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

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Total figures: 6
Supplemental Figures: 3
Supplemental Tables: 6
Word Count: 4,243

Running Title: Costs of HIPV-mediated eavesdropping

Highlight: Long-term exposure to a ubiquitous herbivore-associated volatile cue results in species-specific fitness effects. Differential cue-mediated growth/defense tradeoffs have important implications for plant-herbivore interactions.

Abstract

Herbivore-induced plant volatile (HIPV)-mediated eavesdropping is a well-documented, inducible phenomenon that should—like any inducible phenomenon—incur fitness costs. Yet, ecological costs associated with volatile exposure alone are unclear. 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), a ubiquitous HIPV. *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 defense mechanism in lima bean, was lower in *z3*HAC-exposed leaves, suggesting a growth-defense tradeoffs from *z3*HAC exposure alone. Plant species-specific responses to an identical priming cue have important implications for ecological costs and benefits of volatile-mediated inter-plant communication under field conditions.

Key Words - *cis-3*-hexenyl acetate**,** eavesdropping, lima bean, pepper, plant fitness, plant volatiles

Abbreviations used- *cis*-3-hexenyl acetate (*z3*HAC), Herbivore-induced plant volatile (HIPV), Green-leaf volatile (GLV)

60 **Introduction**

61 Production and utilization of airborne chemical cues is prevalent within the plant
62 kingdom. Plants depend on airborne chemical signaling for pollination (Muhlemann *et al.*,
63 2014), indirect defense (Mumm and Dicke, 2010), protection from pathogens (Farag *et al.*,
64 2013), and herbivore resistance (Frost *et al.*, 2008c). Volatile communication is also pivotal for
65 plant-plant signaling, and selection for such signaling depends on honest cues that reliably confer
66 ecologically relevant information. For example, herbivory is a fundamental ecological
67 interaction that impacts plant fitness, and many plants increase the production and emission of
68 volatile compounds in response to herbivore damage (Turlings *et al.*, 1995). Such herbivore-
69 induced plant volatiles (HIPVs) are potentially reliable cues around which plant-plant
70 eavesdropping could be evolutionarily adaptive(Rodriguez-Saona and Frost, 2010). Undamaged
71 plants (or parts of the same plant (Frost *et al.*, 2007; Heil and Silva Bueno, 2007)) eavesdropping
72 on HIPVs from a plant experiencing herbivory may directly trigger stress responses (Arimura *et*
73 *al.*, 2002; Heil and Karban, 2010; Pearse *et al.*, 2013), or alternatively prime responses for future
74 potential herbivory (Frost and Hunter, 2008).

75 HIPV-mediated signaling appears to be a common phenomenon. For example, HIPVs
76 prime or induce corn (Erb *et al.*, 2015; Farag *et al.*, 2005), tomato (Farag and Paré, 2002), poplar
77 (Frost *et al.*, 2007; Frost *et al.*, 2008c), blueberry (Rodriguez-Saona *et al.*, 2009) and lima bean
78 (Arimura *et al.*, 2008; Sarai Giron-Calva *et al.*, 2012) against herbivory. HIPVs can be diverse
79 and taxa-specific (Arimura *et al.*, 2009; Copolovici *et al.*, 2011), but are often comprised of
80 monoterpenes, sesquiterpenes, benzenoids and green leaf volatiles GLVs (Boggia *et al.*, 2015;
81 Engelberth *et al.*, 2004). In contrast to volatile terpenes and benzenoids (Arimura *et al.*, 2009;
82 Paschold *et al.*, 2006), GLVs are immediately released into the airspace whenever leaves are

83 mechanically damaged (Matsui *et al.*, 2012) serving as early indicators of wounding. GLV
84 exposure alters gene expression profiles related to specialized metabolite production and
85 accumulated secondary metabolite precursors in preparation for inducing resistance (Pastor *et*
86 *al.*, 2014). For example, the GLV *cis*-3-hexenyl acetate (*z*3HAC) induces transcriptional changes
87 in poplar (Frost *et al.*, 2008c) and maize (Engelberth *et al.*, 2004) that prime oxylipin signaling
88 and induced resistance. Among the GLVs, *z*3HAC may represent a reliable cue because it is
89 typically released from herbivore-damaged leaves and not by mechanically damaged leaves in a
90 variety of species (Matsui *et al.*, 2012), including tomato (Farag and Paré, 2002), maize
91 (Engelberth *et al.*, 2004), Arabidopsis (D'Auria *et al.*, 2007), lima bean(Arimura *et al.*, 2008;
92 Heil and Silva Bueno, 2007), pepper(Addesso *et al.*, 2011; Cardoza and Tumlinson, 2006), and
93 poplar (Frost *et al.*, 2008c; McCormick *et al.*, 2014).

