollini, D. (2010) Constitutive expression of methyl jasmonate-inducible responses delays reproduction and constrains fitness responses to nutrients in *Arabidopsis thaliana*. *Evolutionary Ecology,* **24,** 59-68.

Cipollini, D. & Heil, a. M. (2010) Costs and benefits of induced resistance to herbivores and pathogens in plants. *CAB International,* **5,** 1-25.

Cipollini, D., Purrington, C. B. & Bergelson, J. (2003) Costs of induced responses in plants. *Basic and Applied Ecology,* **4,** 79-89.

Copolovici, L., Kannaste, A., Remmel, T., Vislap, V. & Niinemets, U. (2011) Volatile Emissions from *Alnus glutionosa* Induced by Herbivory are Quantitatively Related to the Extent of Damage. *Journal of Chemical Ecology,* **37,** 18-28.

D'Auria, J. C., Pichersky, E., Schaub, A., Hansel, A. & Gershenzon, J. (2007) Characterization of a BAHD acyltransferase responsible for producing the green leaf volatile (Z)-3-hexen-1-yl acetate in *Arabidopsis thaliana*. *Plant Journal,* **49,** 194-207.

Didiano, T. J., Turley, N. E., Everwand, G., Schaefer, H., Crawley, M. J. & Johnson, M. T. J. (2014) Experimental test of plant defence evolution in four species using long-term rabbit exclosures. *Journal of Ecology,* **102,** 584-594.

Douma, J. C., Vermeulen, P. J., Poelman, E. H., Dicke, M. & Anten, N. P. R. (2017) When does it pay off to prime for defense? A modeling analysis. *New Phytologist,* **216,** 782-797.

Eichenberg, D., Purschke, O., Ristok, C., Wessjohann, L. & Bruelheide, H. (2015) Trade-offs between physical and chemical carbon-based leaf defence: of intraspecific variation and trait evolution. *Journal of Ecology,* **103,** 1667-1679.

Engelberth, J., Alborn, H. T., Schmelz, E. A. & Tumlinson, J. H. (2004) Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences of the United States of America,* **101,** 1781-1785.

Engelberth, J., Seidl-Adams, I., Schultz, J. C. & Tumlinson, J. H. (2007) Insect elicitors and exposure to green leafy volatiles differentially upregulate major octadecanoids and transcripts of 12-oxo phytodienoic acid reductases in Zea mays. *Molecular Plant-Microbe Interactions,* **20,** 707-716.

Erb, M., Veyrat, N., Robert, C. A. M., Xu, H., Frey, M., Ton, J. & Turlings, T. C. J. (2015) Indole is an essential herbivore-induced volatile priming signal in maize. pp. 1-10. *Nature communications.*

Farag, M. A., Fokar, M., Zhang, H. A., Allen, R. D. & Pare, P. W. (2005) (Z)-3-Hexenol induces defense genes and downstream metabolites in maize. *Planta,* **220,** 900-909.

Farag, M. A. & Paré, P. W. (2002) C6-Green leaf volatiles trigger local and systemic VOC emissions in tomato. *Phytochemistry,* **61,** 545-554.

Farag, M. A., Zhang, H. M. & Ryu, C. M. (2013) Dynamic Chemical Communication between Plants and Bacteria through Airborne Signals: Induced Resistance by Bacterial Volatiles. *Journal of Chemical Ecology*, **39,** 1007-1018.

Frost, C. J., Appel, M., Carlson, J. E., De Moraes, C. M., Mescher, M. C. & Schultz, J. C. (2007) Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. *Ecology Letters,* **10,** 490-498.

Frost, C. J. & Hunter, M. D. (2004) Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. *Ecology*, **85,** 3335-3347.

Frost, C. J. & Hunter, M. D. (2008) Herbivore-induced shifts in carbon and nitrogen allocation in red oak seedlings. *New Phytologist,* **178,** 835-845.

Frost, C. J., Mescher, M. C., Carlson, J. E. & De Moraes, C. M. (2008a) Plant defense priming against herbivores: Getting ready for a different battle. *Plant Physiology*, **146,** 818-824.

Frost, C. J., Mescher, M. C., Dervinis, C., Davis, J. M., Carlson, J. E. & De Moraes, C. M. (2008b) Priming defense genes and metabolites in hybrid poplar by the green leaf volatile cis-3-hexenyl acetate. *New Phytologist*, **180,** 722-733.

