Dispensing a synthetic green leaf volatile to two plant species in a common garden differentially alters physiological responses and herbivory

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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 (~10ng/hour) of the green leaf volatile *cis*-3-hexenyl acetate (*z3*HAC), which is an HIPV and damage-associated volatile. *z*3HAC-treated pepper plants were shorter, had less aboveground and belowground biomass, and produced fewer flowers and fruits relative to controls while *z*3HAC-treated lima bean plants were taller and produced more leaves and flowers than did controls. Natural herbivory was reduced in *z3*HAC-exposed lima bean plants, but not in pepper. Cyanogenic potential, a putative direct defense mechanism in lima bean, was lower in young *z3*HAC-exposed leaves, suggesting a growth-defense tradeoff from *z3*HAC exposure alone. Plant species-specific responses to an identical volatile cue have important implications for agronomic costs and benefits of volatile-mediated inter-plant communication under field conditions.

Key Words. *Phaseolus lunatus, Capsicum annuum* var. Cayenne, Fabaceae, Solanaceae, common garden, field study, *cis-3*-hexenyl acetate

Introduction

Production and utilization of airborne chemical cues is 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 (*z*3HAC) induces transcriptional changes in poplar [4] and maize [21] that prime oxylipin signaling and induced resistance. Like other GLVs, *z*3HAC can be emitted from wounded leaves alone, but may also represent a

70 reliable cue because it is typically released from herbivore-damaged leaves in a variety of species
71 [23], including tomato [14], maize [21], Arabidopsis [25], lima bean [8,17], pepper [26,27], and
72 poplar [4,28].

73 Costs incurred by plants responding to airborne HIPV cues alone are largely unknown.
74 Plant defense theory posits that induced resistance by plants against herbivores is a cost-savings
75 strategy to restrict the deployment of costly specialized defensive metabolites until necessary
76 [29,30]. However, inducible resistance generates a period of vulnerability between the time of
77 attack and the upregulation of resistance [31]. Perception of early reliable cues may overcome
78 such a vulnerability by allowing a plant to anticipate a probable attack and prime defenses before
79 herbivory occurs. Since HIPV-mediated priming is an inducible phenomenon, theory predicts
80 that responding to reliable cues alone should incur costs that outweigh and select against
81 maintaining a "primed state" [32,33]. In other words, perception of a priming stimulus, such as an
82 HIPV, may induce physiological changes that incur costs that are less expensive than induced
83 resistance itself. Previous work with non-volatile priming agents β-amino butyric acid (BABA)
84 [34] and snail mucus [35] both support this prediction. Similarly, costs associated with volatile
85 perception alone that initiate priming should be less severe than costs of induced resistance to
86 actual herbivory [36]. Yet there is currently limited experimental evidence of such costs with
87 respect to anti-herbivore volatile cues. For example, bacterial-derived volatiles 3-pentanol and
88 2-butanone increased fresh fruit weight in field-grown *Cucumis sativa* [37], wild tobacco
89 (*Nicotiana attenuata*) exposed to airspace of experimentally clipped sagebrush produce more
90 seeds (i.e., higher presumptive fitness) relative to control plants [38]. These results suggest that
91 ecological costs of exposure to volatile cues may be context dependent, but comparative

92 cost/benefit tradeoffs for perception of HIPVs alone among sympatric field-grown plants is
93 currently lacking.
94 Here, we report a common garden field experiment with lima bean (*Phaseolus lunatus*)
95 and chili pepper (*Capsicum annuum*) testing the hypothesis that field plants subject to a
96 persistent dose of an ostensibly reliable volatile cue incur consistent costs reflected in reduced
97 growth and reproduction. We treated individuals of both species to repeated low-dose
98 applications of *z3*HAC and measured their growth, reproduction, and herbivore damage
99 throughout the growing season. We predicted that exposure to *z*3HAC—regardless of plant
100 species identity—would reduce growth and reproductive output, while also reducing natural
101 herbivory.
102
103 **Materials and Methods**
104 *Study Site and Plants*
105 A common garden experiment was established on a 54m^2 plot within Blackacre
106 Conservancy's community garden in Louisville, Kentucky (38°11'33.8"N 85°31'28.3"W;
107 Supplemental Fig. 1). The field site was enclosed in a mesh fence to exclude mammalian
108 herbivores. *Phaseolus lunatus,* Fabaceae, variety Fordham Hook 242 ('lima bean') and the
109 *Capsicum annuum*, Solanaceae, variety Cayenne pepper, Joe Red Long ('pepper') were chosen
110 as phylogenetically distinct model plants with previously established defense profiles [39,40].
111 Seeds were purchased from the Louisville Seed Company (Louisville, KY, USA), and
112 germinated in Metromix 510© in May 2016 in the Biology Department's greenhouse. After
113 reaching ~20cm in height, 132 lima bean plants were transplanted to the field May 30, 2016, at 4
114 weeks old and 98 pepper plants at 8 weeks old were transplanted to the field June 28, 2016.

While both species were started at the same time, peppers were placed in the field later than the lime 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 consequent 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 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 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 10ng/hr of *z*3HAC, a concentration 25% of that which previously primed poplar [4] and maize [21]. A treatment vial contained 50mg of a 30ng/ul *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 photodegradation [47] (Supplemental Fig. 2). These vials were left in the field and replaced every seven days for the duration of the field season. 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 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.