94 Costs incurred by plants exposed to HIPV cues alone are largely unknown. Ecological
95 theory posits that induced resistance by plants against herbivores is a cost-savings strategy to
96 restrict the deployment of costly specialized defensive metabolites until necessary (Accamando
97 and Cronin, 2012; Cipollini *et al.*, 2003). However, inducible resistance generates a period of
98 vulnerability between the time of attack and the upregulation of resistance (Cipollini and Heil,
99 2010). Perception of early reliable cues may overcome such a vulnerability by allowing a plant
100 to anticipate a probable attack. Since priming is an inducible phenomenon, theory predicts that
101 responding to reliable cues alone should incur costs that outweigh and select against maintaining
102 a "primed state" (Douma *et al.*, 2017; Frost *et al.*, 2008b). In other words, perception of a
103 priming stimulus, such as an HIPV, may induce physiological changes that incur costs that are
104 less expensive than induced resistance itself. Non-volatile priming agents β-amino butyric acid
105 (BABA) (van Hulten *et al.*, 2006) and snail mucus (Orrock, 2018) both support this prediction.

106 Similarly, costs associated with volatile perception alone that initiate priming should be less
107 severe than costs of induced resistance to actual herbivory(Martinez-Medina *et al.*, 2016). Yet
108 there is currently limited experimental evidence of such costs with respect to anti-herbivore
109 volatile cues. For example, whereas bacterial-derived volatiles 3-pentanol and 2-butanone
110 decrease reproductive output in field-grown *Cucumis sativa* (Song and Ryu, 2013), wild tobacco
111 (*Nicotiana attenuata*) exposed to airspace of experimentally clipped sagebrush produce more
112 seeds (i.e., higher presumptive fitness) relative to control plants (Karban and Maron, 2002).
113 These results suggest that ecological costs of exposure to volatile cues may be context
114 dependent, but comparative cost/benefit tradeoffs for perception of HIPVs alone among
115 sympatric field-grown plants is currently lacking.
116 Here, we report a common garden field experiment with lima bean (*Phaseolus lunatus*)
117 and chili pepper (*Capsicum annuum*) testing the hypothesis that field plants subject to a
118 persistent dose of an ostensibly reliable volatile cue incur consistent costs reflected in reduced
119 growth and reproduction. We treated individuals of both species to repeated low-dose
120 applications of *z3*HAC and measured their growth, reproduction, and herbivore damage
121 throughout the growing season. We predicted that exposure to *z*3HAC—regardless of plant
122 species identity—would reduce growth and reproductive output, while also reducing natural
123 herbivory.
124

125 **Methods and Materials**

126 *Study Site and Plants*

127 A common garden experiment was established on a 54m^2 plot within Blackacre Conservancy's
128 community garden in Louisville, Kentucky (38°11'33.8"N 85°31'28.3"W; Supplemental Fig. 1).

129 The field site was enclosed in a mesh fence to exclude mammalian herbivores. *Phaseolus*
130 *lunatus,* Fabaceae, variety Fordham Hook 242 ('lima bean') and the *Capsicum annuum*,
131 Solanaceae, variety Cayenne pepper, Joe Red Long ('pepper') were chosen as phylogenetically
132 distinct model plants with previously established defense profiles (Ballhorn *et al.*, 2008;
133 Zachariah *et al.*, 2010). Seeds were purchased from the Louisville Seed Company (Louisville,
134 KY, USA), and germinated in Metromix 510© in May 2016 in the Biology Department's
135 greenhouse. After reaching ~20cm in height, 132 lima bean plants were transplanted to the field
136 May 30, 2016, at 4 weeks old and 98 pepper plants at 8 weeks old were transplanted to the field
137 June 28, 2016.While both species were started at the same time, peppers were placed in the field
138 later than the lime beans because they needed additional maturation time before transferring to
139 the field. Within the field site, plants were planted in alternating rows of twos of lima bean and
140 pepper. Distance limitations exist regarding plant volatile perception(Frost *et al.*, 2008a) and
141 consequent herbivore resistance (Dolch and Tscharntke, 2000; Frost *et al.*, 2007). Previous
142 studies with sagebrush (Karban *et al.*, 2006) and lima bean (Heil and Adame-Alvarez, 2010)
143 indicate that volatile cues are effective over relatively short distances of less than 100cm.
144 Therefore, all plants in our experiment were spaced one meter apart from one another in all
145 directions to reduce the risk of interplant communication and cue crossover.
146
147 *Volatile exposure*
148 Plants were acclimated to the field for one week after planting before volatile treatments began.
149 To simulate a naturally occurring low dose (Engelberth *et al.*, 2007; Shiojiri *et al.*, 2012), plants
150 were exposed to lanolin infused paste equivalent to 10ng/hr of *z*3HAC for seven days. This
151 concentration is 25% of that which previously primed poplar (Frost *et al.*, 2008c) and maize