Gomez, S., Ferrieri, R. A., Schueller, M. & Orians, C. M. (2010) Methyl jasmonate elicits rapid changes in carbon and nitrogen dynamics in tomato. *New Phytologist*, **188,** 835-844.

Halpern, S. L., Adler, L. S. & Wink, M. (2010) Leaf herbivory and drought stress affect floral attractive and defensive traits in *Nicotiana quadrivalvis*. *Oecologia*, **163,** 961-971.

Heath, J. J., Kessler, A., Woebbe, E., Cipollini, D. & Stireman, J. O. (2014) Exploring plant defense theory in tall goldenrod, *Solidago altissima*. *New Phytologist*, **202,** 1357-1370.

Heil, M. (2008) Indirect defence via tritrophic interactions. *New Phytologist*, **178,** 41-61.

Heil, M. & Adame-Alvarez, R. M. (2010) Short signalling distances make plant communication a soliloquy. *Biology Letters,* **6,** 843-845.

Heil, M. & Karban, R. (2010) Explaining evolution of plant communication by airborne signals. *Trends in Ecology & Evolution*, **25,** 137-144.

Heil, M. & Silva Bueno, J. C. (2007) Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proceedings of the National Academy of Sciences of the United States of America*, **104,** 5467-5472.

Hilker, M., Schwachtje, J., Baier, M., Balazadeh, S., Baurle, I., Geiselhardt, S., Hincha, D. K., Kunze, R., Mueller-Roeber, B., Rillig, M. G., Rolff, J., Romeis, R., Schmulling, T., Steppuhn, A., van Dongen, J., Whitcomb, S. J., Wurst, S., Zuther, E. & Kopka, J. (2016) Priming and memory of stress responses in organisms lacking a nervous system. *Biological Reviews,* **91,** 1118-1133.

Hoffmeister, M. & Junker, R. R. (2016) Herbivory-induced changes in the olfactory and visual display of flowers and extrafloral nectaries affect pollinator behavior. *Evolutionary Ecology***,** 1-16.

Holopainen, J. K. & Gershenzon, J. (2010) Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science,* **15,** 176-184.

Hughes, P. W. (2017) Between semelparity and iteroparity: Empirical evidence for a continuum of modes of parity. *Ecology and Evolution,* **7,** 8232-8261.

Huot, B., Yao, J., Montgomery, B. L. & He, S. Y. (2014) Growth-Defense Tradeoffs in Plants: A Balancing Act to Optimize Fitness. *Molecular Plant*, **7,** 1267-1287.

Karban, R. (2017) Tradeoff between resistance induced by volatile communication and over-topping vertical growth. *Plant Signaling & Behavior*, 1-5.

Karban, R. & Maron, J. (2002) The fitness consequences of interspecific eavesdropping between plants. *Ecology*, **83,** 1209-1213.

Karban, R., Shiojiri, K., Huntzinger, M. & McCall, A. C. (2006) Damage-induced resistance in sagebrush: Volatiles are key to intra- and interplant communication. *Ecology*, **87,** 922-930.

Kessler, A. & Baldwin, I. T. (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, **291,** 2141-2144.

Kessler, A., Halitschke, R., Diezel, C. & Baldwin, I. T. (2006) Priming of plant defense responses in nature by airborne signaling between *Artemisia tridentata* and *Nicotiana attenuata*. *Oecologia*, **148,** 280-292.

Kessler, A., Halitschke, R. & Poveda, K. (2011) Herbivory-mediated pollinator limitation: negative impacts of induced volatiles on plant-pollinator interactions. *Ecology*, **92,** 1769-1780.

Koricheva, J. (2002) Meta-analysis of sources of variation in fitness costs of plant antiherbivore defenses. *Ecology*, **83,** 176-190.

Lu, H. B., Xu, S. Y., Zhang, W. J., Xu, C. M., Li, B. X., Zhang, D. X., Mu, W. & Liu, F. (2017) Nematicidal Activity of trans-2-Hexenal against Southern Root-Knot Nematode (*Meloidogyne incognita*) on Tomato Plants. *Journal of Agricultural and Food Chemistry*, **65,** 544-550.

Lucas-Barbosa, D. (2016) Integrating Studies on Plant-Pollinator and Plant-Herbivore Interactions. *Trends in Plant Science*, **21,** 125-133.