160 From the fruits harvested from the final biomass harvest, ~10 randomly selected, mature
161 fruits from each pepper plant were chosen for seed count analysis (188 fruits from z3HAC-
162 treated plants and 210 fruits from controls). Dried fruits were dissected with a scalpel and all
163 seeds were isolated and counted.
164
165 *Herbivory*
166 Leaf chewing damage was assessed for both pepper and lima bean as percent leaf area
167 removed (LAR) using a visual estimation technique [48,49] with the following damage categories:
168 0%, 0-5%, 5-15%, 15-30%, 30-50%, 50-70%, 70-90%, and >90%. For each damage assessment,
169 every leaf on a plant was categorized into one of the damage categories, and an overall percent
170 damage was determined as a weighted average of all leaves. Plants were also routinely
171 monitored for the presence of naturally occurring chewing and piercing/sucking herbivores. In
172 particular, we observed an ephemeral, natural occurrence of the black bean aphid (*Aphis faba*),
173 and recorded its presence/absence on lima bean plants in the field.
174
175 *Leaf collections and cyanide measurements*
176 Since cyanogenic potential (CP) is an inducible herbivore defense in lima bean [50,51], we
177 used CP as a metric for induced responses in the presence of *z3*HAC. We collected source and
178 sink leaves [52,53] on July 10, 2016, which was approximately 6 weeks into the field season. We
179 developed a novel protocol in the lab for microscale colorimetric CP quantification by modifying
180 an existing macro-scale protocol [54,55]. Briefly, 5mg of lyophilized tissue was mixed with
181 200uL of citrate buffer (0.1 M, pH 5.5-6.5) in a 2mL centrifuge tube, into which a 200uL glass
182 vial (Agilent #5183-2090) containing 100uL 1.0M NaOH was also placed and the centrifuge

183 tube caped completely. After 15 hours, a 50uL aliquot of the 1.0M NaOH in the inner glass vial
184 was diluted to 0.1M, and a 30uL aliquot was neutralized with 30uL 0.5M acetic acid in a 96-well
185 reaction plate. Then, 75 μL of Reagent A (5 mg/ml succinimide [VWR, AAA13503] and 0.5
186 mg/ml n-chlorosuccinimide [VWR, AAA10310]) and then 30 μL of Reagent B (30 mg/ml
187 barbituric acid [VWR, BT134930] in 30% pyridine [Sigma, 270970]) was added to each well.
188 After 8 min, absorbance at 580nm was measured on a plate reader (Molecular Devices
189 SpectraMax M2). Quantification of CP was made against a standard curve of NaCN (VWR,
190 BT212960).
191
192 *Statistical analyses*
193 All statistical analyses were performed in R (version 3.4.2) with the lme4 and multcomp
194 packages. Growth data, such as plant height, leaf area removed, and flower counts, were
195 analyzed using repeated measures ANOVA with the aov function with a Gaussian distribution.
196 For repeated measures analyses, we treated date as a within-subjects effect and treatment as a
197 between-subjects effect for all analyses. Differences between treatments at each individual time
198 point, as well as all biomass data, were analyzed using one-way ANOVA (glmer function)
199 followed by a Tukey's post hoc comparison. Cyanogenesis data were log transformed to satisfy
200 assumptions of normality before being analyzed by a glm model followed by a Tukey's post hoc
201 comparison.
202
203 **Results**
204 *z3HAC differentially effects growth of lima bean and pepper plants*

205 Treatment with *z3*HAC differentially affected the growth of lima bean and pepper plants.
206 On average, *z*3HAC-treated lima bean grew 11% taller compared to control plants throughout the
207 field season (Fig.1A; $F_{2,927}$=9.688, *P*=0.002) and produced 17% more leaves overall than did
208 controls (Fig.1B; $F_{1,571}$=4.339, *P*=0.038). In contrast, *z*3HAC-treated pepper plants were 12%
209 shorter relative to controls (Fig.1C; $F_{1,48}$=6.168, *P*=0.017) and produced 23% fewer leaves over
210 the field season (Fig.1D; $F_{1,237}$=21.58, *P*<0.001). Consistent with height and leaf counts, *z3*HAC
211 treatment reduced overall biomass of pepper plants by 24% on average (Fig.2). When we
212 destructively harvested all pepper plant biomass at the end of the season, *z*3HAC-treated pepper
213 plants had lower leaf, stem, and root dry biomass by 21%, 31%, 29%, respectively (Fig.2A-C)
214 (Z=3.379, *P*<0.001*;* Z=-2.035, *P*=0.042; Z=-2.379, *P*=0.017*)*. Despite these *z*3HAC-mediated
215 effects on biomass exposure, the aboveground-to-belowground biomass ratio was similar
216 regardless of treatment (Fig.2D; Z=0.31, *P*=0.757). That is, pepper plants treated with *z*3HAC
217 were smaller relative to control plants.
218
219 *z3HAC reduces reproductive output in pepper plants*
220 *z*3HAC treatment also differentially affected reproductive output between the two
221 species, and lowered fruit output in pepper. Flower production was 30% higher in lima bean
222 plants exposed to *z*3HAC (Fig.3A; $F_{1,576}$=15.044, *P*<0.001), while *z*3HAC-treated peppers
223 produced 37% fewer flowers relative to control plants at the end of the field season (Fig.3B;
224 $F_{1,43}$=14.48, *P*<0.001). *z*3HAC-treated pepper plants also produced 23% fewer fruits overall
225 relative to controls (Fig.4A; Z=-2.035, *P*=0.042), and the fruits that were produced by *z*3HAC-
226 treated plants had lower wet and dry masses (Fig.4B-C; Z=-2.88, *P*=0.004; Z=-2.439, *P*=0.015),
227 and 10% lower total seed counts (Fig.4D; Z=3.524, *P*<0.001) and total seed masses (Fig.4E;

