

Article

Dispensing a Synthetic Green Leaf Volatile to Two Plant Species in a Common Garden Differentially Alters Physiological Responses and Herbivory

Grace E. Freundlich ¹, Maria Shields ¹ and Christopher J. Frost ^{1,2,*}

¹ Department of Biology, University of Louisville, Louisville, KY 40292, USA; grace.freundlich@louisville.edu (G.E.F.); maria.shields@louisville.edu (M.S.)

² BIO5 Institute, University of Arizona, Tucson, AZ 85721, USA

* Correspondence: jasmonate@gmail.com



Citation: Freundlich, G.E.; Shields, M.; Frost, C.J. Dispensing a Synthetic Green Leaf Volatile to Two Plant Species in a Common Garden Differentially Alters Physiological Responses and Herbivory. *Agronomy* **2021**, *11*, 958. <https://doi.org/10.3390/agronomy11050958>

Academic Editors: Patrick Pageat and Andrea Clavijo McCormick

Received: 29 March 2021

Accepted: 5 May 2021

Published: 12 May 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Herbivore-induced plant volatile (HIPV)-mediated eavesdropping by plants is a well-documented, inducible phenomenon that has practical agronomic applications for enhancing plant defense and pest management. However, as with any inducible phenomenon, responding to volatile cues may incur physiological and ecological costs that limit plant productivity. In a common garden experiment, we tested the hypothesis that exposure to a single HIPV would decrease herbivore damage at the cost of reduced plant growth and reproduction. Lima bean (*Phaseolus lunatus*) and pepper (*Capsicum annuum*) plants were exposed to a persistent, low dose (~10 ng/h) of the green leaf volatile *cis*-3-hexenyl acetate (z3HAC), which is a HIPV and damage-associated volatile. z3HAC-treated pepper plants were shorter, had less aboveground and belowground biomass, and produced fewer flowers and fruits relative to controls, while z3HAC-treated lima bean plants were taller and produced more leaves and flowers than did controls. Natural herbivory was reduced in z3HAC-exposed lima bean plants, but not in pepper. Cyanogenic potential, a putative direct defense mechanism in lima bean, was lower in young z3HAC-exposed leaves, suggesting a growth–defense tradeoff from z3HAC exposure alone. Plant species-specific responses to an identical volatile cue have important implications for agronomic costs and benefits of volatile-mediated interplant communication under field conditions.

Keywords: *Phaseolus lunatus*; *Capsicum annuum* var; Cayenne; Fabaceae; Solanaceae; common garden; field study; *cis*-3-hexenyl acetate

1. Introduction

Production and utilization of airborne chemical cues are prevalent within the plant kingdom. Plants depend on airborne chemical signaling for pollination [1], indirect defense [2], protection from pathogens [3], and herbivore resistance [4]. 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 [5]. Such herbivore-induced plant volatiles (HIPVs) are potentially reliable cues around which plant–plant eavesdropping could be evolutionarily adaptive [6]. Undamaged plants (or parts of the same plant [7,8]) eavesdropping on HIPVs from a plant experiencing herbivory may directly trigger stress responses [9–11], or alternatively prime responses for future potential herbivory [4,7].

HIPV-mediated eavesdropping appears to be a common phenomenon. For example, HIPVs prime or induce corn [12,13], tomato [14], poplar [4,7], blueberry [15] and lima bean [16,17] against herbivory. HIPVs can be diverse and taxa specific [18,19], but are often comprised of monoterpenes, sesquiterpenes, benzenoids and green leaf volatiles

(GLVs) [20,21]. In contrast to volatile terpenes and benzenoids [18,22], GLVs are immediately released into the airspace whenever leaves are mechanically damaged [23], serving as early indicators of wounding and herbivory. GLV exposure alters gene expression profiles related to specialized metabolite production and accumulated secondary metabolite precursors in preparation for inducing resistance [24]. For example, the GLV *cis*-3-hexenyl acetate (z3HAC) induces transcriptional changes in poplar [4] and maize [21] that prime oxylipin signaling and induced resistance. Like other GLVs, z3HAC can be emitted from wounded leaves alone, but may also represent a reliable cue because it is typically released from herbivore-damaged leaves in a variety of species [23], including tomato [14], maize [21], *Arabidopsis* [25], lima bean [8,17], pepper [26,27], and poplar [4,28].

Costs incurred by plants responding to airborne HIPV cues alone are largely unknown. Plant defense 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 [29,30]. However, inducible resistance generates a period of vulnerability between the time of attack and the upregulation of resistance [31]. Perception of early reliable cues may overcome such a vulnerability by allowing a plant to anticipate a probable attack and prime defenses before herbivory occurs. Since HIPV-mediated priming is an inducible phenomenon, theory predicts that responding to reliable cues alone should incur costs that outweigh and select against maintaining a “primed state” [32,33]. In other words, perception of a priming stimulus, such as a HIPV, may induce physiological changes that incur costs that are less expensive than induced resistance itself. Previous work with non-volatile priming agents β -amino butyric acid (BABA) [34] and snail mucus [35] both support this prediction. Similarly, costs associated with volatile perception alone that initiate priming should be less severe than costs of induced resistance to actual herbivory [36]. Yet, there is currently limited experimental evidence of such costs with respect to anti-herbivore volatile cues. For example, bacterial-derived volatiles 3-pentanol and 2-butanone increased fresh fruit weight in field-grown *Cucumis sativa* [37], wild tobacco (*Nicotiana attenuata*) exposed to airspace of experimentally clipped sagebrush produce more seeds (i.e., higher presumptive fitness) relative to control plants [38]. 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 annuum*) testing the hypothesis that field plants subject to a persistent dose of an ostensibly reliable volatile cue incur consistent costs reflected in reduced growth and reproduction. We treated individuals of both species to repeated low-dose applications of z3HAC and measured their growth, reproduction, and herbivore damage throughout the growing season. We predicted that exposure to z3HAC—regardless of plant species identity—would reduce growth and reproductive output, while also reducing natural herbivory.

2. Materials and Methods

2.1. Study Site and Plants

A common garden experiment was established on a 54 m² plot within Blackacre Conservancy’s community garden in Louisville, Kentucky (38°11′33.8″ N 85°31′28.3″ W; Supplemental Figure S1). 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 [39,40]. 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 ~20 cm in height, 132 lima bean plants were transplanted to the field 30 May 2016, at 4 weeks old and 98 pepper plants at 8 weeks old were transplanted to the field on 28 June 2016. While both species were started at the same time, peppers were placed in the field later than the lima beans because they needed additional maturation

time before transferring to the field. Within the field site, plants were planted in alternating rows of twos of lima bean and pepper. Distance limitations exist regarding plant volatile perception [41] and herbivore resistance [7,42]. Previous studies with sagebrush [43] and lima bean [44] indicate that volatile cues are effective over relatively short distances of less than 100 cm. 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.

2.2. Volatile Exposure Manipulations

Plants were acclimated to the field for one week after planting before volatile treatments began. To simulate a naturally occurring low dose [45,46], plants were exposed to lanolin infused with the equivalent of “headspace” z3HAC concentrations of 10 ng/h, a concentration 25% of that which previously primed poplar [4] and maize [21]. A treatment vial contained 50 mg of a 30 ng/ μ L z3HAC/lanolin, while a control vial contained 50 mg of lanolin. Each glass vial had a 9 mm aperture and was maintained at -80°C until use. Each week, both the z3HAC-infused lanolin vials and lanolin-only control vials were placed at the bottom of their respective plants. Because of the growth habit of the lima bean and pepper plants, we opted to place the vials closer to the ground ($\sim 3\text{--}5$ cm) than has been done with previous VOC dispensing studies (e.g., 70 cm stakes with maize [47]). Each vial was inverted and supported with a wire stand and each vial was wrapped in aluminum foil to reduce photodegradation [47] (Supplemental Figure S2). These vials were left in the field and replaced every seven days for the duration of the field season (May–October 2016). Plants were randomly assigned to either z3HAC 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 initially included as a random factor in statistical models, but was not a significant factor.

We confirmed our z3HAC treatment and 1 m plant spacing experimental design by conducting “recovery” open-air volatile collections without plants using four independent vials containing z3HAC (Figure 1). These vials were placed in the field in 4 separate locations and open-air collections were made with VOC filters placed 0.1 m and 0.5 m from each vial. The glass/teflon filters contained ~ 30 mg PorapakQ, and were collected “pull only” with a 12V diaphragm vacuum pump (Karlsson Robotics) with ~ 3 L/min. In the lab, each filter was eluted with 150 μ L dichloromethane containing 10 ng/ μ L nonyl acetate as an internal standard [7,15], and analyzed with an Agilent 7890B Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA) in splitless mode with an inlet temperature of 250°C and a DB-5 column (30 m length, 0.25 mm diameter, with a built-in 10 m DuraGuard pre-column). The initial oven temperature was 35°C for sample injection and then increased 15°C per minute to 250°C with helium as a carrier gas at an average velocity of 22.5 cm per second. z3HAC and the internal standard were detected with an Agilent 5977A Mass Spectrometer with an EI ion source with the MS in scanning mode (50–550 m/z) and transfer line and ion source temperatures set at 230 and 150°C . Peak areas within a sample were determined after peak deconvolution using the MassHunter software suite (Agilent).