Lucchi, A., Loni, A., Gandini, L. M., Scaramozzino, P., Ioriatti, C., Ricciardi, R. & Shearer, P. W. (2017) Using herbivore-induced plant volatiles to attract lacewings, hoverflies and parasitoid wasps in vineyards: achievements and constraints. *Bulletin of Insectology*, **70,** 273-282.

Machado, R. A. R., Ferrieri, A. P., Robert, C. A. M., Glauser, G., Kallenbach, M., Baldwin, I. T. & Erb, M. (2013) Leaf-herbivore attack reduces carbon reserves and regrowth from the roots via jasmonate and auxin signaling. *New Phytologist*, **200,** 1234-1246.

Martinez-Medina, A., Flors, V., Heil, M., Mauch-Mani, B., Pieterse, C. M. J., Pozo, M. J., Ton, J., van Dam, N. M. & Conrath, U. (2016) Recognizing Plant Defense Priming. *Trends in Plant Science*, **21,** 818-822.

Matsui, K., Sugimoto, K., Mano, J. i., Ozawa, R. & Takabayashi, J. (2012) Differential Metabolisms of Green Leaf Volatiles in Injured and Intact Parts of a Wounded Leaf Meet Distinct Ecophysiological Requirements. *Plos One*, **7**.

Mauricio, R. (1998) Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana*. *American Naturalist*, **151,** 20-28.

McCormick, A. C., Irmisch, S., Reinecke, A., Boeckler, G. A., Veit, D., Reichelt, M., Hansson, B. S., Gershenzon, J., Kollner, T. G. & Unsicker, S. B. (2014) Herbivore-induced volatile emission in black poplar: regulation and role in attracting herbivore enemies. *Plant Cell and Environment*, **37,** 1909-1923.

Miller, T. E. X., Tenhumberg, B. & Louda, S. M. (2008) Herbivore-mediated ecological costs of reproduction shape the life history of an iteroparous plant. *American Naturalist*, **171,** 141-149.

Muhlemann, J. K., Klempien, A. & Dudareva, N. (2014) Floral volatiles: from biosynthesis to function. *Plant Cell and Environment,* **37,** 1936-1949.

Mumm, R. & Dicke, M. (2010) Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **88,** 628-667.

Ninkovic, V. (2003) Volatile communication between barley plants affects biomass allocation. *Journal of Experimental Botany,* **54,** 1931-1939.

Orrock, J., Connolly, B., Choi, W., Guiden, P., Swanson, S., & Gilroy, S. (2018) Plants eavesdrop on cues produced by snails and induce costly defenses that affect insect herbivores. *Oecologia*, **186,** 703-710.

Paschold, A., Halitschke, R. & Baldwin, I. T. (2006) Using 'mute' plants to translate volatile signals. *Plant Journal,* **45,** 275-291.

Pashalidou, F. G., Lucas-Barbosa, D., van Loon, J. J. A., Dicke, M. & Fatouros, N. E. (2013) Phenotypic plasticity of plant response to herbivore eggs: effects on resistance to caterpillars and plant development. *Ecology,* **94,** 702-713.

Pastor, V., Balmer, A., Gamir, J., Flors, V. & Mauch-Mani, B. (2014) Preparing to fight back: generation and storage of priming compounds. *Frontiers in Plant Science*, **5**.

Pearse, I. S., Hughes, K., Shiojiri, K., Ishizaki, S. & Karban, R. (2013) Interplant volatile signaling in willows: revisiting the original talking trees. *Oecologia,* **172,** 869-875.

Peñaflor, M. F. G. V. & Bento, J. M. S. (2013) Herbivore-induced plant volatiles to enhance biological control in agriculture. *Neotropical Entomology,* **42,** 331-343.

Peñaflor, M. F. G. V., Gonçalves, F. G., Colepicolo, C., Sanches, P. A. & Bento, J. M. S. (2017) Effects of single and multiple herbivory by host and non-host caterpillars on the attractiveness of herbivore-induced volatiles of sugarcane to the generalist parasitoid *Cotesia flavipes*. *Entomologia Experimentalis et Applicata*, **165,** 83-93.

Pilson, D. & Decker, K. L. (2002) Compensation for herbivory in wild sunflower: Response to simulated damage by the head-clipping weevil. *Ecology,* **83,** 3097-3107.

Rasmann, S., De Vos, M., Casteel, C. L., Tian, D. L., Halitschke, R., Sun, J. Y., Agrawal, A. A., Felton, G. W. & Jander, G. (2012) Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiology,* **158,** 854-863.