228 Z=3.334, P<0.001), relative to controls. That said, the ratio of seed mass to fruit mass was
229 similar between *z*3HAC-treated and control plants (Fig.4F; Z=0.588, P=0.807), as was mass of
230 an individual pepper seed (Supplemental Fig.3). There was no apparent difference in lima bean
231 pod production (Supplemental Fig.3), though an unexpected field-wide premature pod drop
232 independent of treatment prevented us from fully determining lima bean pod/seed production
233 with confidence.
234
235 *z3HAC exposure reduces herbivory on lima bean*
236 *z*3HAC exposure reduced natural herbivory in lima bean but not pepper plants. Chewing
237 herbivory on pepper plants was low overall and statistically higher in *z*3HAC-treated plants only
238 during the first sampling date (Fig.5A, $F_{1,193}$=5.627, P=0.019). In contrast, chewing damage to
239 lima bean leaves increased as the field season progressed, with *z*3HAC-treated plants having
240 overall 26% less chewing damage than did control plants (Fig.5B; $F_{1,539}$=21.745, P<0.001). In
241 addition to chewing herbivory, black bean aphids (*Aphis faba*) colonized 87% of the *z*3HAC-
242 treated lima bean plants, compared with only 21% of control plants (Fig.5C; χ^2 = 50.11, df=1,
243 P<0.001). *A.faba* colonized early in the season and was only observed June 15-31 (Julian dates
244 166-181) because a heavy rainfall event reduced their population to undetectable levels.
245 Piercing/sucking herbivores were rare for the remainder of the experiment.
246
247 *Cyanogenic potential in lima bean is decreased by z3HAC exposure*
248 Exposure to *z3*HAC reduced cyanogenic potential in lima bean plants. *z3*HAC exposure
249 alone reduced cyanide concentration by 50% in sink leaves (Figure 6A; Z= -0.3257, P=0.003),

250 but not significantly in source leaves (Fig. 6B; Z=-0.14387, *P*=0.146). Baseline cyanogenic
251 potential was ~2x greater in sink leaves compared to source leaves (Figure 6).
252
253 **Discussion**
254 We show that a persistent, low-dose application of *z*3HAC differentially affects growth
255 and reproduction of two plant species grown in the same field. Based on previous work on plant
256 and sensory perception of volatiles [7,21], we hypothesized *z3*HAC application would decrease
257 growth and reproductive fitness in both plant species. The rationale for this hypothesis was a
258 central assumption of induced resistance theory that ecological costs modulate the deployment
259 particular defensive phenotypes until necessary [30,31,56-60]. Volatile-mediated priming, even if
260 regulated by a different mechanism from resistance [61], is an inducible phenomenon that
261 theoretically should incur such fitness costs [36]. Yet, our results clearly indicate that pepper and
262 lima bean had divergent fitness outcomes when subjected to a single GLV under identical field
263 conditions. Whereas *z3*HAC-treated pepper plants had reduced growth (Fig. 1) and no effect on
264 herbivore resistance (Fig. 5A) relative to controls, *z3*HAC-treated lima bean plants grew more
265 and produced more flowers (Fig. 1,3), and suffered less chewing herbivory (Fig. 5B) compared
266 to controls. This result—that some plants experience costs while others have minimal or even
267 positive effects when exposed to the same HIPV—has important implications for how volatile
268 cues may structure interspecific competition and ecological communities. HIPVs alone may be
269 sufficient to result in differential fitness effects among species. Moreover, the positive effects of
270 *z3*HAC on lima bean growth and flowering opens agronomic opportunities to exploit plant
271 volatiles to enhance both growth and pest resistance of important crop plants.

272 What might affect the response of plants to volatile exposure? One possibility is that the
273 signal integrity of HIPVs varies among plant species, which influences plant sensory perception
274 and the outcome of defense priming. That is, *z*3HAC provides different information to different
275 plants. Previous work on the role of HIPVs in plant anti-herbivore resistance focused on
276 priming-mediated defense with consistent results in wheat [62,63], corn [12,45], lima bean [8,11,64],
277 tomato [65], blueberry [15], sagebrush [43], Arabidopsis [66] and poplar [4]. In contrast, we
278 specifically focused on indicators of plant fitness in lima bean and pepper in a common garden
279 experiment. *z*3HAC treatment alone increased growth and flowering in lima bean, while
280 reducing growth and reproductive output in pepper (Fig. 3 and 5). This season-long evidence
281 illustrates that informational integrity on an HIPV varies between these two plant species. Such
282 divergent fitness effects from exposure to a single ubiquitous herbivore-associated cue
283 underscore the potential for functional similarity in the mechanisms by which plants modulate
284 responses to herbivory and volatile indicators of herbivory.