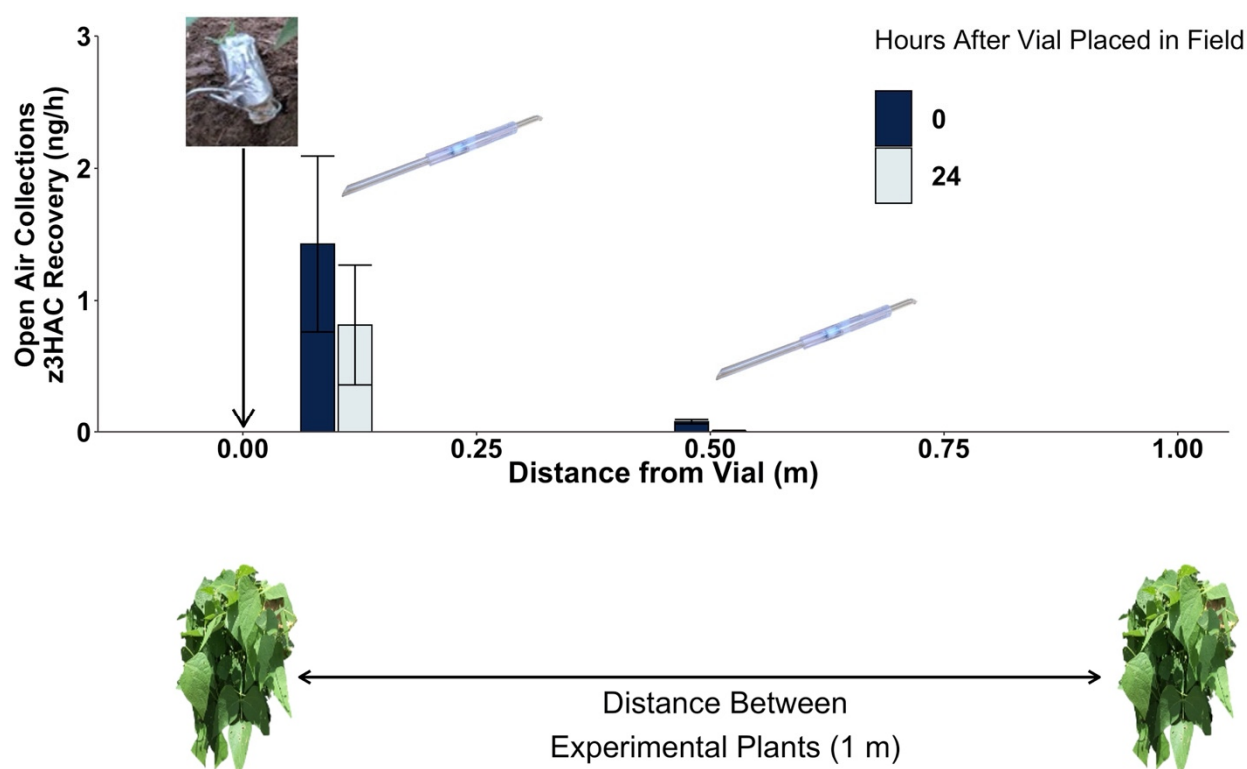


Figure 1. Recovery of *cis*-3-hexenyl acetate (z3HAC) in open-air collections. Volatile collection filters were placed 0.1 m and 0.5 m from experimental z3HAC vials ($n = 4$). Open-air collections were made immediately (0 h) and 24 h after the vials were placed in the field. z3HAC was recovered at the close sampling range of 10 cm even after 24 h of the vial but only minimally detected 0.5 m from the vial. For comparison, experimental plants were spaced 1 m apart.

2.3. Growth, Biomass, and Reproduction Measurements

We measured height and total leaf counts routinely on the experimental plants. 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. Leaves were only counted if they were wider than 2 cm across for both species to exclude immature developing leaves. 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 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 h 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 in both species. 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.

2.4. Herbivory

Leaf chewing damage was assessed for both pepper and lima bean as percent leaf area removed (LAR) using a visual estimation technique [48,49] 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 observed an ephemeral, natural occurrence of the black bean aphid (*Aphis faba*), and recorded its presence/absence on lima bean plants in the field.

2.5. Leaf Collections and Cyanide Measurements

Since cyanogenic potential (CP) is an inducible herbivore defense in lima bean [50,51], we used CP as a metric for induced responses in the presence of z3HAC. We collected source and sink leaves [52,53] on 10 July 2016, which was approximately 6 weeks into the field season. We developed a novel protocol in the lab for microscale colorimetric CP quantification by modifying an existing macro-scale protocol [54,55]. Briefly, 5 mg of lyophilized tissue was mixed with 200 μ L of citrate buffer (0.1 M, pH 5.5–6.5) in a 2 mL centrifuge tube, into which a 200 μ L glass vial (Agilent #5183–2090) containing 100 μ L 1.0 M NaOH was also placed and the centrifuge tube capped completely. After 15 h, a 50 μ L aliquot of the 1.0 M NaOH in the inner glass vial was diluted to 0.1 M, and a 30 μ L aliquot was neutralized with 30 μ L 0.5 M acetic acid in a 96-well reaction plate. Then, 75 μ L of Reagent A (5 mg/mL succinimide (VWR, AAA13503) and 0.5 mg/mL n-chlorosuccinimide (VWR, AAA10310)) and then 30 μ L of Reagent B (30 mg/mL barbituric acid (VWR, BT134930) in 30% pyridine (Sigma, 270970)) was added to each well. After 8 min, absorbance at 580 nm was measured on a plate reader (Molecular Devices SpectraMax M2). Quantification of CP was made against a standard curve of NaCN (VWR, BT212960).

2.6. Statistical Analyses

All statistical analyses were performed in R (version 4.0.3) implemented in RStudio (version 1.3.1093). Plant height and leaf counts, flower counts, and leaf area removed were first analyzed using repeated-measures ANOVA (aov function), with date as a within-subjects effect and treatment as a between-subjects effect. Differences between treatments and controls for these variables were also determined for individual time points using one-way ANOVA followed by Tukey HSD post hoc contrasts. Pepper biomass and fruit/seed data were likewise analyzed by one-way ANOVA. Aphid presence/absence was analyzed with Chi-Squared (chisq_test function). Cyanogenic potential data were analyzed with two-way ANOVA assessing independent and interactive effects of leaf developmental stage (sink vs. source) and treatment (control vs. z3HAC), with pairwise Tukey HSD contrasts. Data were transformed as necessary to satisfy assumptions of normality of model residuals. Graphs were made using ggplot2 in R.

3. Results

3.1. z3HAC Differentially Affects Growth of Lima Bean and Pepper Plants

Treatment with z3HAC differentially affected the growth of lima bean and pepper plants. On average, z3HAC-treated lima bean grew taller compared to control plants throughout the field season (Figure 2a; $F_{1,128} = 5.314$, $p = 0.022$), particularly during the last three time points. The z3HAC-treated lima bean also produced more leaves overall than did controls (Figure 2b; Treatment*Date $F_{2,253} = 3.272$, $p = 0.040$). In contrast, z3HAC-treated pepper plants grew noticeably shorter relative to controls (Figure 2c; Treatment*Date $F_{2,114} = 6.602$, $p = 0.002$) and produced fewer leaves over the field season (Figure 2d; Treatment*Date $F_{2,105} = 5.063$, $p = 0.008$). Consistent with height and leaf counts, z3HAC treatment reduced the overall biomass of pepper plants (Figure 3). When we destructively harvested all pepper plant biomass at the end of the season, z3HAC-treated pepper plants

had lower leaf, stem, and root dry biomass by 32%, 37%, 39%, respectively (Figure 3a–c) ($Z = -3.296$, $p = 0.002$; $Z = -3.584$, $p = 0.001$; $Z = -3.410$, $p = 0.001$). Despite these z3HAC-mediated effects on biomass exposure, the aboveground-to-belowground biomass ratio was similar between treatment and controls (Figure 3d; $Z = -0.560$, $p = 0.578$).