Rodriguez-Saona, C. R., Rodriguez-Saona, L. E. & Frost, C. J. (2009) Herbivore-induced volatiles in the perennial shrub, *Vaccinium corymbosum*, and their role in inter-branch signaling. *Journal of Chemical Ecology,* **35,** 163-175.

Sarai Giron-Calva, P., Molina-Torres, J. & Heil, M. (2012) Volatile dose and exposure time impact perception in neighboring plants. *Journal of Chemical Ecology,* **38,** 226-228.

Schweiger, R., Heise, A. M., Persicke, M. & Muller, C. (2014) Interactions between the jasmonic and salicylic acid pathway modulate the plant metabolome and affect herbivores of different feeding types. *Plant Cell and Environment*, **37,** 1574-1585.

Shiojiri, K., Ozawa, R., Matsui, K., Sabelis, M. W. & Takabayashi, J. (2012) Intermittent exposure to traces of green leaf volatiles triggers a plant response. *Scientific Reports,* **2**.

Song, G. C. & Ryu, C. M. (2013) Two volatile organic compounds trigger plant self-defense against a bacterial pathogen and a sucking insect in cucumber under open field conditions. *International Journal of Molecular Sciences*, **14,** 9803-9819.

Stenberg, J. A., Heil, M., Ahman, I. & Bjorkman, C. (2015) Optimizing crops for biocontrol of pests and disease. *Trends in Plant Science,* **20,** 698-712.

Turlings, T. C. J., Loughrin, J. H., McCall, P. J., Rose, U. S. R., Lewis, W. J. & Tumlinson, J. H. (1995) How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America,* **92,** 4169-4174.

van Hulten, M., Pelser, M., van Loon, L. C., Pieterse, C. M. J. & Ton, J. (2006) Costs and benefits of priming for defense in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, **103,** 5602-5607.

von Merey, G., Veyrat, N., Mahuku, G., Valdez, R. L., Turlings, T. C. J. & D'Alessandro, M. (2011) Dispensing synthetic green leaf volatiles in maize fields increases the release of sesquiterpenes by the plants, but has little effect on the attraction of pest and beneficial insects. *Phytochemistry,* **72,** 1838-1847.

Walters, D. R., Paterson, L., Walsh, D. J. & Havis, N. D. (2008) Priming for plant defense in barley provides benefits only under high disease pressure. *Physiological and Molecular Plant Pathology*, **73,** 95-100.

Webster, B., Bruce, T., Dufour, S., Birkemeyer, C., Birkett, M., Hardie, J. & Pickett, J. (2008) Identification of volatile compounds used in host location by the black bean aphid, *Aphis fabae*. *Journal of Chemical Ecology*, **34,** 1153-1161.

Wei, J., van Loon, J. J. A., Gols, R., Menzel, T. R., Li, N., Kang, L. & Dicke, M. (2014) Reciprocal crosstalk between jasmonate and salicylate defence-signalling pathways modulates plant volatile emission and herbivore host-selection behaviour. *Journal of Experimental Botany*, **65,** 3289-3298.

Zachariah, T. J., Safeer, A. L., Jayarajan, K., Leela, N. K., Vipin, T. M., Saji, K. V., Shiva, K. N., Parthasarathy, V. A. & Mammootty, K. P. (2010) Correlation of metabolites in the leaf and berries of selected black pepper varieties. *Scientia Horticulturae,* **123,** 418-422.