152 (Engelberth *et al.*, 2004). A treatment vial contained 50mg of a 30ng/ul *z*3HAC/lanolin, while a
153 control vial contained 50mg of lanolin. Each glass vial had a 9mm aperture and was maintained
154 at -80°C until use. Each week, both the *z*3HAC-infused lanolin vials and lanolin-only controls
155 were placed at the bottom of their respective plants. Each vial was inverted and supported with a
156 wire stand and each vial was wrapped in aluminum foil to reduce photo degradation (von Merey
157 *et al.*, 2011) (Supplemental Fig. 2). Plants were randomly assigned to either *z*3HAC treatment
158 (lima bean n=63; pepper n=35) or lanolin control (lima bean n=72; pepper n=43). The unit of
159 replication was an individual plant and each plant received its own vial. Random assignment of
160 treatments was made using blocks of 4 adjacent plants; block was included as a random factor in
161 statistical models, and was not a significant factor in any of the models.
162
163 *Growth, biomass, and reproduction measurements*
164 We measured height and total leaf counts routinely on the experimental plants. Leaves were only
165 counted if they were wider than 2cm across for both species while height measurements for both
166 species were recorded from the base of the plant to the uppermost branching point. For lima
167 bean, height was determined by measuring the longest runner within the bush, while pepper
168 plants were measured from the base of the main stalk to the highest branching point. Along with
169 height, the total number of leaves per plant was measured throughout the field season. A
170 complete biomass harvest was conducted on pepper for leaves, roots, and stems at the end of the
171 field season. All leaves and fruits were separated into paper bags before individual plants were
172 extracted from of the ground. After removal, roots and stems were separated, roots were washed
173 with water to remove dirt, and placed into separate paper bags. All materials were dried at 60°C
174 for 24 hours and then weighed. A biomass harvest for lima bean was not performed because an

175 *Epliachna varivestis* (Mexican Bean Beetle) outbreak late in the season removed much of the
176 leaf tissue before we could determine reliable biomass measurements.
177
178 We measured total flower and fruit production as proxies for reproductive fitness in both species.
179 Flowers were recorded if they were true flowers with fully mature pistils and stamen. If a flower
180 was not fully mature, it was recorded as a flower bud. Fruits were recorded as soon as fruit
181 development was observed with either initial pod or exocarp development. Throughout the field
182 season, fruit and flower counts per plant were recorded along with the number of mature and
183 immature fruits.
184
185 From the fruits harvested from the final biomass harvest, ~10 randomly selected, mature fruits
186 from each pepper plant were chosen for seed count analysis (188 fruits from z3HAC-treated
187 plants and 210 fruits from controls). Dried fruits were dissected with a scalpel and all seeds were
188 isolated and counted.
189
190 *Herbivory*
191 Leaf chewing damage was assessed for both pepper and lima bean as percent leaf area removed
192 (LAR) using a visual estimation technique (Frost and Hunter, 2004, 2008) with the following
193 damage categories: 0%, 0-5%, 5-15%, 15-30%, 30-50%, 50-70%, 70-90%, and >90%. For each
194 damage assessment, every leaf on a plant was categorized into one of the damage categories, and
195 an overall percent damage was determined as a weighted average of all leaves. Plants were also
196 routinely monitored for the presence of naturally occurring chewing and piercing/sucking

197 herbivores. In particular, we noted a natural occurrence of the black bean aphid (*Aphis faba*),
198 and recorded its presence/absence on lima bean plants in the field.
199
200 *Leaf collections and cyanide measurements*
201 Since cyanogenic potential (CP) is an inducible herbivore defense in lima bean (Ballhorn *et al.*,
202 2005; Gleadow and Moller, 2014), we used CP as a metric for induced responses in the presence
203 of *z3*HAC. We collected source and sink leaves (Ochoa-Lopez *et al.*, 2015; Schultz *et al.*, 2013)
204 on July 10, 2016, which was approximately 6 weeks into the field season. We modified a
205 colorometric protocol (Brinker and Seigler, 1989; Gleadow *et al.*, 2011) for microscale
206 measurements. Briefly, 5mg of lyophilized tissue was mixed with 200uL of citrate buffer (0.1 M,
207 pH 5.5-6.5) in a 2mL centrifuge tube, into which a 200uL glass vial (Agilent part number 5183-
208 2090) containing 100uL 1.0M NaOH was also placed. After 15 hours, 50uL of the 1.0M NaOH
209 was diluted to 0.1M, and a 30uL aliquot was neutralized (30uL 0.5M acetic acid) and reacted
210 with two reagents (A: 75 uL of $5.0x10^{-3}$g ml^{-1} succinimide and $5.0x10^{-4}$g ml^{-1} n-
211 chlorosuccinimide; B: 30uL of 0.03g mL^{-1} barbituric acid in 30% pyridine). After 8 min,
212 absorbance at 580nm was determined on a plate reader (SpectraMax M2). Quantification of CP
213 was made against a standard curve of NaCN.
214
215 *Statistical analyses*
216 All statistical analyses were performed in R (version 3.4.2) with the lme4 and multcomp
217 packages. Growth data, such as plant height, leaf area removed, and flower counts, were
218 analyzed using repeated measures ANOVA with the aov function with a Gaussian distribution.
219 For repeated measures analyses, we treated date as a within-subjects effect and treatment as a