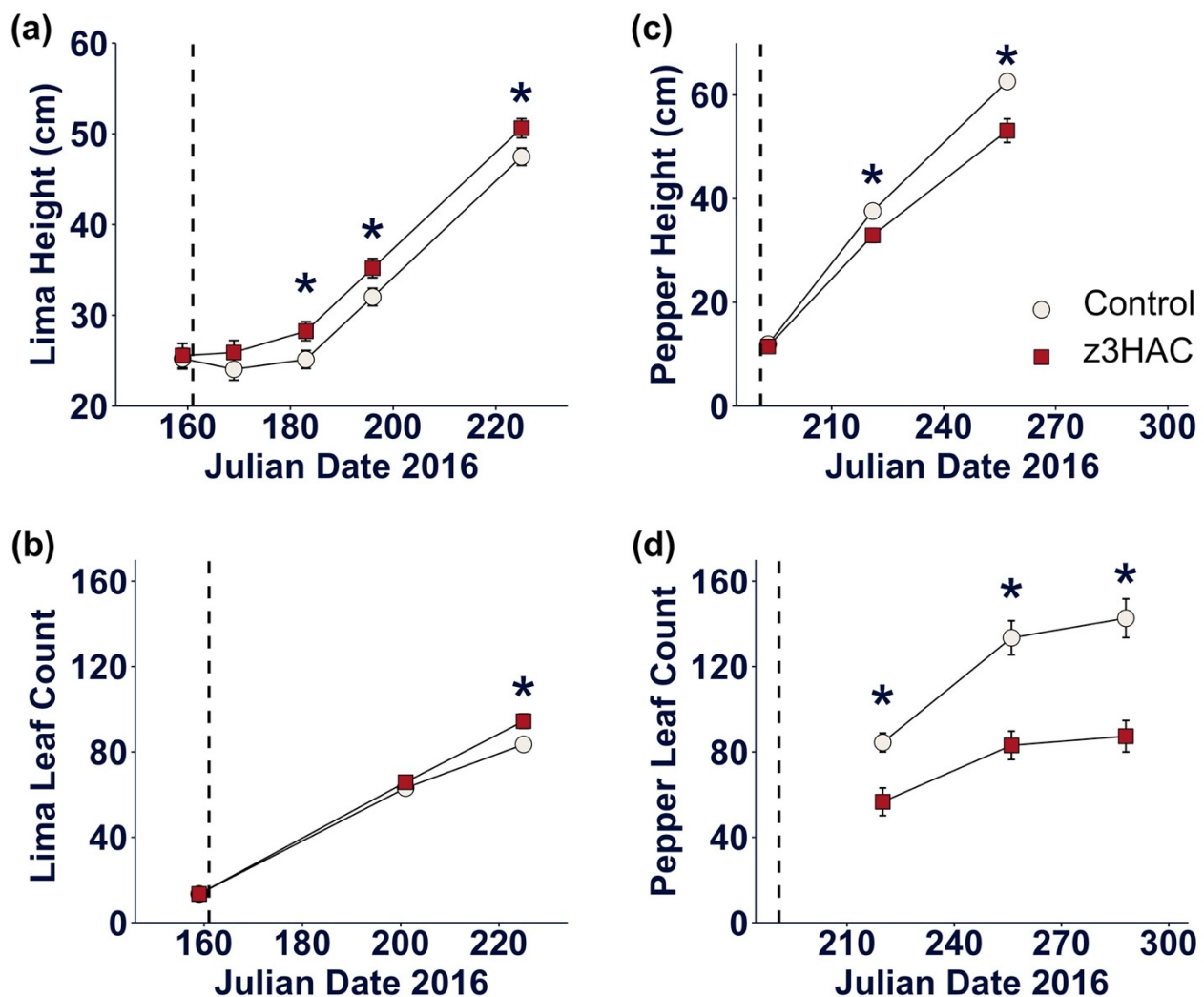


Figure 2. Height measurements and leaf counts for *Phaseolus lunatus* (lima bean) and *Capsicum annuum* (pepper) grown in a common garden field experiment. Height for (a) lima bean was measured from the base of the longest runner to the uppermost branching point; (c) pepper height was measured from the 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 10 ng/hr *cis*-3-hexenyl acetate (z3HAC) dissolved in lanolin. Dropdown lines indicate the initial application of z3HAC treatment: lima bean and pepper plants were first exposed on 10 June 2016 (Julian date 161) and 11 July 2016 (Julian date 192), respectively. Points represent the averages $\pm$ 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 S1 for statistics.

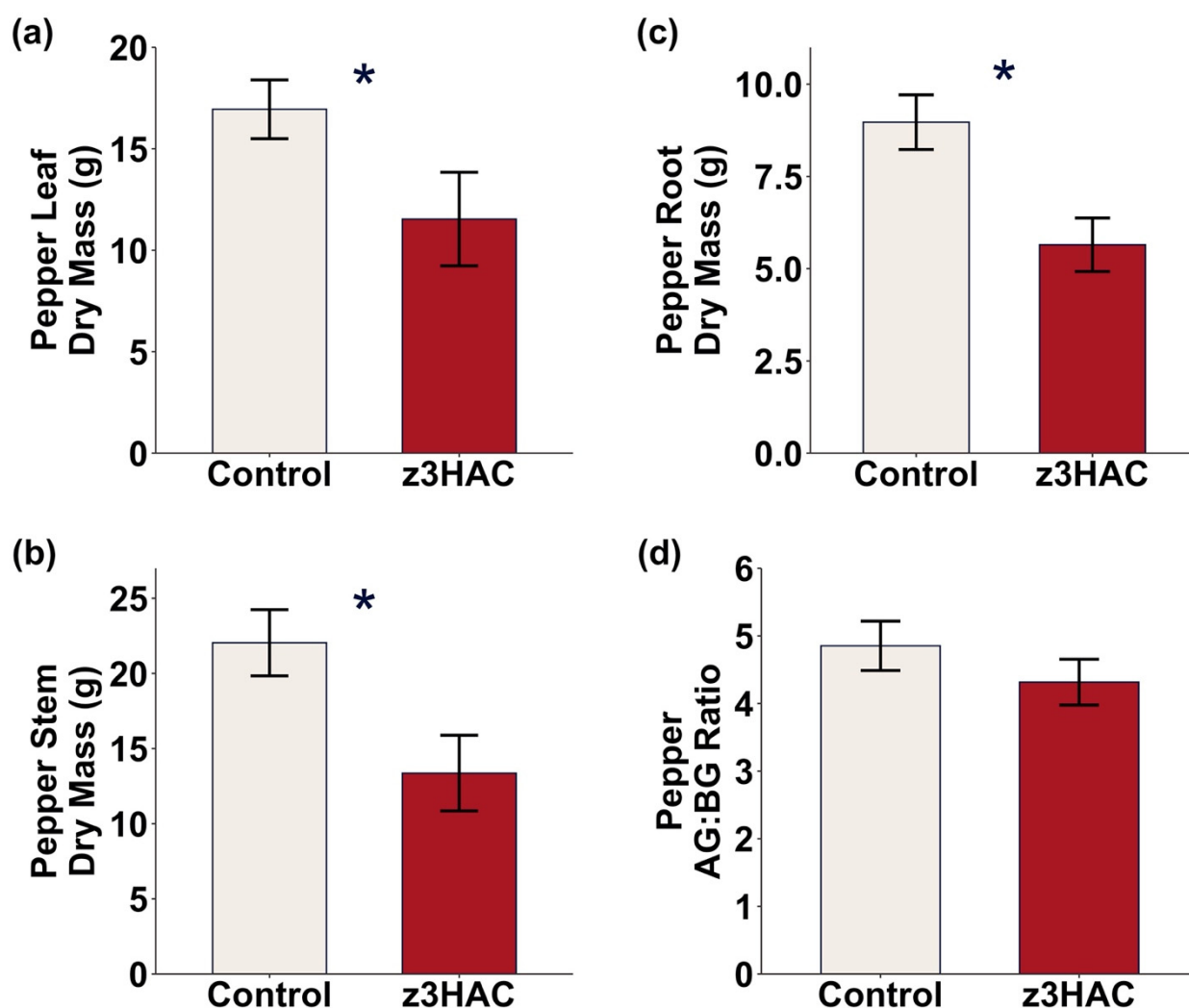


Figure 3. Biomass measurements of field-grown *Capsicum annuum* (pepper) plants. (a) Leaf, (b) stem, (c) root biomass, and (d) the aboveground:belowground biomass ratio in *C. annuum* plants were determined at the end of the field season following destructive harvest. Bars represent the means $\pm$ S.E.M. Asterisks (*) represent $p < 0.05$. See Supplemental Table S2 for statistics.

3.2. z3HAC Reduces Reproductive Output in Pepper Plants

z3HAC 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 z3HAC (Figure 4a; Treatment*Date $F_{2,259} = 4.027$, $p = 0.019$), while z3HAC-treated peppers produced 37% fewer flowers relative to control plants at the end of the field season (Figure 4b; Treatment $F_{1,70} = 4.455$, $p = 0.038$; Oct 14 (JD 288) $F_{1,45} = 13.4$, $p < 0.001$). z3HAC-treated pepper plants statistically similar fruits overall relative to controls (Figure 5a; $t = -0.956$, $p = 0.342$), but the fruits that were produced by z3HAC-treated plants had lower wet and dry masses (Figure 5b–c; $t = -2.487$, $p = 0.013$; $t = -4.245$, $p < 0.001$), and lower total seed counts (Figure 5d; $t = -2.496$, $p = 0.013$) and total seed masses (Figure 5e; $t = -2.644$, $p = 0.008$), relative to controls. However, the ratio of seed mass to fruit mass was similar between z3HAC-treated and control plants (Figure 5f; $t = 0.300$, $p = 0.764$), as was mass of an individual pepper seed (Supplemental Figure S3). There was no apparent difference in lima bean pod production (Supplemental Figure S3), though an unexpected field-wide premature pod drop independent of treatment prevented us from fully determining lima bean pod/seed production with confidence.