Figures

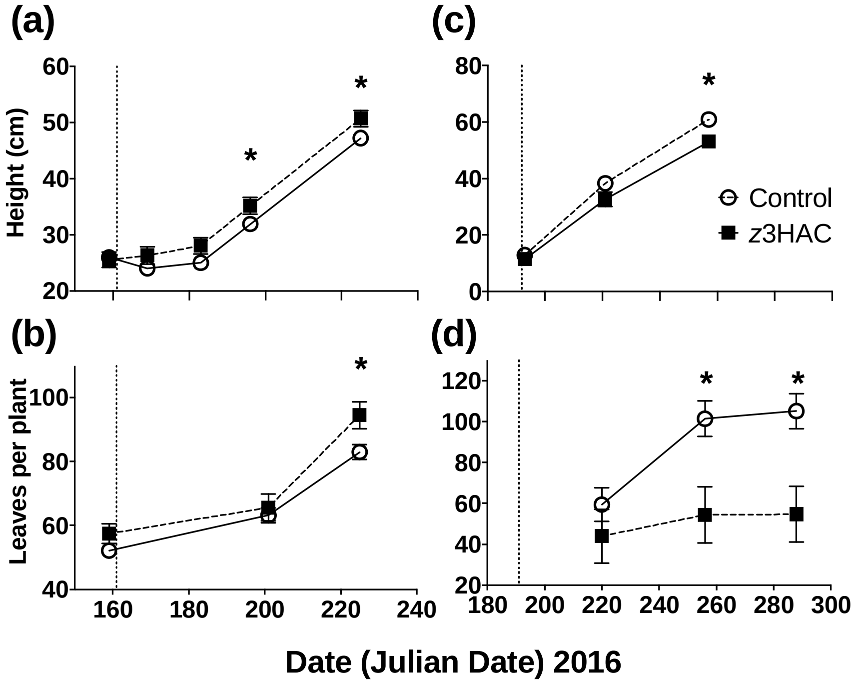

Figure 1. Height measurements and leaf counts for *Phaseolus lunatus* (lima bean) and *Capsicum annuum* (pepper) grown in a common garden field experiment. Height for (a) lima bean was measured from the base of the longest runner to the uppermost branching point; (c) pepper height was measured from the base of the main stalk to the uppermost branching point. Leaf counts for (b) lima bean and (d) pepper included all mature leaves on each plant. Open circles represent control plants (receiving lanolin-filled vials); filled squares represent plants receiving a persistent application of vials containing 10ng/hr *cis*-3-hexenyl acetate (*z*3HAC) dissolved in lanolin. Dropdown lines indicate the initial application of *z*3HAC treatment: lima bean and pepper plants were first exposed on June 10, 2016 (Julian date 161) and July 11, 2016 (Julian date 192), respectively. Points represent averages +/- SE. Repeated measures ANOVAs (aov in R) were followed by one-way ANOVAs at each time-point. Asterisks (*) represent *P*<0.05 between treatment and control at each time point. See Supplemental Table 1 for complete statistics.

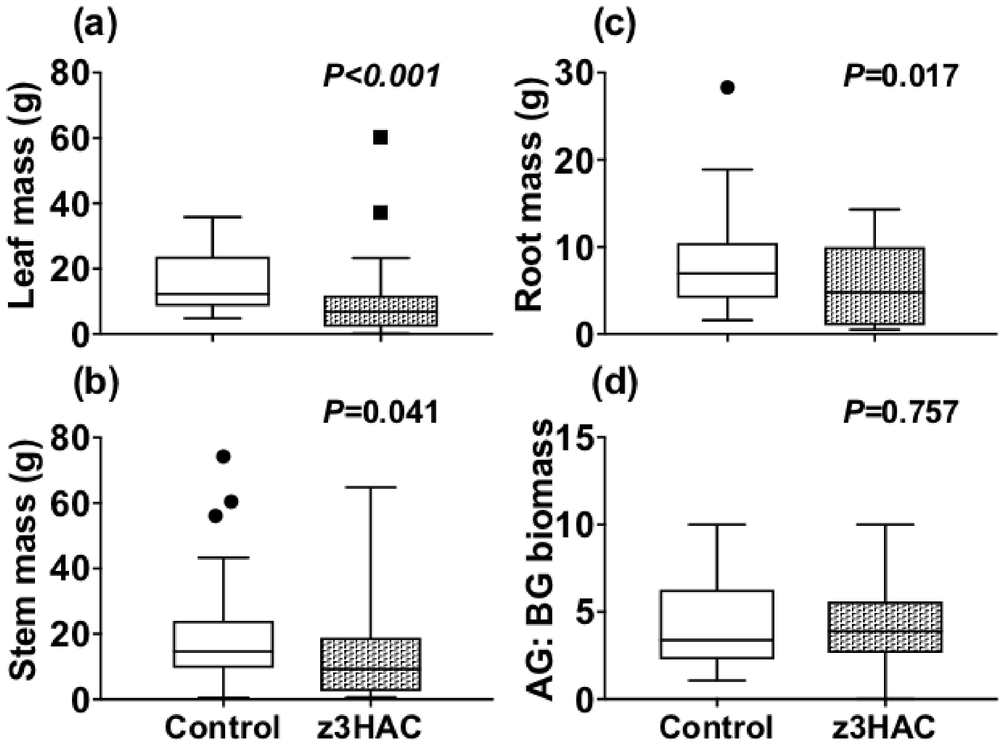

Figure 2. Biomass measurements of field-grown *Capsicum annuum* (pepper) plants. (a) Leaf, (b) stem, (c) root biomass, and (d) the aboveground:belowground biomass ratio in *C.annuum* plants were determined at the end of the field season following destructive harvest. Box plots represent the raw data ranging from the upper to the lower quartiles and the median. Error bars represent the 5% and 95% of the data, and individual dots are observations that fell outside of those parameters. *P*-values represent Tukey's HSD comparisons. See Supplemental Table 2 for complete statistics.