220 between-subjects effect for all analyses. Differences between treatments at each individual time
221 point, as well as all biomass data, were analyzed using one-way ANOVA (glmer function)
222 followed by a Tukey's post hoc comparison. Cyanide measurements were normalized via log
223 transform before running a glm model followed by a Tukey's post hoc comparison.
224

225 **Results**

226 Treatment with *z3*HAC differentially affected the growth of lima bean and pepper plants.
227 On average, *z*3HAC-treated lima bean grew 11% taller compared to control plants throughout the
228 field season (Fig.1A; $F_{2,927}$=9.688, *P*=0.002) and produced 17% more leaves overall than did
229 controls (Fig.1B; $F_{1,571}$=4.339, *P*=0.038). In contrast, *z*3HAC-treated pepper plants were 12%
230 shorter relative to controls (Fig.1C; $F_{1,48}$=6.168, *P*=0.017) and produced 23% fewer leaves over
231 the field season (Fig.1D; $F_{1,237}$=21.58, *P*<0.001). Consistent with height and leaf counts, *z3*HAC
232 treatment reduced overall biomass of pepper plants by 24% on average (Fig.2). When we
233 destructively harvested all pepper plant biomass at the end of the season, *z*3HAC-treated pepper
234 plants had lower leaf, stem, and root dry biomass by 21%, 31%, 29%, respectively (Fig.2A-C)
235 (Z=3.379, *P*<0.001*;* Z=-2.035, *P*=0.042; Z=-2.379, *P*=0.017*)*. Despite these *z*3HAC-mediated
236 effects on biomass exposure, the aboveground-to-belowground biomass ratio was similar
237 regardless of treatment (Fig.2D; Z=0.31, *P*=0.757). That is, pepper plants treated with *z*3HAC
238 were smaller relative to control plants.

239 *z*3HAC treatment also differentially affected reproductive output between the two
240 species, and lowered fruit output in pepper. Flower production was 30% higher in lima bean
241 plants exposed to *z*3HAC (Fig.3A; $F_{1,576}$=15.044, *P*<0.001), while *z*3HAC-treated peppers
242 produced 37% fewer flowers relative to control plants at the end of the field season (Fig.3B;

243 $F_{1,43}$=14.48, *P*<0.001). *z*3HAC-treated pepper plants also produced 23% fewer fruits overall
244 relative to controls (Fig.4A; Z=-2.035, *P*=0.042), and the fruits that were produced by *z*3HAC-
245 treated plants had lower wet and dry masses (Fig.4B-C; Z=-2.88, *P*=0.004; Z=-2.439, *P*=0.015),
246 and 10% lower total seed counts (Fig.4D; Z=3.524, *P*<0.001) and total seed masses (Fig.4E;
247 Z=3.334, *P*<0.001), relative to controls. Even though total fruit and seed production was reduced
248 by *z*3HAC treatment, the ratio of seed mass to fruit mass was similar between *z*3HAC-treated
249 and control plants (Fig.4F; Z=0.588, *P*=0.807). Moreover, the estimated mass of an individual
250 seed was similar between *z*3HAC-treated plants and controls in pepper (Supplemental Fig.3).
251 There was no apparent difference in lima bean pod production (Supplemental Fig.3). However,
252 an unexpected field-wide premature pod drop that was independent of treatment prevented us
253 from determining lima bean seed production with confidence.
254 *z*3HAC exposure reduced natural herbivory in lima bean but not pepper plants. Chewing
255 herbivory on pepper plants was low throughout the season and statistically higher in *z*3HAC-
256 treated plants; however, this effect was driven by only the first sampling date (Fig.5A,
257 $F=_{1,193}$=5.627, *P*=0.019). In contrast, chewing damage to lima bean leaves increased as the field
258 season progressed, with *z*3HAC-treated plants having overall 26% less chewing damage than did
259 control plants (Fig.5B; $F_{1,539}$=21.745, *P*<0.001). In addition to chewing herbivory, black bean
260 aphids (*Aphis faba*) colonized 87% of the *z*3HAC-treated lima bean plants, compared with only
261 21% of control plants (Fig.5C; χ^2 = 50.11, df=1, *P*<0.001). *A.faba* colonized early in the season
262 and was only observed June 15-31 (Julian dates 166-181) because a heavy rainfall event reduced
263 their population to undetectable levels. Piercing/sucking herbivores were rare for the remainder
264 of the experiment.