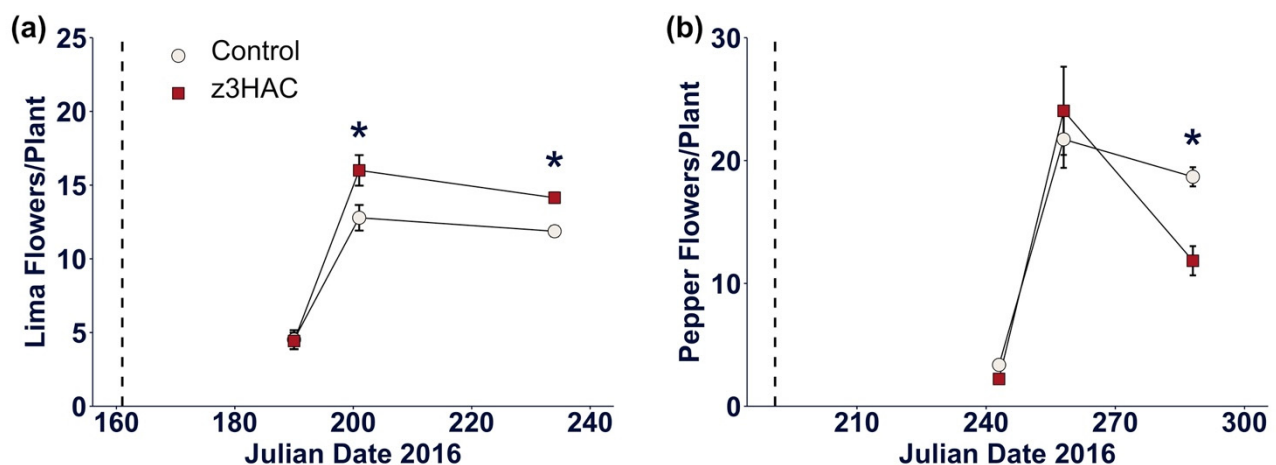


Figure 4. Total flower production in (a) *Phaseolus lunatus* (lima bean) and (b) *Capsicum annuum* (pepper). Open circles represent control plants (receiving lanolin-only vials); filled squares represent plants receiving a persistent application of vials containing 10 ng/h *cis*-3-hexenyl acetate (z3HAC) dissolved in lanolin. Dropdown lines indicate the initial application of z3HAC treatment: lima bean and pepper plants were first exposed on 10 June 2016 (Julian date 161) and 11 July 2016 (Julian date 192), respectively. Points represent the averages $\pm$ SE. Asterisks (*) represent $p < 0.05$ between treatment and control at each time point. See Supplemental Table S3 for statistics.

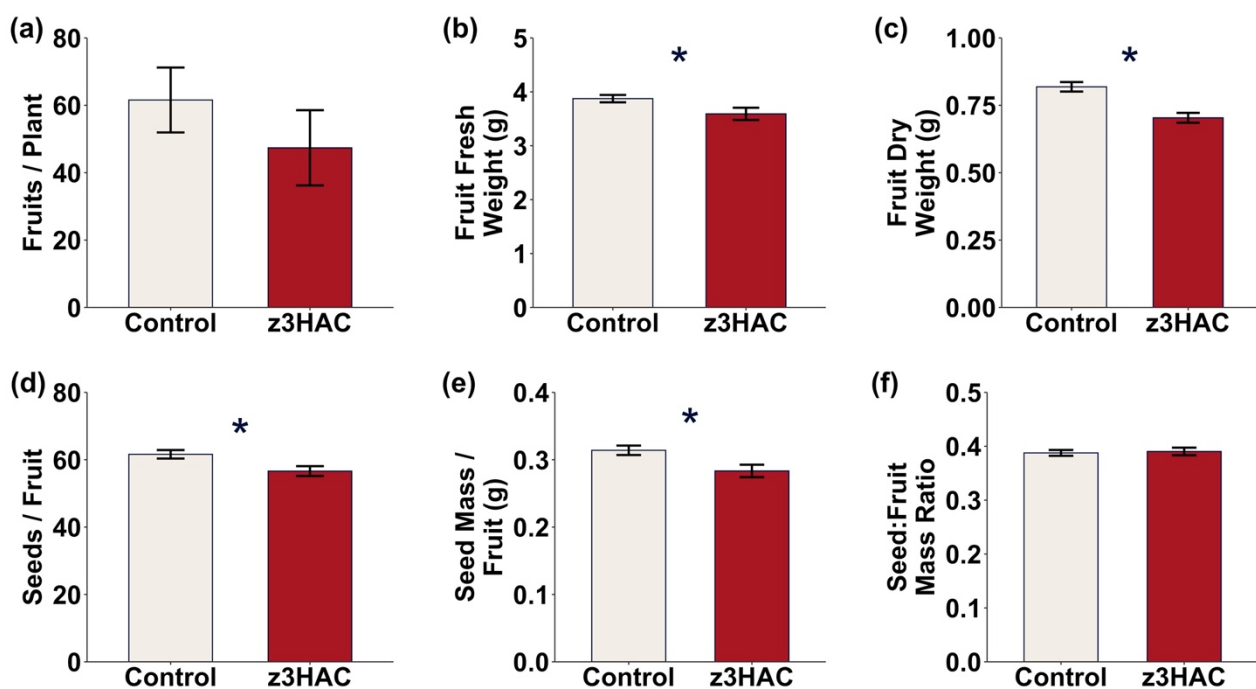


Figure 5. Fruit and seed production in *Capsicum annuum* (pepper) plants grown in a common garden experiment treated with a persistent application of the green leaf volatile *cis*-3-hexenyl acetate (z3HAC). The (a) total number of fruits were counted in the field, and (b) wet and (c) dry masses fruit 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. Bars represent the means $\pm$ S.E.M. Grey bars represent control plants; red bars represent plants treated with z3HAC. Asterisks (*) represent $p < 0.05$ between treatment and control. See Supplemental Table S4 for statistics.

3.3. z3HAC Exposure Reduces Herbivory on Lima Bean

z3HAC exposure reduced natural herbivory in lima bean but not pepper plants. Chewing herbivory to lima bean leaves increased as the field season progressed, with

z3HAC-treated plants having less chewing damage than did control plants (Figure 6a; Treatment $F_{1,130} = 20.692$, $p < 0.001$; Treatment*Date $F_{2,232} = 15.66$, $p < 0.001$). Damage to the lima bean plants in the final observation included *E. varivestis* feeding, at which point they more severely damaged control plants. In contrast, chewing herbivory on pepper plants was low overall and similar between z3HAC-treated and control plants (Figure 6b, Treatment $F_{1,59} = 0.454$, $p = 0.503$; Treatment*Date $F_{2,105} = 2.46$, $p = 0.090$). In addition to chewing herbivory, black bean aphids (*Aphis faba*) colonized 84% of the z3HAC-treated lima bean plants, compared with only 24% of control plants (Figure 6c; $\chi^2 = 59.3$, $df = 1$, $p < 0.001$). *A. faba* colonized early in the season and was only observed in the period 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.

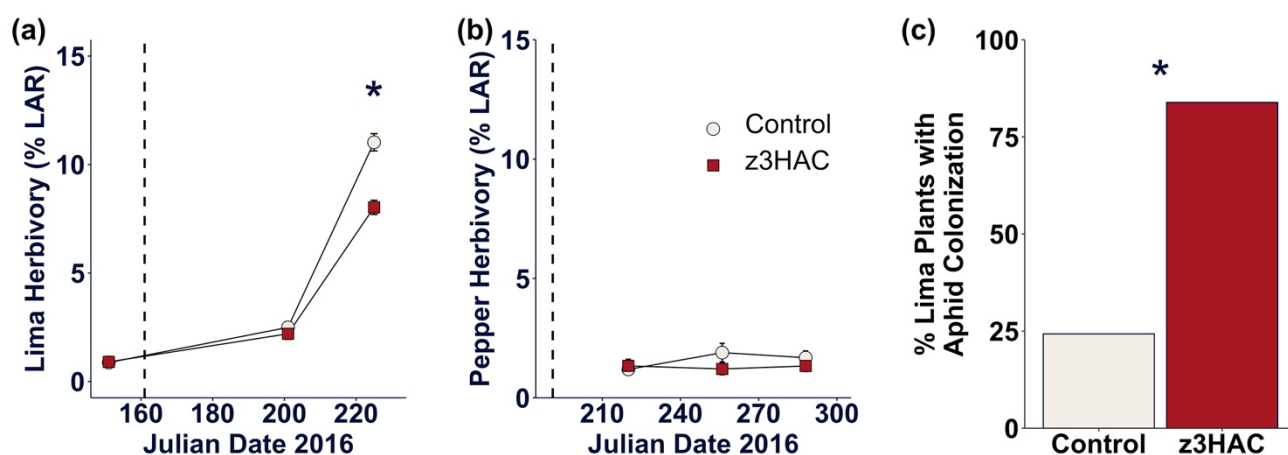


Figure 6. Herbivore damage on *Capsicum annuum* (pepper) and *Phaseolus lunatus* (lima bean) plants in a common garden field experiment. Chewing damage on (a) pepper and (b) lima bean plants was determined using a visual estimation technique (see Methods). Open circles represent control plants (receiving lanolin-filled vials); filled squares represent plants receiving a persistent application 10 ng/h *cis*-3-hexenyl acetate (z3HAC) dissolved in lanolin. Dropdown lines indicate the initial application of z3HAC treatment. Points represent the averages $\pm$ SE. Repeated-measures ANOVAs (aov in R) were followed by Tukey HSD contrasts. (c) *Aphis faba* colonization on lima bean plants. Bars represent the percentage of plants in each group where *A. faba* were observed. Asterisks (*) represent $p < 0.05$ between treatment and controls. See Supplemental Table S5 for complete statistics.

3.4. Cyanogenic Potential in Lima Bean Is Decreased by z3HAC Exposure

Exposure to z3HAC reduced cyanogenic potential in lima bean plants. z3HAC exposure alone reduced cyanide concentration by 28% in sink leaves (Figure 7; $F_{1,97} = 9.058$, $p = 0.003$), but not in source leaves (Figure 7; $F_{1,113} = 2.111$, $p = 0.149$). Baseline cyanogenic potential was ~2-fold greater in sink leaves compared to source leaves (Figure 7; $F_{1,210} = 126.9$, $p < 0.001$).