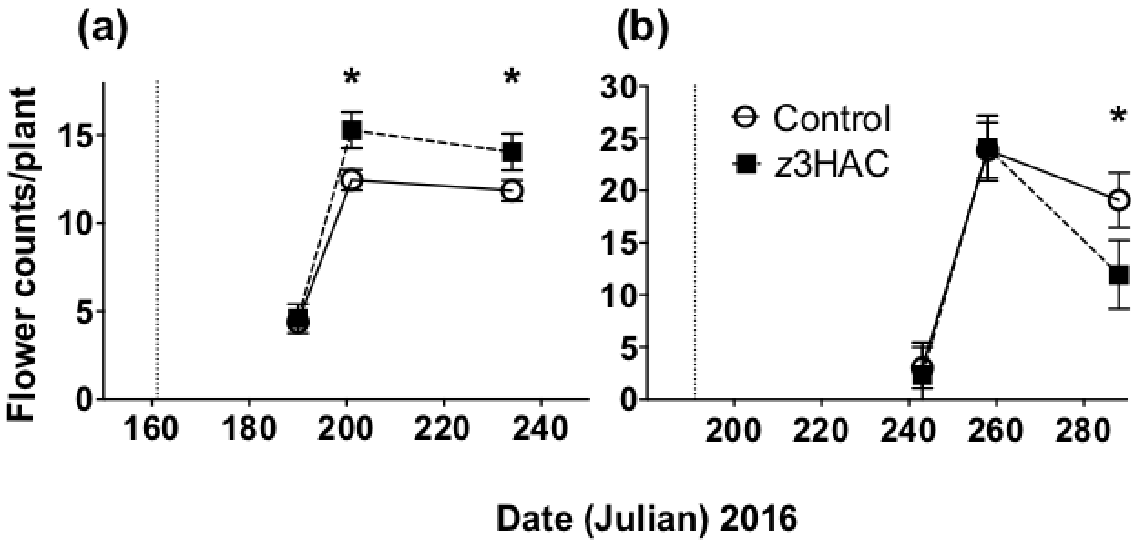

Figure 3. Total flower production in (a) *Phaseolus lunatus* (lima bean) and (b) *Capsicum annuum* (pepper). Open circles represent control plants (receiving lanolin-only vials); filled squares represent plants receiving a persistent application of vials containing 10ng/hr *cis*-3-hexenyl acetate (*z*3HAC) dissolved in lanolin. Dropdown lines indicate the initial application of z3HAC treatment: lima bean and pepper plants were first exposed on June 10, 2016 (Julian date 161) and July 11, 2016 (Julian date 192), respectively. Points represent averages +/- SE. Asterisks (*) represent *P*<0.05 between treatment and control at each time point. See Supplemental Table 3 for complete statistics.