265 Exposure to *z3*HAC reduced cyanogenic potential in lima bean plants. *z3*HAC exposure
266 alone reduced cyanide concentration by 50% in sink leaves (Figure 6A; Z= -0.3257, *P*=0.003),
267 but not significantly in source leaves (Fig. 6C; Z=-0.14387, *P*=0.146). Additionally, baseline
268 levels of cyanide were nearly 2x greater in sink leaves compared to source leaves (Figure 6)
269 regardless of *z3*HAC exposure.
270
271 **Discussion**
272 We show that a persistent, low-dose application of *z*3HAC differentially affects growth
273 and reproduction in two plant species under identical field conditions. Based on previous work
274 on plant and sensory perception of volatiles (Engelberth *et al.*, 2004; Frost *et al.*, 2007), we
275 hypothesized *z3*HAC application would decrease growth and reproductive fitness in both plant
276 species. The rationale for this hypothesis was a central assumption of induced resistance theory
277 that ecological costs modulate the deployment particular defensive phenotypes until necessary
278 (Agrawal, 1999; Baldwin, 1998; Cipollini and Heil, 2010; Cipollini *et al.*, 2003; Didiano *et al.*,
279 2014; Koricheva, 2002; Mauricio, 1998). Volatile-mediated priming, even if regulated by a
280 different mechanism from resistance (Hilker *et al.*, 2016), is an inducible phenomenon that
281 theoretically should incur such fitness costs (Martinez-Medina *et al.*, 2016). Yet, our results
282 clearly indicate that pepper and lima bean had divergent fitness outcomes when subjected to a
283 single GLV under identical field conditions. Whereas *z3*HAC-treated pepper plants had reduced
284 growth (Fig. 1) and no effect on herbivore resistance (Fig. 5) relative to controls, *z3*HAC-treated
285 lima bean plants grew more and produced more flowers (Fig. 1), and suffered less chewing
286 herbivory (Fig. 5B) compared to controls. This result—that some plants experience costs while
287 others have minimal or even positive effects when exposed to the same HIPV—has important

288 implications for how volatile cues may structure interspecific competition and ecological
289 communities. HIPVs alone may be sufficient to result in differential fitness effects among
290 species.

291 What might affect the response of plants to volatile exposure? One possibility is that the
292 signal integrity of HIPVs varies among plant species, which influences plant sensory perception
293 and the outcome of defense priming. Previous work on the role of HIPVs in plant anti-herbivore
294 resistance focused on priming-mediated defense with consistent results in wheat (Ameye *et al.*,
295 2015; Walters *et al.*, 2008), corn (Engelberth *et al.*, 2007; Farag *et al.*, 2005), lima bean (Arimura
296 *et al.*, 2002; Choh and Takabayashi, 2006; Heil and Silva Bueno, 2007), tomato (Acevedo *et al.*,
297 2017), blueberry (Rodriguez-Saona *et al.*, 2009), sagebrush (Karban *et al.*, 2006), Arabidopsis
298 (Bissmeyer *et al.*, 2018) and poplar (Frost *et al.*, 2008c). In contrast, we specifically focused on
299 indicators of plant fitness in lima bean and pepper in a common garden experiment. *z*3HAC
300 treatment alone increased growth and flowering in lima bean, while reducing growth and
301 reproductive output in pepper (Fig. 3 and 5). This long-term evidence illustrates that
302 informational integrity varies among species. Such divergent fitness effects from exposure to a
303 single ubiquitous herbivore-associated cue underscore the potential for functional similarity in
304 the mechanisms by which plants modulate responses to herbivory and volatile indicators of
305 herbivory.

306 Flower and fruit production is a key component of plant fitness potential. We show that
307 *z*3HAC treatment alone differentially affected flower production in lima bean and pepper (Fig.
308 3). Insect herbivory can increase or decrease floral production depending on the system and
309 environmental conditions (Agrawal *et al.*, 1999; Lucas-Barbosa, 2016; Pashalidou *et al.*, 2013).
310 Whereas increased flower production is a strategy assumed to ameliorate fitness losses in the

311 presence of an environmental stress (Agrawal, 1999, 2000), decreased flower production may be
312 related to costs of chemically mediated defense (Heath *et al.*, 2014). Additionally, herbivory
313 affects floral attractiveness (Halpern *et al.*, 2010; Hoffmeister and Junker, 2016), which may
314 ultimately influence fitness (Kessler *et al.*, 2011). Our data indicate that a volatile cue alone is
315 sufficient to trigger changes in floral biology, but the magnitude and direction of those changes
316 are plant species-specific. Additionally, *z*3HAC-treated pepper produced fewer fruits (Fig. 4)
317 but lima bean did not (Supplemental Fig. 3). The mechanisms underlying *z*3HAC-mediated
318 effects on flower and fruit production are unknown, but may be similar to those induced by
319 herbivory (Lucas-Barbosa, 2016).