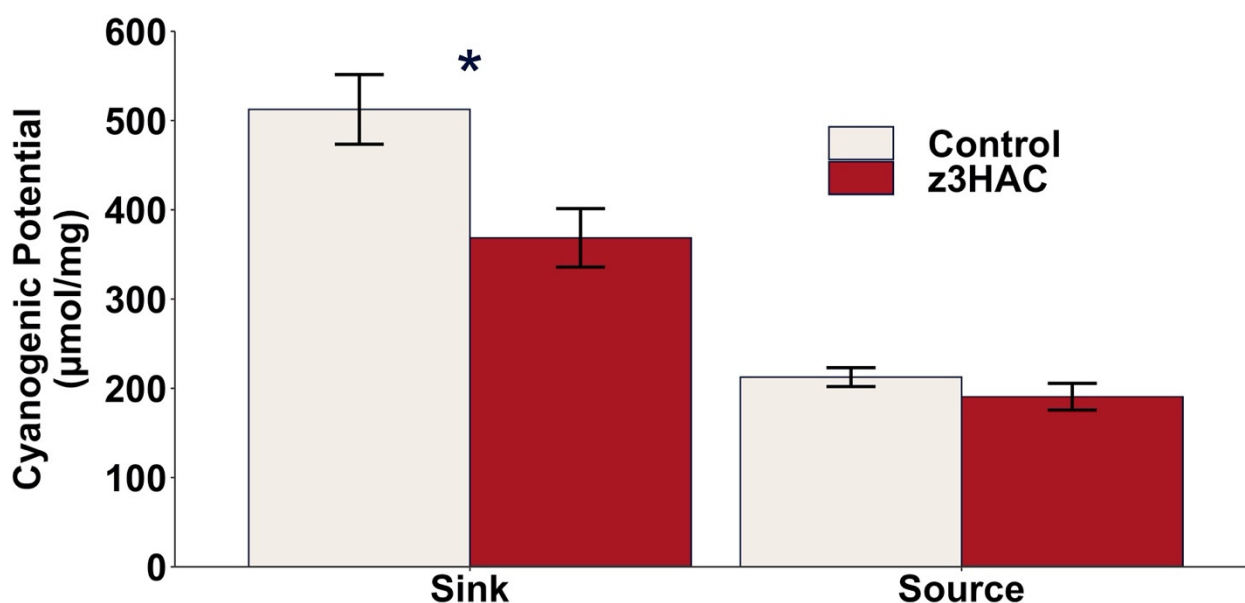


Figure 7. Cyanogenic potential in *Phaseolus lunatus* (Lima bean) as affected by z3HAC exposure. Sink (immature) and source leaves were exposed to 10 ng/h z3HAC (red bars) or left as a controls (grey bars). Points represent the averages $\pm$ SE and * indicates a $p < 0.05$ between control and z3HAC sink leaves.

4. Discussion

We show that a persistent, low-dose application of z3HAC differentially affects growth and reproduction of two plant species grown in the same field. Based on previous work on plant and sensory perception of volatiles [7,21], we hypothesized that z3HAC 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 [30,31,56–60]. Volatile-mediated priming, even if regulated by a different mechanism from resistance [61], is an inducible phenomenon that theoretically should incur such fitness costs [36]. Such physiological costs were observed in maize seedlings over a 3-day period following z3HAC treatment [62], and in field-grown tall goldenrod (*Solidago altissima*) plants exposed to volatile cues from specialist herbivore [63]. 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 z3HAC-treated pepper plants had reduced growth (Figure 2) and no effect on herbivore resistance (Figure 6a) relative to controls, z3HAC-treated lima bean plants grew more and produced more flowers (Figures 2 and 4), and suffered less chewing herbivory (Figure 6b) compared to controls. This result—that some plants experience 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. HIPVs alone may be sufficient to result in differential fitness effects among species. Moreover, the positive effects of z3HAC on lima bean growth and flowering opens agronomic opportunities to exploit plant volatiles to enhance both growth and pest resistance of important crop plants.

What might affect the response of plants to volatile exposure? One possibility is that the signal integrity of HIPVs varies among plant species, which influences plant sensory perception and the outcome of defense priming. That is, z3HAC provides different information to different plants. Previous work on the role of HIPVs in plant anti-herbivore resistance focused on priming-mediated defense with consistent results in wheat [64,65], corn [12,45], lima bean [8,11,66], tomato [67], blueberry [15], sagebrush [43], Arabidopsis [68] and poplar [4]. In contrast, we specifically focused on indicators of plant fitness in lima bean and pepper in a common garden experiment. z3HAC treatment alone increased

growth and flowering in lima bean, while reducing growth and reproductive output in pepper (Figures 4 and 6). This season-long evidence illustrates that informational integrity on a HIPV varies between these two plant species. Such divergent fitness effects from exposure to a single ubiquitous herbivore-associated cue underscore the potential for functional similarity in the mechanisms by which plants modulate responses to herbivory and volatile indicators of herbivory. That said, it would be valuable to consider how other priming VOCs, such as z-3-hexenol [45] or blends [47] affect plant species-specific information integrity.

Flower and fruit production is a key component of plant fitness potential. We show that z3HAC treatment alone differentially affected flower production in lima bean and pepper (Figure 4). Insect herbivory can increase or decrease floral production depending on the system and environmental conditions [69–71]. Whereas increased flower production is a strategy assumed to ameliorate fitness losses in the presence of an environmental stress [56,72], decreased flower production may be related to costs of chemically mediated defense [73]. Previous work with lima bean has demonstrated stress-mediated compensation [74,75]. Our result that z3HAC alone was sufficient to trigger increased flowering is consistent with this observation, suggesting that z3HAC alone may stimulate a long-term stress response similar to herbivory. Therefore, even though the mechanisms underlying z3HAC-mediated effects on flower and fruit production are not yet known, they may be similar to those induced by herbivory [69].

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 [49,76,77], as does application of the phytohormone jasmonic acid (JA) [78,79]. 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 [80], while exposure to volatiles decreases aboveground biomass in other systems [81,82]. In our case, volatile treatment reduced overall aboveground and belowground biomass in pepper, but did not appear to alter overall biomass allocation patterns. Simply put, z3HAC-treated pepper plants were smaller overall, and therefore produced fewer seeds.

Differential investment between growth and defense is vital for maximizing limited resources and, ultimately, fitness. Inducible defenses in plants against herbivores and pathogens modulate such growth/defense tradeoffs in a number of plant species [31]. In some cases, exposure to VOCs alone is sufficient to affect such tradeoffs. For example, sagebrush exposed to VOCs from damaged conspecifics had decreased growth [83], while HIPV-exposed tobacco had increased herbivore resistance but decreased seed set [38]. In our experiment, z3HAC exposure decreased cyanogenic potential in sink leaves (Figure 6), which were 20–30% higher than in source leaves. These results suggest that persistent exposure to z3HAC led to allocation shifts from cyanogenic potential (a putative defense) towards growth (Figures 2 and 4), as well as supporting ontogeny-mediated cyanogenic potential [52,84]. That said, z3HAC-treated plants also experienced less natural herbivory primarily on mature source leaves than did controls (Figure 6, personal observations), suggesting that the herbivory was deterred by defense mechanism other than cyanogenic potential in source leaves.

Volatile cues may impact ecological communities in both expected and pleiotropic ways. HIPVs are well-established mediators of multi-trophic antagonistic and mutualistic interactions [85–87], and manipulations of chemical signals and volatile blends have been used for biological control in a wide range of systems [88,89]. For example, HIPV-infused sticky traps in a grape (*Vitis vinifera*) orchard differentially attracted lacewings, hoverflies, and parasitoids [90]. Exogenous GLV manipulation using “dispensars” under field conditions altered the arthropod community composition in maize [47]. In our study, *A. faba* were clearly and unexpectedly attracted to z3HAC-exposed plants (Figure 6). Under glasshouse conditions, *A. faba* were repelled by z3HAC alone [91], 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 z3HAC effects on JA and salicylic acid (SA) signaling, which would be consistent with a JA-SA tradeoff [92,93]. Ultimately, however, the utility of GLVs (or other VOCs) in field applications will depend on understanding community-level effects of volatile exposure.

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 [94,95], which can influence fitness in neighboring plants [83,96,97]. Plant-derived compounds associated with herbivory include GLVs [4,12,81], shikimate derivatives [13], and terpenes [98]. However, individual compounds within a blend can affect plant defense and priming as much as the blend itself. We used z3HAC in this study because it is released herbivore-damaged leaves [21], which ostensibly allows z3HAC to confer reliable ecological information. That said, z3HAC can also be released from wounded leaves without herbivores [23,94]. However, plants detect and respond specifically to z3HAC [4], and the costs associated with that response in the context of pest resistance were the focus of this investigation. Additionally, concentration of a cue may influence plant resistance [68,81]. For example, a repeated, low-dose exposure to a GLV blend enhances plant resistance compared to a single application [46], while z3HAC emissions can be as high as 66 ng/cm³ after herbivory [20]. For these reasons, we chose to use a low-dose exposure to z3HAC (25% of the concentration that primed poplar [4] and maize [21]), and still observed divergent fitness effects between the two plant species (Figures 2, 4 and 6).