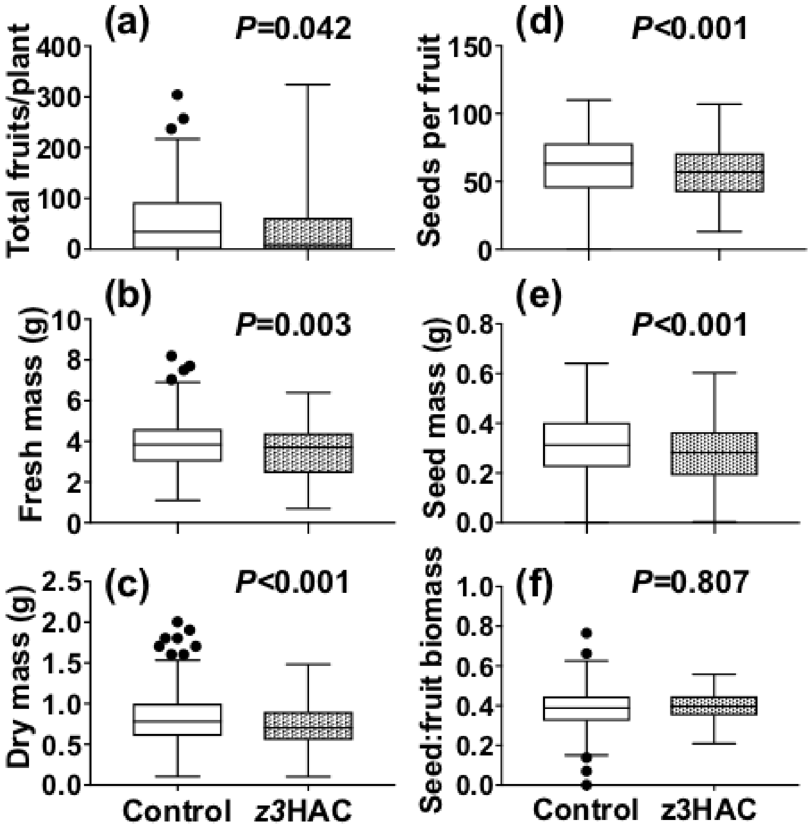

Figure 4. Fruit and seed production in *Capsicum annuum* (pepper) plants grown in a common garden experiment treated with a persistent application of the green leaf volatile *cis*-3-hexenyl acetate (*z*3HAC). The (a) total number of fruits were counted in the field, and (b) wet and (c) dry masses fruit masses were determined in the lab. (d) The total number of seeds per fruit and (e) the estimated mass per seed were determined from a subset of the fruits produced. (f) The ratio of seed mass to fruit mass was calculated to assess the efficiency of seed production. Box plots represent the raw data ranging from the upper to the lower quartiles and the median. White boxes represent control plants; gray boxes represent plants treated with *z*3HAC. Error bars represent the 5% and 95% of the data, and individual dots are observations that fell outside of those parameters. *P*-values represent Tukey's HSD comparisons. See Supplemental Table 4 for complete statistics.

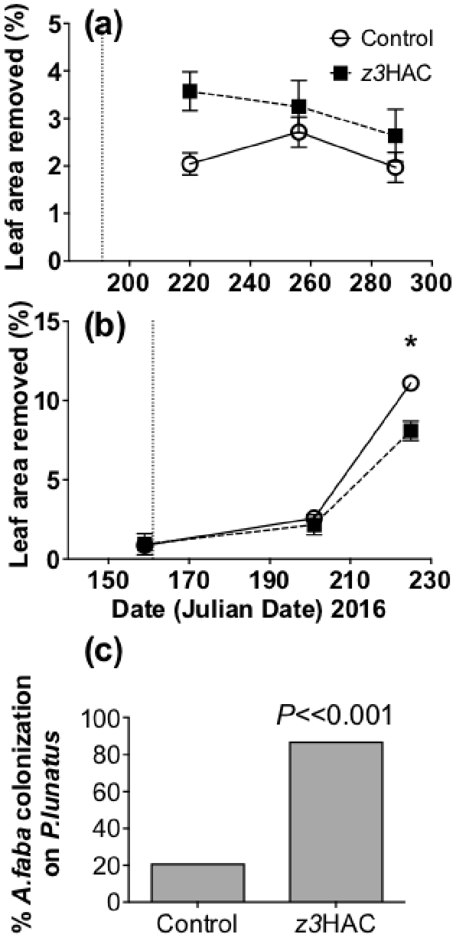

Figure 5. Herbivore damage on *Capsicum annuum* (pepper) and *Phaseolus lunatus* (lima bean) plants in a common garden field experiment. Chewing damage on (a) pepper and (b) lima bean plants was determined using a visual estimation technique (see Methods). Open circles represent control plants (receiving lanolin-filled vials); filled squares represent plants receiving a persistent application 10ng/hr *cis*-3-hexenyl acetate (z3HAC) dissolved in lanolin. Dropdown lines indicate the initial application of z3HAC treatment. Points represent averages +/- SE. Repeated measures ANOVAs (aov in R) were followed by one-way ANOVAs at each time-point. Asterisks (*) represent *P*<0.05 between treatment and control at each time point. See Supplemental Table 5 for complete statistics. (c) *Aphis faba* colonization on lima bean plants. Bars represent the percentage of plants in each group where *A.faba* were observed ($\chi^2 = 50.11$, df=1, *P*<0.001).