285 Flower and fruit production is a key component of plant fitness potential. We show that
286 *z*3HAC treatment alone differentially affected flower production in lima bean and pepper (Fig.
287 3). Insect herbivory can increase or decrease floral production depending on the system and
288 environmental conditions [67-69]. Whereas increased flower production is a strategy assumed to
289 ameliorate fitness losses in the presence of an environmental stress [56,70], decreased flower
290 production may be related to costs of chemically mediated defense [71]. Previous work with lima
291 bean has demonstrated stress-mediated compensation [72]. Our result that *z*3HAC alone was
292 sufficient to trigger increased flowering is consistent with this observation, suggesting that
293 *z*3HAC alone may stimulate a long-term stress response similar to herbivory. Therefore, even

294 though the mechanisms underlying *z*3HAC-mediated effects on flower and fruit production are
295 not yet known, they may be similar to those induced by herbivory [67].
296 Resource allocation between different tissues is pivotal for growth, reproduction, and
297 defense, and can be influenced by environmental stress. For example, direct herbivory alters
298 resource allocation between aboveground tissue and belowground tissue [49,73,74], as does
299 application of the anti-herbivore phytohormone jasmonic acid (JA) [75,76]. Volatile cues can also
300 affect biomass allocation. For example, barley exposed to volatiles from unwounded neighboring
301 plants of different cultivars increases root and leaf biomass [77], while exposure to volatiles
302 decreases aboveground biomass in other systems [78,79]. In our case, volatile treatment reduced
303 overall aboveground and belowground biomass in pepper, but did not appear to alter overall
304 biomass allocation patterns. Simply put, *z*3HAC-treated pepper plants were smaller overall, and
305 therefore produced fewer seeds.
306 Differential investment between growth and defense is vital for maximizing limited
307 resources and, ultimately, fitness. Inducible defenses in plants against herbivores and pathogens
308 modulate such growth/defense tradeoffs in a number of plant species [31]. In some cases,
309 exposure to VOCs alone is sufficient to affect such tradeoffs. For example, sagebrush exposed to
310 VOCs from damaged conspecifics had decreased growth [80], while HIPV-exposed tobacco had
311 increased herbivore resistance but decreased seed set [38]. In our experiment, *z*3HAC exposure
312 decreased cyanogenic potential in sink leaves (Fig. 6), which were 20-30% higher than in source
313 leaves. These results suggest that persistent exposure to *z*3HAC led to allocation shifts from
314 cyanogenic potential (a putative defense) towards growth (Fig. 1 and 3), as well as supporting
315 ontogeny-mediated cyanogenic potential [52,81].. That said, *z*3HAC-treated plants also
316 experienced less natural damage primarily on mature source leaves than did controls (Fig. 5,

317 personal observations), suggesting that the herbivory was deterred by defense mechanism other
318 than cyanogenic potential in source leaves.

319 Volatile cues may impact ecological communities in both expected and pleiotropic ways.
320 HIPVs are well-established mediators of multi-trophic antagonistic and mutualistic interactions
321 [82-84], and manipulations of chemical signals and volatile blends have been used for biological
322 control in a wide range of systems [85,86]. For example, HIPV-infused sticky traps in a grape
323 (*Vitis vinifera*) orchard differentially attracted lacewings, hoverflies, and parasitoids [87].
324 Exogenous GLV manipulation using "dispensers" under field conditions altered the arthropod
325 community composition in maize [47]. In our study, *A.faba* were clearly and unexpectedly
326 attracted to *z*3HAC-exposed plants (Fig. 5). Under glasshouse conditions, *A.faba* were repelled
327 by *z*3HAC alone [88], which suggests that the cue that mediated attraction was not our treatment
328 alone. It is tempting to speculate that aphid attraction combined with reduced chewing herbivory
329 in lima bean may be reflective of *z*3HAC effects on JA and salicylic acid (SA) signaling, which
330 would be consistent with a JA-SA tradeoff [89,90]. Ultimately, however, the utility of GLVs (or
331 other VOCs) in field applications will depend on understanding community-level effects of
332 volatile exposure.

333 As a caveat, volatile identity, concentration, and duration may affect the reliability of a
334 cue and therefore the costs associated with eavesdropping. Plants experiencing insect herbivory
335 frequently generate species-specific blends of volatile compounds [91,92], which can influence
336 fitness in neighboring plants [80,93,94]. Plant-derived compounds associated with herbivory
337 include GLVs [4,12,78], shikimate derivatives [13], and terpenes [95]. However, individual
338 compounds within a blend can affect plant defense and priming as much as the blend itself. We
339 used *z*3HAC in this study because it is released herbivore-damaged leaves [21], which ostensibly

340 allows *z*3HAC to confer reliable ecological information. That said, *z*3HAC can also be released
341 from wounded leaves without herbivores [91] [23]. However, plants detect and respond
342 specifically to *z*3HAC [4], and the costs associated with that response in the context of pest
343 resistance were the focus of this investigation. Additionally, concentration of a cue may
344 influence plant resistance [66,78]. For example, a repeated, low-dose exposure to a GLV blend
345 enhances plant resistance compared to a single application [46] while *z3*HAC emissions can be as
346 high as 66 ng/cm^3 after herbivory [20]. For these reasons, we chose to use a low-dose exposure to
347 *z*3HAC (25% of the concentration that primed poplar [4] and maize [21]), and still observed
348 divergent fitness effects between the two plant species (Figs.1, 3, and 5).
349 In summary, our key finding is that persistent application of a low dose of a single
350 volatile compound *z*3HAC, a common HIPV and GLV, in field conditions leads to divergent
351 growth and reproductive fitness effects between two plant species. This result underscores the
352 variable nature of volatile-mediated eavesdropping, and that plants may have evolved species-
353 specific mechanisms for responding to volatile cues. Given natural variation among species,
354 future work must assess costs with other species as well as within accessions and landraces of
355 our model plants over multiple years. Ultimately, the adaptive significance of eavesdropping for
356 enhancing plant immunity will depend on plant life history, physiology, and other ecological
357 factors to determine whether a plant will benefit from eavesdropping VOCs or not, and therefore
358 what impact volatile-mediated eavesdropping might have on plants and their insect pests.
359
360 **Supplemental Material**. Supplemental Tables describing the outputs of statistical analyses are
361 listed as tabs in a single spreadsheet ("Supp_Tables"), with tab names corresponding to each
362 table. Supplemental Figure 1 ("Supp_Fig1"). Aerial view of the field site within the Blackacre

Community Garden. The research area is outlined and the original picture was acquired from Google Earth. Supplemental Figure 2 ("Supp_Fig2"). Volatile administration setup. Each 2mL glass vial was inverted and supported by wire stands to prevent rain water accumulation. Additionally, each vial was wrapped in aluminum foil to prevent photodegradation [47]. Supplemental Figure 3 ("Supp_Fig3"). Calculated individual seed mass for *C.annuum* (A) and total pod production for *P.lunatus* (B). 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.