In summary, our key finding is that persistent application of a low dose of a single volatile compound z3HAC, 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 volatile-mediated eavesdropping, and that plants may have evolved species-specific mechanisms for responding to volatile cues. Given natural variation among species, future work must assess costs with other species as well as within accessions and landraces of our model plants over multiple years. 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 volatile-mediated eavesdropping might have on plants and their insect pests.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11050958/s1>. Supplemental Tables describing the outputs of statistical analyses are listed as tabs in a single spreadsheet ("Supplemental_Tables"), with tab names corresponding to each table. Three Supplemental Figures are in a single file (Supplemental_Figures).

Author Contributions: Conceptualization, C.J.F.; Methodology, C.J.F., G.E.F. and M.S.; Validation, C.J.F. and G.E.F.; Formal Analysis, C.J.F. and G.E.F.; Investigation, C.J.F. and G.E.F.; Resources, C.J.F.; Data Curation, C.J.F. and G.E.F.; Writing, C.J.F., G.E.F. and M.S.; Visualization, C.J.F. and G.E.F.; Supervision, C.J.F.; Project Administration, C.J.F.; Funding Acquisition, C.J.F. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Science Foundation grants IOS-2101059 and IOS-1656625 to C.J.F.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available at DOI:10.25422/azu.data.14575260.

Acknowledgments: We are grateful to Andrea Clavijo-McCormick for the invitation to submit our work to this Special Issue. We thank Allie Peot and Abhinav Maurya for field assistance, and A. Peot for assistance processing the plant materials in the laboratory. Comments from Heidi Appel, Amy Austin, Susan Frost, Raysun Frost, and anonymous reviewers 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 the Blackacre Conservancy.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

- Muhlemann, J.K.; Klempien, A.; Dudareva, N. Floral volatiles: From biosynthesis to function. *Plant Cell Environ.* **2014**, *37*, 1936–1949. [[CrossRef](#)] [[PubMed](#)]
- Mumm, R.; Dicke, M. Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense: The present review is one in the special series of reviews on animal–plant. *Can. J. Zool.* **2010**, *88*, 628–667. [[CrossRef](#)]
- Farag, M.A.; Zhang, H.; Ryu, C.-M. Dynamic Chemical Communication between Plants and Bacteria through Airborne Signals: Induced Resistance by Bacterial Volatiles. *J. Chem. Ecol.* **2013**, *39*, 1007–1018. [[CrossRef](#)]
- Frost, C.J.; Mescher, M.C.; Dervinis, C.; Davis, J.M.; Carlson, J.E.; de Moraes, C.M. Priming defense genes and metabolites in hybrid poplar by the green leaf volatile cis-3-hexenyl acetate. *New Phytol.* **2008**, *180*, 722–734. [[CrossRef](#)]
- Turlings, T.C.; Loughrin, J.H.; McCall, P.J.; Rose, U.S.; Lewis, W.J.; Tumlinson, J.H. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 4169–4174. [[CrossRef](#)] [[PubMed](#)]
- Rodriguez-Saona, C.R.; Frost, C.J. New evidence for a multi-functional role of herbivore-induced plant volatiles in defense against herbivores. *Plant Signal. Behav.* **2010**, *5*, 58–60. [[CrossRef](#)] [[PubMed](#)]
- Frost, C.J.; Appel, H.M.; Carlson, J.E.; de Moraes, C.M.; Mescher, M.C.; Schultz, J.C. Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. *Ecol. Lett.* **2007**, *10*, 490–498. [[CrossRef](#)] [[PubMed](#)]
- Heil, M.; Bueno, J.C.S. Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 5467–5472. [[CrossRef](#)]
- Heil, M.; Karban, R. Explaining evolution of plant communication by airborne signals. *Trends Ecol. Evol.* **2010**, *25*, 137–144. [[CrossRef](#)]
- Pearse, I.S.; Hughes, K.; Shiojiri, K.; Ishizaki, S.; Karban, R. Interplant volatile signaling in willows: Revisiting the original talking trees. *Oecologia* **2013**, *172*, 869–875. [[CrossRef](#)]
- Arimura, G.-I.; Ozawa, R.; Nishioka, T.; Boland, W.; Koch, T.; Kühnemann, F.; Takabayashi, J. Herbivore-induced volatiles induce the emission of ethylene in neighboring lima bean plants. *Plant J.* **2002**, *29*, 87–98. [[CrossRef](#)]
- Farag, M.A.; Fokar, M.; Abd, H.; Zhang, H.; Allen, R.D. (Z)-3-Hexenol induces defense genes and downstream metabolites in maize. *Planta* **2004**, *220*, 900–909. [[CrossRef](#)]
- Erb, M.; Veyrat, N.; Robert, C.A.M.; Xu, H.; Frey, M.; Ton, J.; Turlings, T.C.J. Indole is an essential herbivore-induced volatile priming signal in maize. *Nat. Commun.* **2015**, *6*, 6273. [[CrossRef](#)]
- Farag, M.A.; Paré, P.W. C6-Green leaf volatiles trigger local and systemic VOC emissions in tomato. *Phytochemistry* **2002**, *61*, 545–554. [[CrossRef](#)]
- Rodriguez-Saona, C.R.; Rodriguez-Saona, L.E.; Frost, C.J. Herbivore-Induced Volatiles in the Perennial Shrub, *Vaccinium corymbosum*, and Their Role in Inter-branch Signaling. *J. Chem. Ecol.* **2009**, *35*, 163–175. [[CrossRef](#)]
- Girón-Calva, P.S.; Molina-Torres, J.; Heil, M. Volatile Dose and Exposure Time Impact Perception in Neighboring Plants. *J. Chem. Ecol.* **2012**, *38*, 226–228. [[CrossRef](#)]
- Arimura, G.-I.; Köpke, S.; Kunert, M.; Volpe, V.; David, A.; Brand, P.; Dabrowska, P.; Maffei, M.E.; Boland, W. Effects of Feeding *Spodoptera littoralis* on Lima Bean Leaves: IV. Diurnal and Nocturnal Damage Differentially Initiate Plant Volatile Emission. *Plant Physiol.* **2008**, *146*, 965–973. [[CrossRef](#)]
- Arimura, G.-I.; Matsui, K.; Takabayashi, J. Chemical and Molecular Ecology of Herbivore-Induced Plant Volatiles: Proximate Factors and Their Ultimate Functions. *Plant Cell Physiol.* **2009**, *50*, 911–923. [[CrossRef](#)]
- Coplovici, L.; Kännaste, A.; Remmel, T.; Vislav, V.; Niinemets, Ü. Volatile Emissions from *Alnus glutinosa* Induced by Herbivory are Quantitatively Related to the Extent of Damage. *J. Chem. Ecol.* **2011**, *37*, 18–28. [[CrossRef](#)]
- Boggia, L.; Sgorbini, B.; Berteau, C.M.; Cagliero, C.; Bicchi, C.; Maffei, M.E.; Rubiolo, P. Direct Contact—Sorbptive Tape Extraction coupled with Gas Chromatography—Mass Spectrometry to reveal volatile topographical dynamics of lima bean (*Phaseolus lunatus* L.) upon herbivory by *Spodoptera littoralis* Bois. *BMC Plant Biol.* **2015**, *15*, 102. [[CrossRef](#)]
- Engelberth, J.; Alborn, H.T.; Schmelz, E.A.; Tumlinson, J.H. Airborne signals prime plants against insect herbivore attack. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 1781–1785. [[CrossRef](#)] [[PubMed](#)]
- Paschold, A.; Halitschke, R.; Baldwin, I.T. Using ‘mute’ plants to translate volatile signals. *Plant J.* **2005**, *45*, 275–291. [[CrossRef](#)] [[PubMed](#)]
- Matsui, K.; Sugimoto, K.; Mano, J.; Ozawa, R.; Takabayashi, J. Differential Metabolisms of Green Leaf Volatiles in Injured and Intact Parts of a Wounded Leaf Meet Distinct Ecophysiological Requirements. *PLoS ONE* **2012**, *7*, e36433. [[CrossRef](#)] [[PubMed](#)]
- Pastor, V.; Balmer, A.; Gamir, J.; Flors, V.; Mauch-Mani, B. Preparing to fight back: Generation and storage of priming compounds. *Front. Plant Sci.* **2014**, *5*, 295. [[CrossRef](#)]
- D’Auria, J.C.; Pichersky, E.; Schaub, A.; Hansel, A.; Gershenzon, J. Characterization of a BAHD acyltransferase responsible for producing the green leaf volatile (Z)-3-hexen-1-yl acetate in *Arabidopsis thaliana*. *Plant J.* **2006**, *49*, 194–207. [[CrossRef](#)]