320 Resource allocation between different tissues is pivotal for growth, reproduction, and
321 defense, and can be influenced by environmental stress. For example, direct herbivory alters
322 resource allocation between aboveground tissue and belowground tissue (Eichenberg *et al.*,
323 2015; Frost and Hunter, 2008; Machado *et al.*, 2013), as does application of the anti-herbivore
324 phytohormone jasmonic acid (JA) (Gomez *et al.*, 2010; Schweiger *et al.*, 2014). Volatile cues
325 can also affect biomass allocation. For example, barley exposed to volatiles from unwounded
326 neighboring plants of different cultivars increases root and leaf biomass (Ninkovic, 2003), while
327 exposure to volatiles decreases aboveground biomass in other systems (Cipollini, 2010; Lu *et al.*,
328 2017). In our case, volatile treatment reduced overall aboveground and belowground biomass in
329 pepper, but did not appear to alter overall biomass allocation patterns. Simply put, *z*3HAC-
330 treated pepper plants were smaller overall, and therefore produced fewer seeds.

331 Differential investment between growth and defense is vital for maximizing limited
332 resources and, ultimately, fitness. Inducible defenses in plants against herbivores and pathogens
333 modulate such growth/defense tradeoffs in a number of plant species (Cipollini and Heil, 2010).

334 In some cases, exposure to VOCs alone is sufficient to affect such tradeoffs. For example,
335 sagebrush exposed to VOCs from damaged conspecifics had decreased growth (Karban, 2017),
336 while HIPV-exposed tobacco had increased herbivore resistance but decreased seed set (Karban
337 and Maron, 2002). In our experiment, *z*3HAC exposure decreased cyanogenic potential in sink
338 leaves (Fig. 6), which were 20-30% higher than in source leaves. These results suggest that
339 persistent exposure to *z*3HAC led to allocation shifts from cyanogenic potential (a putative
340 defense) towards growth (Fig. 1 and 3), as well as supporting ontogeny-mediated cyanogenic
341 potential (Ballhorn *et al.*, 2006; Ochoa-Lopez *et al.*, 2015).. That said, z3HAC-treated plants also
342 experienced less natural damage primarily on mature source leaves than did controls (Fig. 5,
343 personal observations), suggesting that the herbivory was deterred by defense mechanism other
344 than cyanogenic potential in source leaves.

345 Volatile cues may impact ecological communities in both expected and pleiotropic ways.
346 HIPVs are well-established mediators of multi-trophic antagonistic and mutualistic interactions
347 (Heil, 2008; Kessler and Baldwin, 2001; Peñaflor *et al.*, 2017), and manipulations of chemical
348 signals and volatile blends have been used for biological control in a wide range of systems
349 (Peñaflor and Bento, 2013; Stenberg *et al.*, 2015). For example, HIPV-infused sticky traps in a
350 grape (*Vitis vinifera*) orchard differentially attracted lacewings, hoverflies, and parasitoids
351 (Lucchi *et al.*, 2017). Exogenous GLV manipulation using "dispensers" under field conditions
352 altered the arthropod community composition in maize (von Merey *et al.*, 2011). In our study,
353 *A.faba* were clearly and unexpectedly attracted to *z*3HAC-exposed plants (Fig. 5). Under
354 glasshouse conditions, *A.faba* were repelled by *z*3HAC alone (Webster *et al.*, 2008), which
355 suggests that the cue that mediated attraction was not our treatment alone. It is tempting to
356 speculate that aphid attraction combined with reduced chewing herbivory in lima bean may be

357 reflective of *z*3HAC effects on JA and salicylic acid (SA) signaling, which would be consistent
358 with a JA-SA tradeoff (Huot *et al.*, 2014; Wei *et al.*, 2014). Ultimately, however, the utility of
359 GLVs (or other VOCs) in field applications will depend on understanding community-level
360 effects of volatile exposure.

361 As a caveat, volatile identity, concentration, and duration may affect the reliability of a
362 cue and therefore the costs associated with eavesdropping. Plants experiencing insect herbivory
363 frequently generate species-specific blends of volatile compounds (Ameye *et al.*, 2017;
364 Holopainen and Gershenzon, 2010), which can influence fitness in neighboring plants
365 (Caparrotta *et al.*, 2018; Karban, 2017; Kessler *et al.*, 2006). Plant-derived compounds associated
366 with herbivory include GLVs (Farag *et al.*, 2005; Frost *et al.*, 2008c; Lu *et al.*, 2017),
367 phenylpropanoid derivatives (Erb *et al.*, 2015), and terpenes (Arimura *et al.*, 2012). However,
368 individual compounds within a blend can affect plant defense and priming as much as the blend.
369 We used *z*3HAC in this study because it is released primarily from herbivore-damaged leaves
370 and not just wounded leaves (Engelberth *et al.*, 2004), which differs from other wound-released
371 GLVs (Ameye *et al.*, 2017) (Matsui *et al.*, 2012) and ostensibly allows *z*3HAC to confer reliable
372 ecological information. Moreover, plants detect and respond specifically to *z*3HAC (Frost *et al.*,
373 2008c), and the costs associated with that response were the focus of this investigation.
374 Additionally, concentration of a cue may influence plant resistance (Bissmeyer *et al.*, 2018; Lu *et*
375 *al.*, 2017). For example, a repeated, low-dose exposure to a GLV blend enhances plant resistance
376 compared to a single application (Shiojiri *et al.*, 2012) while *z3*HAC emissions can be as high as
377 66 ng/cm^3 after herbivory (Boggia *et al.*, 2015). For these reasons, we chose to use a low-dose
378 exposure to *z*3HAC (25% of the concentration that primed poplar (Frost *et al.*, 2008c) and maize