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686 **Figure Captions**
687
688 **Fig. 1**. **Height measurements and leaf counts for *Phaseolus lunatus* (lima bean) and**
689 ***Capsicum annuum* (pepper) grown in a common garden field experiment.** Height for (A)
690 lima bean was measured from the base of the longest runner to the uppermost branching point;
691 (C) pepper height was measured from the base of the main stalk to the uppermost branching
692 point. Leaf counts for (B) lima bean and (D) pepper included all mature leaves on each plant.
693 Open circles represent control plants (receiving lanolin-filled vials); filled squares represent
694 plants receiving a persistent application of vials containing 10ng/hr *cis*-3-hexenyl acetate
695 (*z*3HAC) dissolved in lanolin. Dropdown lines indicate the initial application of *z*3HAC
696 treatment: lima bean and pepper plants were first exposed on June 10, 2016 (Julian date 161) and
697 July 11, 2016 (Julian date 192), respectively. Points represent averages +/- SE. Repeated
698 measures ANOVAs (aov in R) were followed by one-way ANOVAs at each time-point.
699 Asterisks (*) represent $P<0.05$ between treatment and control at each time point. See
700 Supplemental Table 1 for complete statistics.
701
702 **Fig. 2**. **Biomass measurements of field-grown *Capsicum annuum* (pepper) plants.** (A) Leaf,
703 (B) stem, (C) root biomass, and (D) the aboveground:belowground biomass ratio in *C.annuum*
704 plants were determined at the end of the field season following destructive harvest. Bars
705 represent means +/- S.E.M.. *P*-values represent Tukey's HSD comparisons. See Supplemental
706 Table 2 for complete statistics.
707
708 **Fig. 3**. **Total flower production in (A) *Phaseolus lunatus* (lima bean) and (B) *Capsicum***
709 ***annuum* (pepper).** Open circles represent control plants (receiving lanolin-only vials); filled
710 squares represent plants receiving a persistent application of vials containing 10ng/hr *cis*-3-
711 hexenyl acetate (*z*3HAC) dissolved in lanolin. Dropdown lines indicate the initial application of
712 *z*3HAC treatment: lima bean and pepper plants were first exposed on June 10, 2016 (Julian date
713 161) and July 11, 2016 (Julian date 192), respectively. Points represent averages +/- SE.
714 Asterisks (*) represent $P<0.05$ between treatment and control at each time point. See
715 Supplemental Table 3 for complete statistics.
716
717 **Fig. 4**. **Fruit and seed production in *Capsicum annuum* (pepper) plants grown in a common**
718 **garden experiment treated with a persistent application of the green leaf volatile *cis*-3-**
719 **hexenyl acetate (*z*3HAC).** The (A) total number of fruits were counted in the field, and (B) wet
720 and (C) dry masses fruit masses were determined in the lab. (D) The total number of seeds per
721 fruit and (E) the estimated mass per seed were determined from a subset of the fruits produced.
722 (F) The ratio of seed mass to fruit mass was calculated to assess the efficiency of seed
723 production. Bars represent means +/- S.E.M.. White boxes represent control plants; gray boxes
724 represent plants treated with *z*3HAC. *P*-values represent Tukey's HSD comparisons. See
725 Supplemental Table 4 for complete statistics.
726
727 **Fig. 5**. **Herbivore damage on *Capsicum annuum* (pepper) and *Phaseolus lunatus* (lima**
728 **bean) plants in a common garden field experiment.** Chewing damage on (A) pepper and (B)
729 lima bean plants was determined using a visual estimation technique (see Methods). Open
730 circles represent control plants (receiving lanolin-filled vials); filled squares represent plants
731 receiving a persistent application 10ng/hr *cis*-3-hexenyl acetate (*z*3HAC) dissolved in lanolin.