26. Addesso, K.M.; McAuslane, H.J.; Alborn, H.T. Attraction of pepper weevil to volatiles from damaged pepper plants. *Entomol. Exp. Appl.* **2010**, *138*, 1–11. [[CrossRef](#)]
27. Cardoza, Y.J.; Tumlinson, J.H. Compatible and Incompatible Xanthomonas Infections Differentially Affect Herbivore-Induced Volatile Emission by Pepper Plants. *J. Chem. Ecol.* **2006**, *32*, 1755–1768. [[CrossRef](#)]
28. McCormick, A.C.; Irmisch, S.; Reinecke, A.; Boeckler, G.A.; Veit, D.; Reichelt, M.; Hansson, B.S.; Gershenzon, J.; Köllner, T.G.; Unsicker, S.B. Herbivore-induced volatile emission in black poplar: Regulation and role in attracting herbivore enemies. *Plant Cell Environ.* **2014**, *37*, 1909–1923. [[CrossRef](#)]
29. Accamando, A.K.; Cronin, J.T. Costs and Benefits of Jasmonic Acid Induced Responses in Soybean. *Environ. Entomol.* **2012**, *41*, 551–561. [[CrossRef](#)]
30. Cipollini, D.; Purrington, C.B.; Bergelson, J. Costs of induced responses in plants. *Basic Appl. Ecol.* **2003**, *4*, 79–89. [[CrossRef](#)]
31. Cipollini, D.; Heil, A.M. Costs and benefits of induced resistance to herbivores and pathogens in plants. *CAB Int.* **2010**, *5*, 1–25. [[CrossRef](#)]
32. Douma, J.C.; Vermeulen, P.J.; Poelman, E.H.; Dicke, M.; Anten, N.P.R. When does it pay off to prime for defense? A modeling analysis. *New Phytol.* **2017**, *216*, 782–797. [[CrossRef](#)]
33. Frost, C.J.; Mescher, M.C.; Carlson, J.E.; de Moraes, C.M. Plant Defense Priming against Herbivores: Getting Ready for a Different Battle: Figure 1. *Plant Physiol.* **2008**, *146*, 818–824. [[CrossRef](#)]
34. Van Hulten, M.; Pelser, M.; van Loon, L.C.; Pieterse, C.M.J.; Ton, J. Costs and benefits of priming for defense in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 5602–5607. [[CrossRef](#)]
35. Orrock, J.L.; Connolly, B.M.; Choi, W.-G.; Guiden, P.W.; Swanson, S.J.; Gilroy, S. Plants eavesdrop on cues produced by snails and induce costly defenses that affect insect herbivores. *Oecologia* **2018**, *186*, 703–710. [[CrossRef](#)]
36. Martinez-Medina, A.; Flors, V.; Heil, M.; Mauch-Mani, B.; Pieterse, C.M.; Pozo, M.J.; Ton, J.; van Dam, N.M.; Conrath, U. Recognizing Plant Defense Priming. *Trends Plant Sci.* **2016**, *21*, 818–822. [[CrossRef](#)]
37. Song, G.C.; Ryu, C.-M. Two Volatile Organic Compounds Trigger Plant Self-Defense against a Bacterial Pathogen and a Sucking Insect in Cucumber under Open Field Conditions. *Int. J. Mol. Sci.* **2013**, *14*, 9803–9819. [[CrossRef](#)]
38. Karban, R.; Maron, J. The fitness consequences of interspecific eavesdropping between plants. *Ecology* **2002**, *83*, 1209–1213. [[CrossRef](#)]
39. Ballhorn, D.J.; Schiwy, S.; Jensen, M.; Heil, M. Quantitative Variability of Direct Chemical Defense in Primary and Secondary Leaves of Lima Bean (*Phaseolus lunatus*) and Consequences for a Natural Herbivore. *J. Chem. Ecol.* **2008**, *34*, 1298–1301. [[CrossRef](#)]
40. Zachariah, T.J.; Safeer, A.; Jayarajan, K.; Leela, N.; Vipin, T.; Saji, K.; Shiva, K.; Parthasarathy, V.; Mammooty, K. Correlation of metabolites in the leaf and berries of selected black pepper varieties. *Sci. Hortic.* **2010**, *123*, 418–422. [[CrossRef](#)]
41. Frost, C.J.; Mescher, M.C.; Carlson, J.E.; de Moraes, C.M. Why do distance limitations exist on plant-plant signaling via airborne volatiles? *Plant Signal. Behav.* **2008**, *3*, 466–468. [[CrossRef](#)]
42. Dolch, R.; Tschardt, T. Defoliation of alders (*Alnus glutinosa*) affects herbivory by leaf beetles on undamaged neighbours. *Oecologia* **2000**, *125*, 504–511. [[CrossRef](#)]
43. Karban, R.; Shiojiri, K.; Huntzinger, M.; McCall, A.C. Damage-induced resistance in sagebrush: Volatiles are key to intra- and interplant communication. *Ecology* **2006**, *87*, 922–930. [[CrossRef](#)]
44. Heil, M.; Adame-Álvarez, R.M. Short signalling distances make plant communication a soliloquy. *Biol. Lett.* **2010**, *6*, 843–845. [[CrossRef](#)]
45. Engelberth, J.; Seidl-Adams, I.; Schultz, J.C.; Tumlinson, J.H. Insect Elicitors and Exposure to Green Leafy Volatiles Differentially Upregulate Major Octadecanoids and Transcripts of 12-Oxo Phytodienoic Acid Reductases in *Zea mays*. *Mol. Plant Microbe Interact.* **2007**, *20*, 707–716. [[CrossRef](#)]
46. Shiojiri, K.; Ozawa, R.; Matsui, K.; Sabelis, M.W.; Takabayashi, J. Intermittent exposure to traces of green leaf volatiles triggers a plant response. *Sci. Rep.* **2012**, *2*, 378. [[CrossRef](#)]
47. Von Mérey, G.; Veyrat, N.; Mahuku, G.; Valdez, R.L.; Turlings, T.C.; D'Alessandro, M. 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* **2011**, *72*, 1838–1847. [[CrossRef](#)]
48. Frost, C.J.; Hunter, M.D. Insect Canopy Herbivory and Frass Deposition Affect Soil Nutrient Dynamics and Export in Oak Mesocosms. *Ecology* **2004**, *85*, 3335–3347. [[CrossRef](#)]
49. Frost, C.J.; Hunter, M.D. Herbivore-induced shifts in carbon and nitrogen allocation in red oak seedlings. *New Phytol.* **2008**, *178*, 835–845. [[CrossRef](#)]
50. Gleadow, R.M.; Möller, B.L. Cyanogenic Glycosides: Synthesis, Physiology, and Phenotypic Plasticity. *Annu. Rev. Plant Biol.* **2014**, *65*, 155–185. [[CrossRef](#)]
51. Ballhorn, D.J.; Lieberei, R.; Ganzhorn, J.U. Plant Cyanogenesis of *Phaseolus lunatus* and its Relevance for Herbivore–Plant Interaction: The Importance of Quantitative Data. *J. Chem. Ecol.* **2005**, *31*, 1445–1473. [[CrossRef](#)] [[PubMed](#)]
52. Ochoa-López, S.; Villamil, N.; Zedillo-Avelleyra, P.; Boege, K. Plant defence as a complex and changing phenotype throughout ontogeny. *Ann. Bot.* **2015**, *116*, 797–806. [[CrossRef](#)]
53. Schultz, J.C.; Appel, H.M.; Ferrieri, A.P.; Arnold, T.M. Flexible resource allocation during plant defense responses. *Front. Plant Sci.* **2013**, *4*, 324. [[CrossRef](#)] [[PubMed](#)]