379 (Engelberth *et al.*, 2004), and still observed divergent fitness effects between the two plant
380 species (Figs.1, 3, and 5).
381 In summary, our key finding is that persistent application of a low dose of a single
382 volatile compound *z*3HAC, a common HIPV and GLV, in field conditions leads to divergent
383 growth and reproductive fitness effects between two plant species. This result underscores the
384 variable nature of HIPV-eavesdropping, and that plants may have evolved species-specific
385 mechanisms for responding to volatile cues. Given natural variation among species, future work
386 must assess costs with other species as well as within ascensions and landraces of our model
387 plants over multiple years. Ultimately, the adaptive significance of eavesdropping for enhancing
388 plant immunity will depend on plant life history, physiology, and other ecological factors to
389 determine whether a plant will benefit from eavesdropping VOCs or not, and therefore what
390 impact priming might have on plant communities.
391
392 **Description of Supporting Information**.
393 Fig. S1. An aerial view of the field site.
394 Fig. S2. Volatile administration setup.
395 Fig. S3. Estimated individual seed mass in pepper and total pod production in lima bean.
396 Table S1-S6 describe the complete statistics for their corresponding figures.
397
398 **Acknowledgments** - We are grateful to Allie Peot and Abhinav Maurya for field assistance, and
399 to A.Peot for assistance processing the plant materials in the laboratory. Comments from Heidi
400 Appel and Amy Austin greatly improved the manuscript. We are grateful to A. Dale Josey and

Susan Ballerstedt for permission and logistical support to work in the community gardens at Blackacre Conservancy.

Funding Sources- This work was supported by the National Science Foundation grant IOS-1656625 to C.J.F.

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662 **Figure Captions**
663
664 **Fig. 1**. **Height measurements and leaf counts for *Phaseolus lunatus* (lima bean) and**
665 ***Capsicum annuum* (pepper) grown in a common garden field experiment.** Height for (A)
666 lima bean was measured from the base of the longest runner to the uppermost branching point;
667 (C) pepper height was measured from the base of the main stalk to the uppermost branching
668 point. Leaf counts for (B) lima bean and (D) pepper included all mature leaves on each plant.
669 Open circles represent control plants (receiving lanolin-filled vials); filled squares represent
670 plants receiving a persistent application of vials containing 10ng/hr *cis*-3-hexenyl acetate
671 (*z*3HAC) dissolved in lanolin. Dropdown lines indicate the initial application of *z*3HAC
672 treatment: lima bean and pepper plants were first exposed on June 10, 2016 (Julian date 161) and
673 July 11, 2016 (Julian date 192), respectively. Points represent averages +/- SE. Repeated
674 measures ANOVAs (aov in R) were followed by one-way ANOVAs at each time-point.
675 Asterisks (*) represent $P<0.05$ between treatment and control at each time point. See
676 Supplemental Table 1 for complete statistics.
677
678 **Fig. 2**. **Biomass measurements of field-grown *Capsicum annuum* (pepper) plants.** (A) Leaf,
679 (B) stem, (C) root biomass, and (D) the aboveground:belowground biomass ratio in *C.annuum*
680 plants were determined at the end of the field season following destructive harvest. Box plots
681 represent the raw data ranging from the upper to the lower quartiles and the median. Error bars
682 represent the 5% and 95% of the data, and individual dots are observations that fell outside of
683 those parameters. *P*-values represent Tukey's HSD comparisons. See Supplemental Table 2 for
684 complete statistics.
685
686 **Fig. 3**. **Total flower production in (A) *Phaseolus lunatus* (lima bean) and (B) *Capsicum***
687 ***annuum* (pepper).** Open circles represent control plants (receiving lanolin-only vials); filled
688 squares represent plants receiving a persistent application of vials containing 10ng/hr *cis*-3-
689 hexenyl acetate (*z*3HAC) dissolved in lanolin. Dropdown lines indicate the initial application of
690 *z*3HAC treatment: lima bean and pepper plants were first exposed on June 10, 2016 (Julian date
691 161) and July 11, 2016 (Julian date 192), respectively. Points represent averages +/- SE.
692 Asterisks (*) represent $P<0.05$ between treatment and control at each time point. See
693 Supplemental Table 3 for complete statistics.
694
695 **Fig. 4**. **Fruit and seed production in *Capsicum annuum* (pepper) plants grown in a common**
696 **garden experiment treated with a persistent application of the green leaf volatile *cis*-3-**
697 **hexenyl acetate (*z*3HAC).** The (A) total number of fruits were counted in the field, and (B) wet
698 and (C) dry masses fruit masses were determined in the lab. (D) The total number of seeds per
699 fruit and (E) the estimated mass per seed were determined from a subset of the fruits produced.
700 (F) The ratio of seed mass to fruit mass was calculated to assess the efficiency of seed
701 production. Box plots represent the raw data ranging from the upper to the lower quartiles and
702 the median. White boxes represent control plants; gray boxes represent plants treated with
703 *z*3HAC. Error bars represent the 5% and 95% of the data, and individual dots are observations
704 that fell outside of those parameters. *P*-values represent Tukey's HSD comparisons. See
705 Supplemental Table 4 for complete statistics.
706