732 Dropdown lines indicate the initial application of *z*3HAC treatment. Points represent averages
733 +/- SE. Repeated measures ANOVAs (aov in R) were followed by one-way ANOVAs at each
734 time-point. Asterisks (*) represent $P<0.05$ between treatment and control at each time point. See
735 Supplemental Table 5 for complete statistics. (C) *Aphis faba* colonization on lima bean plants.
736 Bars represent the percentage of plants in each group where *A.faba* were observed ($\chi^2 = 50.11$,
737 df=1, $P<0.001$).
738
739 **Fig. 6. Cyanide induction in *Phaseolus lunatus* (Lima bean) as affected by *z3*HAC exposure**.
740 Cyanide concentration was determined by trapping volatile HCN in 1.0M NaOH followed by
741 colorometric quantification. Sink leaves (A) and source leaves (B) were exposed long-term
742 exposure of 10ng/hour *z3*HAC or left as a control. Points represent averages +/- SE and *
743 indicate a p-value <0.05.
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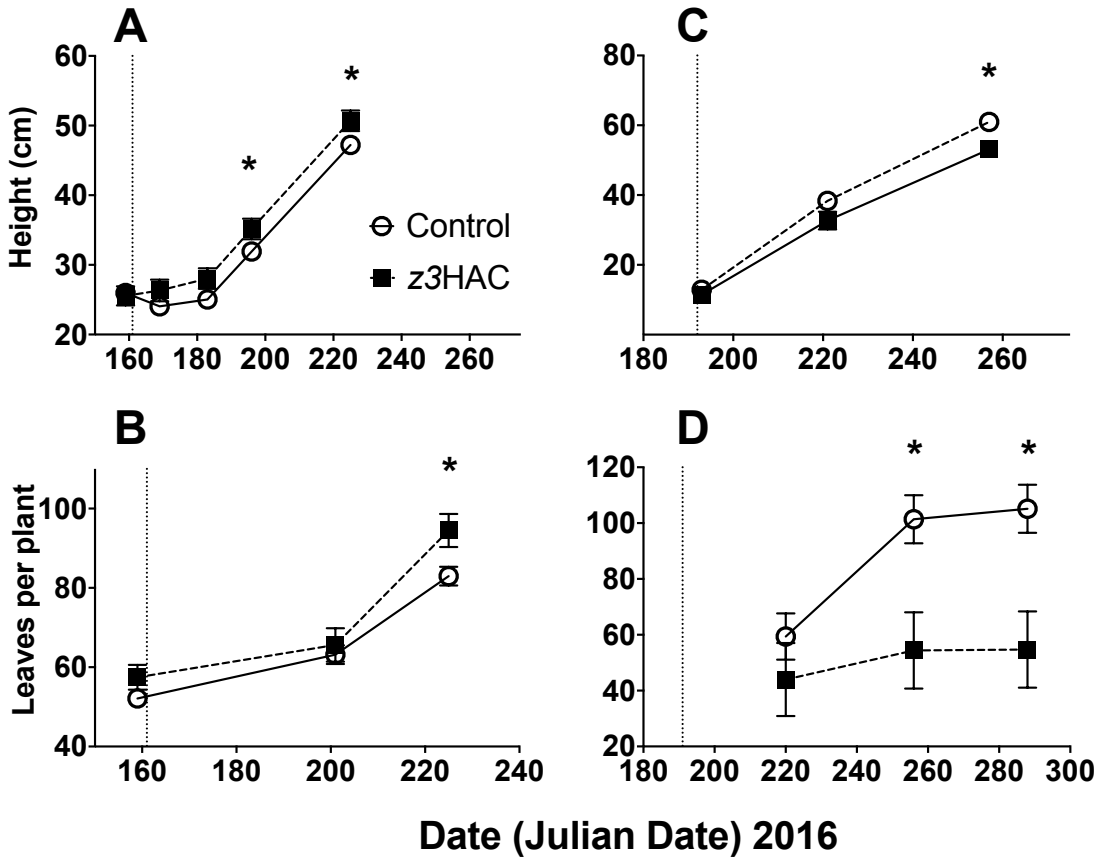
Figure 1


A
B
C
D
Height (cm)
Leaves per plant
Control
z3HAC
Date (Julian Date) 2016

768 **Figure 2**

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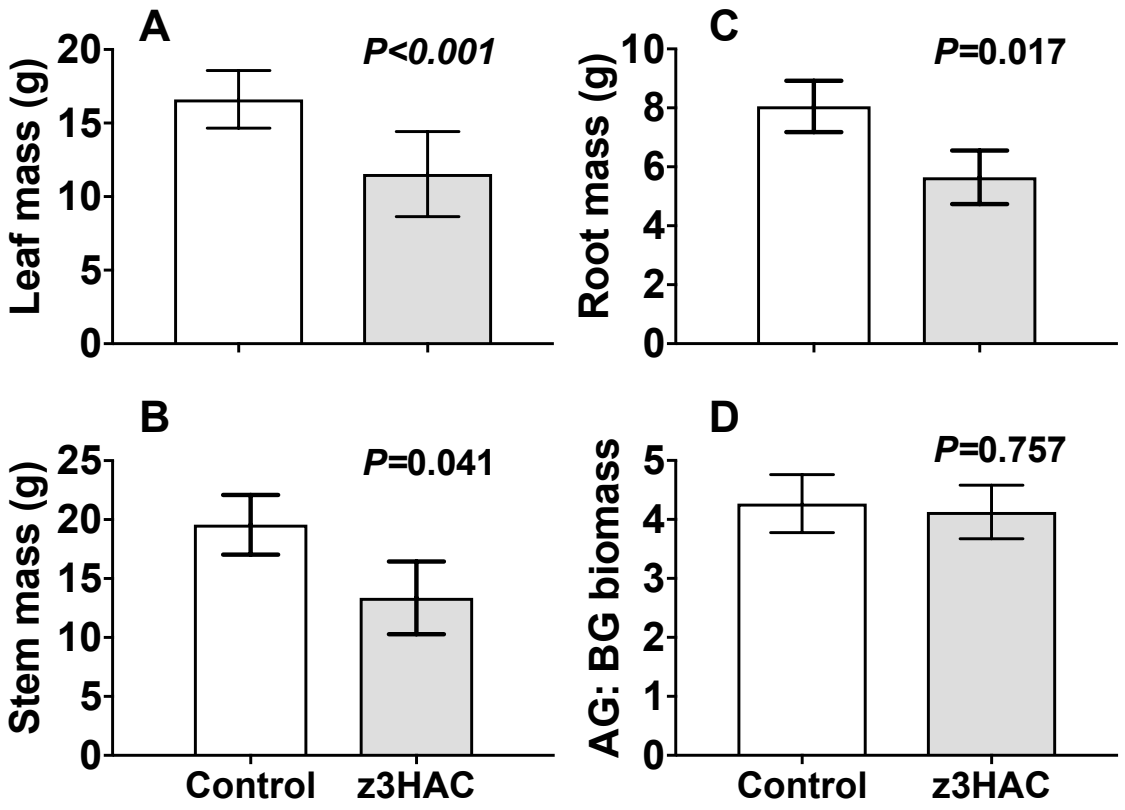