54. Brinker, A.M.; Seigler, D. Methods for the detection and quantitative determination of cyanide in plant materials. *Phyto Chem. Bull.* **1989**, *21*, 24–31.
55. Gleadow, R.; Bjarnholt, N.; Jørgensen, K.; Fox, J. Cyanogenic Glycosides. In *Soil Allelochemicals*; Narwal, S.S., Ed.; Studium Press LLC: New Delhi, India, 2011; Volume 1, pp. 283–310.
56. Agrawal, A.A. Induced Responses to Herbivory in Wild Radish: Effects on Several Herbivores and Plant Fitness. *Ecology* **1999**, *80*, 1713. [[CrossRef](#)]
57. Baldwin, I.T. Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 8113–8118. [[CrossRef](#)] [[PubMed](#)]
58. Didiano, T.J.; Turley, N.E.; Everwand, G.; Schaefer, H.; Crawley, M.J.; Johnson, M.T.J. Experimental test of plant defence evolution in four species using long-term rabbit exclosures. *J. Ecol.* **2014**, *102*, 584–594. [[CrossRef](#)]
59. Koricheva, J. Meta-Analysis of Sources of Variation in Fitness Costs of Plant Antiherbivore Defenses. *Ecology* **2002**, *83*, 176–190. [[CrossRef](#)]
60. Mauricio, R. Costs of Resistance to Natural Enemies in Field Populations of the Annual Plant *Arabidopsis thaliana*. *Am. Nat.* **1998**, *151*, 20–28. [[CrossRef](#)]
61. Hilker, M.; Schwachtje, J.; Baier, M.; Balazadeh, S.; Bäurle, I.; Geiselhardt, S.; Hinch, D.K.; Kunze, R.; Mueller-Roeber, B.; Rillig, M.C.; et al. Priming and memory of stress responses in organisms lacking a nervous system. *Biol. Rev.* **2015**, *91*, 1118–1133. [[CrossRef](#)]
62. Engelberth, J.; Engelberth, M. The costs of green leaf volatile-induced defense priming: Temporal diversity in growth re-sponses to mechanical wounding and insect herbivory. *Plants* **2019**, *8*, 23. [[CrossRef](#)]
63. Yip, E.C.; de Moraes, C.M.; Mescher, M.C.; Tooker, J.F. The volatile emission of a specialist herbivore alters patterns of plant defence, growth and flower production in a field population of goldenrod. *Funct. Ecol.* **2017**, *31*, 1062–1070. [[CrossRef](#)]
64. Ameye, M.; Audenaert, K.; de Zutter, N.; Steppe, K.; van Meulebroek, L.; Vanhaecke, L.; de Vleeschauwer, D.; Haesaert, G.; Smaghe, G. Priming of Wheat with the Green Leaf Volatile Z-3-Hexenyl Acetate Enhances Defense against *Fusarium graminearum* But Boosts Deoxynivalenol Production. *Plant Physiol.* **2015**, *167*, 1671–1684. [[CrossRef](#)]
65. Walters, D.R.; Paterson, L.; Walsh, D.J.; Havis, N.D. Priming for plant defense in barley provides benefits only under high disease pressure. *Physiol. Mol. Plant Pathol.* **2008**, *73*, 95–100. [[CrossRef](#)]
66. Choh, Y.; Takabayashi, J. Herbivore-induced extrafloral nectar production in lima bean plants enhanced by previous exposure to volatiles from infested conspecifics. *J. Chem. Ecol.* **2006**, *32*, 2073–2077. [[CrossRef](#)]
67. Acevedo, F.E.; Peiffer, M.; Tan, C.-W.; Stanley, B.A.; Stanley, A.; Wang, J.; Jones, A.G.; Hoover, K.; Rosa, C.; Luthe, D.; et al. Fall Armyworm-Associated Gut Bacteria Modulate Plant Defense Responses. *Mol. Plant Microbe Interact.* **2017**, *30*, 127–137. [[CrossRef](#)]
68. Bissmeyer, S.; Freundlich, G.; Frost, C. The influence of dose of a plant-derived volatile cue on *Arabidopsis thaliana* resistance against insect herbivores. *Ky. J. Undergrad. Res.* **2018**, *2*, 84–95.
69. Lucas-Barbosa, D. Integrating Studies on Plant–Pollinator and Plant–Herbivore Interactions. *Trends Plant Sci.* **2016**, *21*, 125–133. [[CrossRef](#)]
70. Agrawal, A.A.; Strauss, S.Y.; Stout, M.J. Costs of Induced Responses and Tolerance to Herbivory in Male and Female Fitness Components of Wild Radish. *Evolution* **1999**, *53*, 1093–1104. [[CrossRef](#)]
71. Pashalidou, F.G.; Lucas-Barbosa, D.; van Loon, J.J.A.; Dicke, M.; Fatouros, N.E. Phenotypic plasticity of plant response to herbivore eggs: Effects on resistance to caterpillars and plant development. *Ecology* **2013**, *94*, 702–713. [[CrossRef](#)]
72. Agrawal, A.A. Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *Trends Plant Sci.* **2000**, *5*, 309–313. [[CrossRef](#)]
73. Heath, J.J.; Kessler, A.; Woebbe, E.; Cipollini, D.; Stireman, J.O. Exploring plant defense theory in tall goldenrod, *Solidago altissima*. *New Phytol.* **2014**, *202*, 1357–1370. [[CrossRef](#)] [[PubMed](#)]
74. Cuny, M.A.C.; Gendry, J.; Hernández-Cumplido, J.; Benrey, B. Changes in plant growth and seed production in wild lima bean in response to herbivory are attenuated by parasitoids. *Oecologia* **2018**, *187*, 447–457. [[CrossRef](#)] [[PubMed](#)]
75. Godschalx, A.L.; Stady, L.; Watzig, B.; Ballhorn, D.J. Is protection against florivory consistent with the optimal defense hypothesis? *BMC Plant Biol.* **2016**, *16*, 1–9. [[CrossRef](#)]
76. Machado, R.A.R.; Ferrieri, A.P.; Robert, C.A.M.; Glauser, G.; Kallenbach, M.; Baldwin, I.T.; Erb, M. Leaf-herbivore attack reduces carbon reserves and regrowth from the roots via jasmonate and auxin signaling. *New Phytol.* **2013**, *200*, 1234–1246. [[CrossRef](#)]
77. Eichenberg, D.; Purschke, O.; Ristok, C.; Wessjohann, L.A.; Bruehlheide, H. Trade-offs between physical and chemical carbon-based leaf defence: Of intraspecific variation and trait evolution. *J. Ecol.* **2015**, *103*, 1667–1679. [[CrossRef](#)]
78. Gómez, S.; Ferrieri, R.A.; Schueller, M.; Orians, C.M. Methyl jasmonate elicits rapid changes in carbon and nitrogen dynamics in tomato. *New Phytol.* **2010**, *188*, 835–844. [[CrossRef](#)]
79. Schweiger, R.; Heise, A.-M.; Persicke, M.; Müller, C. Interactions between the jasmonic and salicylic acid pathway modulate the plant metabolome and affect herbivores of different feeding types. *Plant Cell Environ.* **2014**, *37*, 1574–1585. [[CrossRef](#)]
80. Ninkovic, V. Volatile communication between barley plants affects biomass allocation. *J. Exp. Bot.* **2003**, *54*, 1931–1939. [[CrossRef](#)]
81. Lu, H.; Xu, S.; Zhang, W.; Xu, C.; Li, B.; Zhang, D.; Mu, W.; Liu, F. Nematicidal Activity of trans-2-Hexenal against Southern Root-Knot Nematode (*Meloidogyne incognita*) on Tomato Plants. *J. Agric. Food Chem.* **2017**, *65*, 544–550. [[CrossRef](#)]
82. Cipollini, N. Constitutive expression of methyl jasmonate-inducible responses delays reproduction and constrains fitness responses to nutrients in *Arabidopsis thaliana*. *Evol. Ecol.* **2008**, *24*, 59–68. [[CrossRef](#)]

-
83. Karban, R. Tradeoff between resistance induced by volatile communication and over-topping vertical growth. *Plant Signal. Behav.* **2017**, *12*, e1309491. [[CrossRef](#)]
 84. Ballhorn, D.J.; Heil, M.; Lieberei, R. Phenotypic Plasticity of Cyanogenesis in Lima Bean *Phaseolus lunatus*—Activity and Activation of β -Glucosidase. *J. Chem. Ecol.* **2006**, *32*, 261–275. [[CrossRef](#)]
 85. Kessler, A. Defensive Function of Herbivore-Induced Plant Volatile Emissions in Nature. *Science* **2001**, *291*, 2141–2144. [[CrossRef](#)]
 86. Heil, M. Indirect defence via tritrophic interactions. *New Phytol.* **2008**, *178*, 41–61. [[CrossRef](#)]
 87. Peñaflor, M.F.G.; Gonçalves, F.G.; Colepicolo, C.; Sanches, P.A.; Bento, J.M.S. 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*. *Entomol. Exp. Appl.* **2017**, *165*, 83–93. [[CrossRef](#)]
 88. Stenberg, J.A.; Heil, M.; Åhman, I.; Björkman, C. Optimizing Crops for Biocontrol of Pests and Disease. *Trends Plant Sci.* **2015**, *20*, 698–712. [[CrossRef](#)]
 89. Peñaflor, M.F.G.V.; Bento, J.M.S. Herbivore-Induced Plant Volatiles to Enhance Biological Control in Agriculture. *Neotrop. Entomol.* **2013**, *42*, 331–343. [[CrossRef](#)]
 90. Lucchi, A.; Loni, A.; Gandini, L.M.; Scaramozzino, P.; Ioriatti, C.; Ricciardi, R.; Shearer, P.W. Using herbivore-induced plant volatiles to attract lacewings, hoverflies and parasitoid wasps in vineyards: Achievements and constraints. *Bull. Insectol.* **2017**, *70*, 273–282.
 91. Webster, B.; Bruce, T.; Dufour, S.; Birkemeyer, C.; Birkett, M.; Hardie, J.; Pickett, J. Identification of Volatile Compounds Used in Host Location by the Black Bean Aphid, *Aphis fabae*. *J. Chem. Ecol.* **2008**, *34*, 1153–1161. [[CrossRef](#)]
 92. Huot, B.; Yao, J.; Montgomery, B.L.; He, S.Y. Growth–Defense Tradeoffs in Plants: A Balancing Act to Optimize Fitness. *Mol. Plant* **2014**, *7*, 1267–1287. [[CrossRef](#)]
 93. Wei, J.; van Loon, J.J.A.; Gols, R.; Menzel, T.R.; Li, N.; Kang, L.; Dicke, M. Reciprocal crosstalk between jasmonate and salicylate defence-signalling pathways modulates plant volatile emission and herbivore host-selection behaviour. *J. Exp. Bot.* **2014**, *65*, 3289–3298. [[CrossRef](#)]
 94. Ameye, M.; Allmann, S.; Verwaeren, J.; Smagghe, G.; Haesaert, G.; Schuurink, R.C.; Audenaert, K. Green leaf volatile production by plants: A meta-analysis. *New Phytol.* **2017**, *220*, 666–683. [[CrossRef](#)]
 95. Holopainen, J.K.; Gershenzon, J. Multiple stress factors and the emission of plant VOCs. *Trends Plant Sci.* **2010**, *15*, 176–184. [[CrossRef](#)]
 96. Caparrotta, S.; Boni, S.; Taiti, C.; Palm, E.; Mancuso, S.; Pandolfi, C. Induction of priming by salt stress in neighboring plants. *Environ. Exp. Bot.* **2018**, *147*, 261–270. [[CrossRef](#)]
 97. Kessler, A.; Halitschke, R.; Diezel, C.; Baldwin, I.T. Priming of plant defense responses in nature by airborne signaling between *Artemisia tridentata* and *Nicotiana attenuata*. *Oecologia* **2006**, *148*, 280–292. [[CrossRef](#)]
 98. Arimura, G.-I.; Muroi, A.; Nishihara, M. Plant–plant–plant communications, mediated by (E)- β -ocimene emitted from transgenic tobacco plants, prime indirect defense responses of lima beans. *J. Plant Interact.* **2012**, *7*, 193–196. [[CrossRef](#)]