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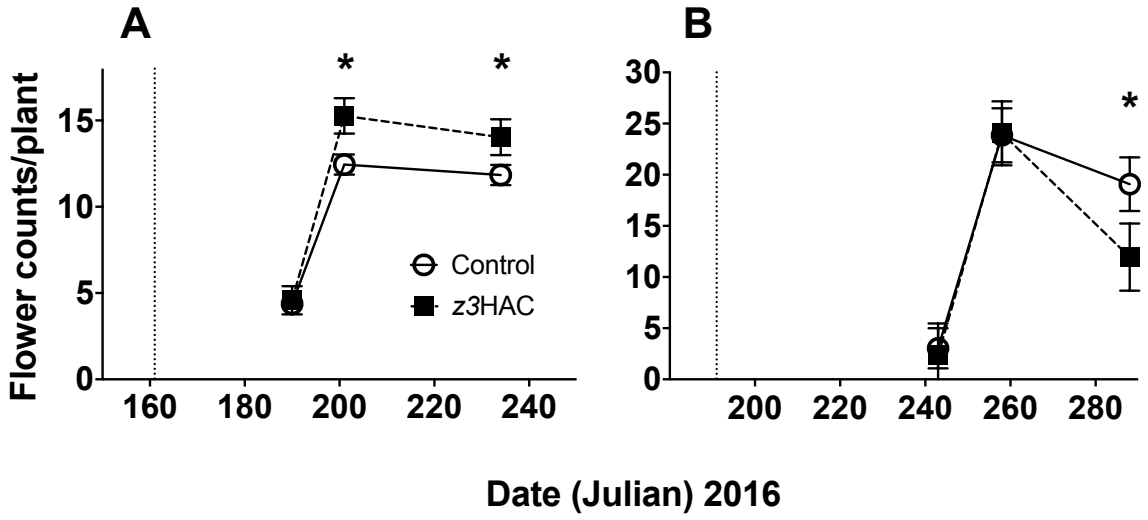
773 **Figure 3**



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792 **Figure 4**

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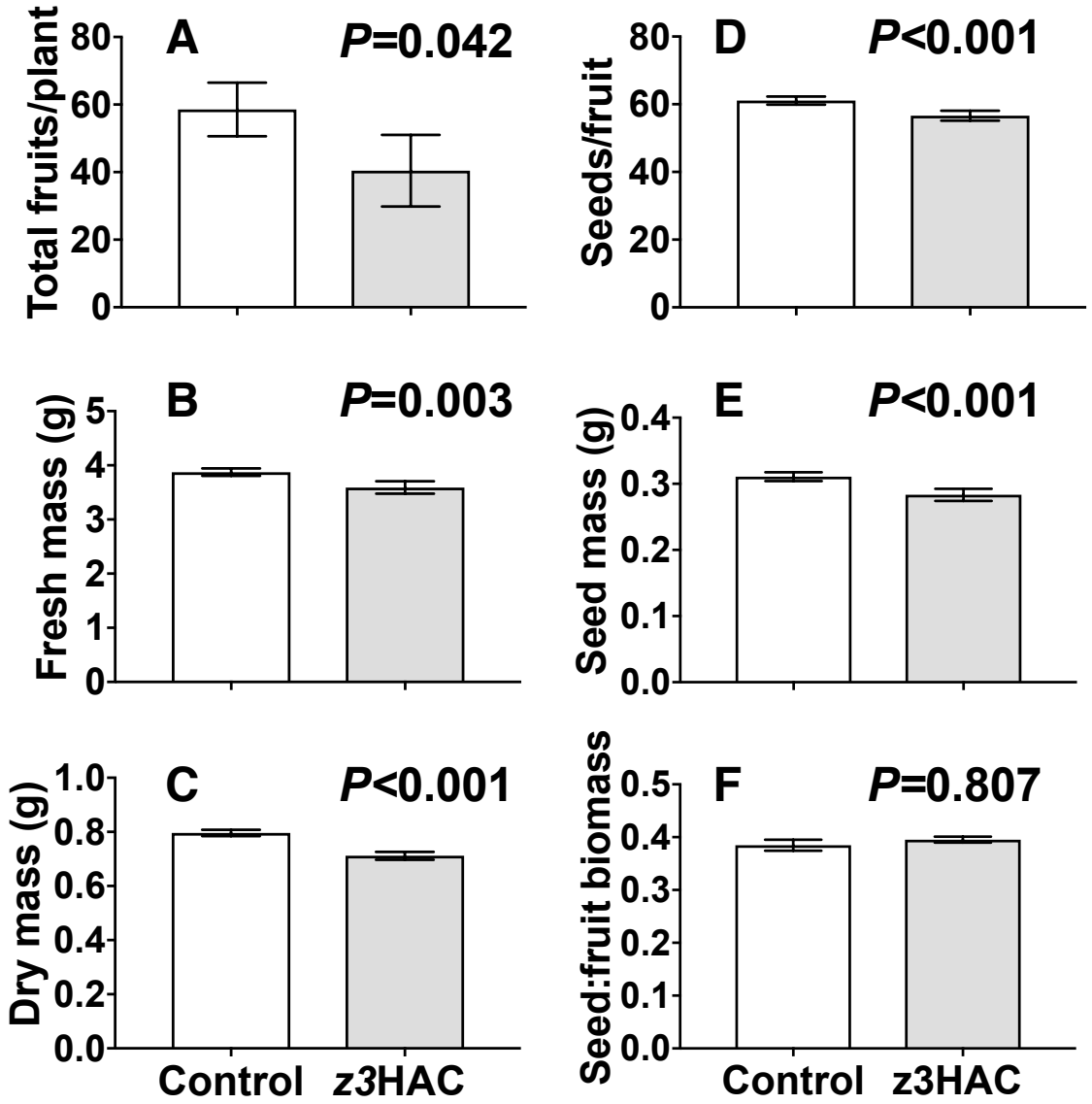

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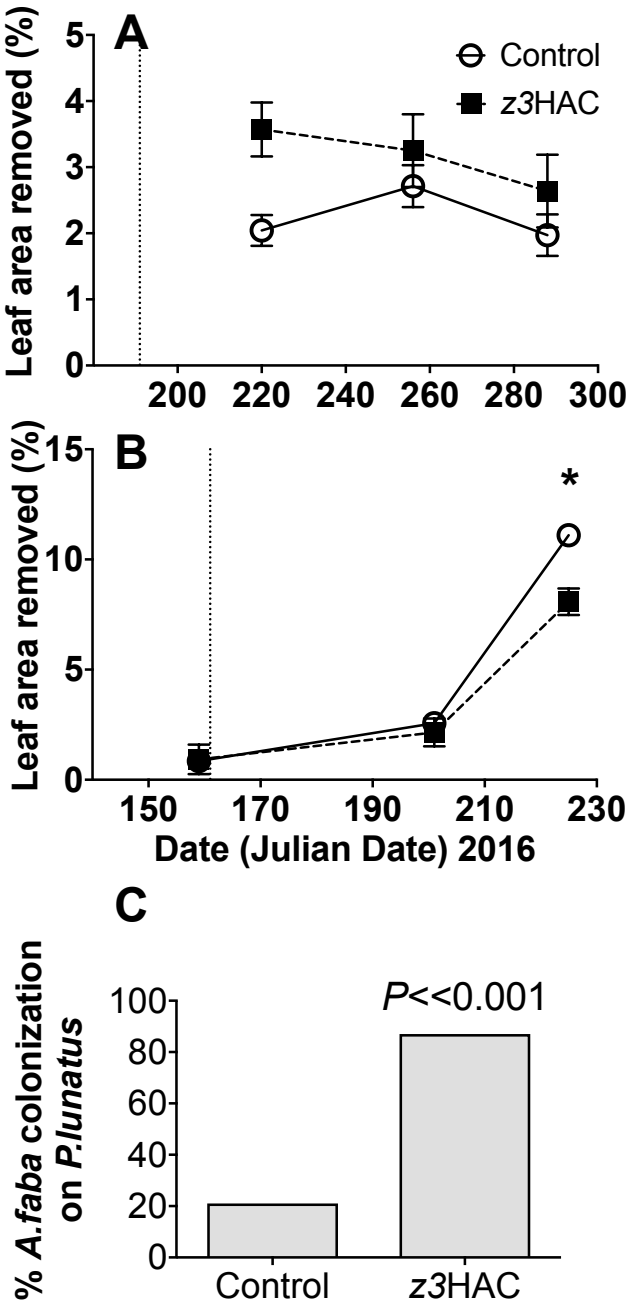
796 **Figure 5**



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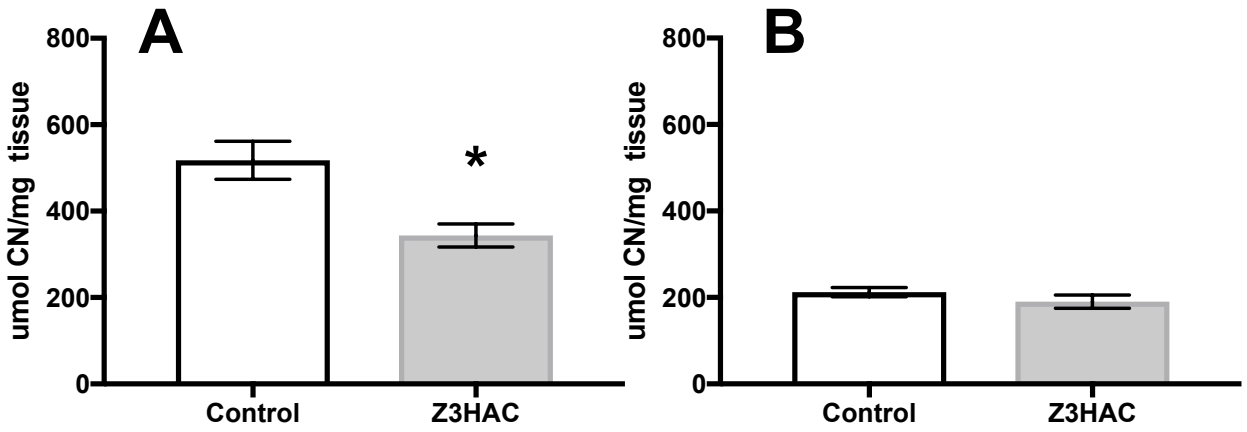
799 **Figure 6**



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