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

Fig. 6. Cyanide induction in *Phaseolus lunatus* (Lima bean) as affected by *z3*HAC exposure. Cyanide concentration was determined by trapping volatile HCN in 1.0M NaOH followed by colorometric quantification. Sink leaves (A) and source leaves (B) were exposed long-term exposure of 10ng/hour *z3*HAC or left as a control. Points represent averages +/- SE and * indicate a p-value <0.05.

Supplemental Figure 1. 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. 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 (von Merey *et al.*, 2011).

Supplemental Figure 3. 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.

747 **Figure 1**

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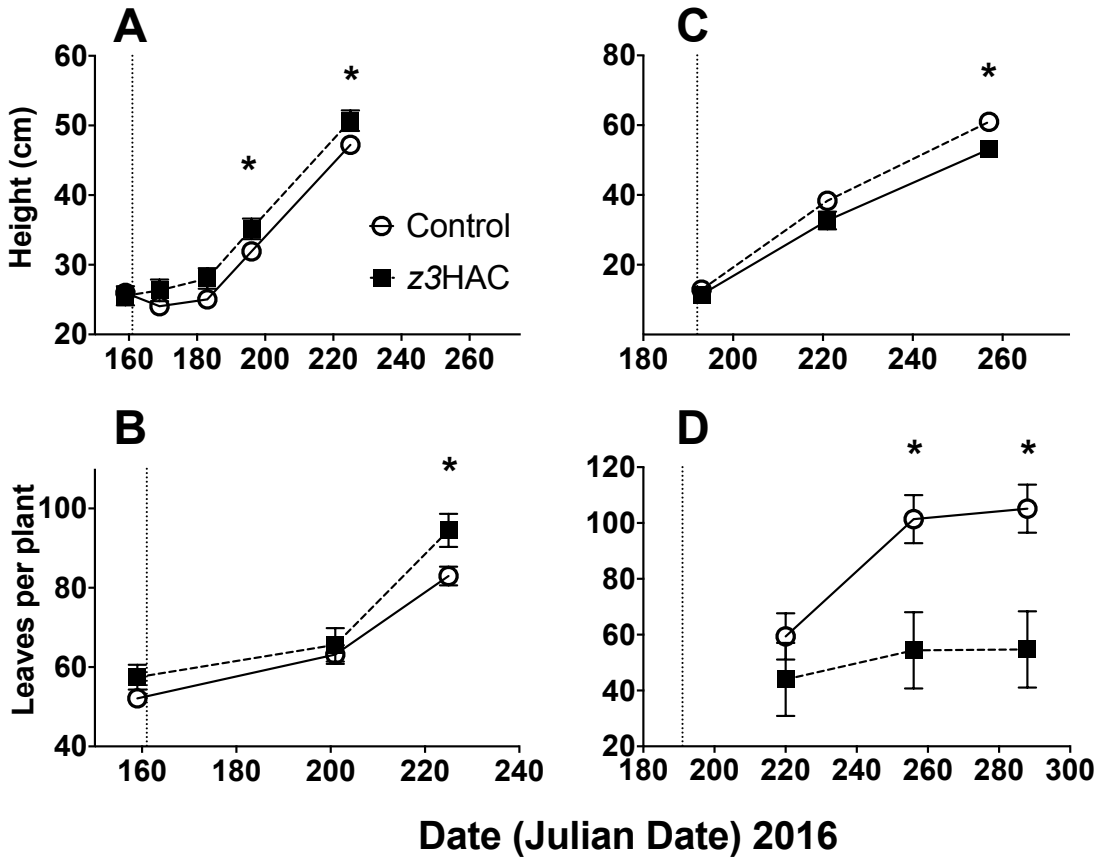

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761 **Figure 2**

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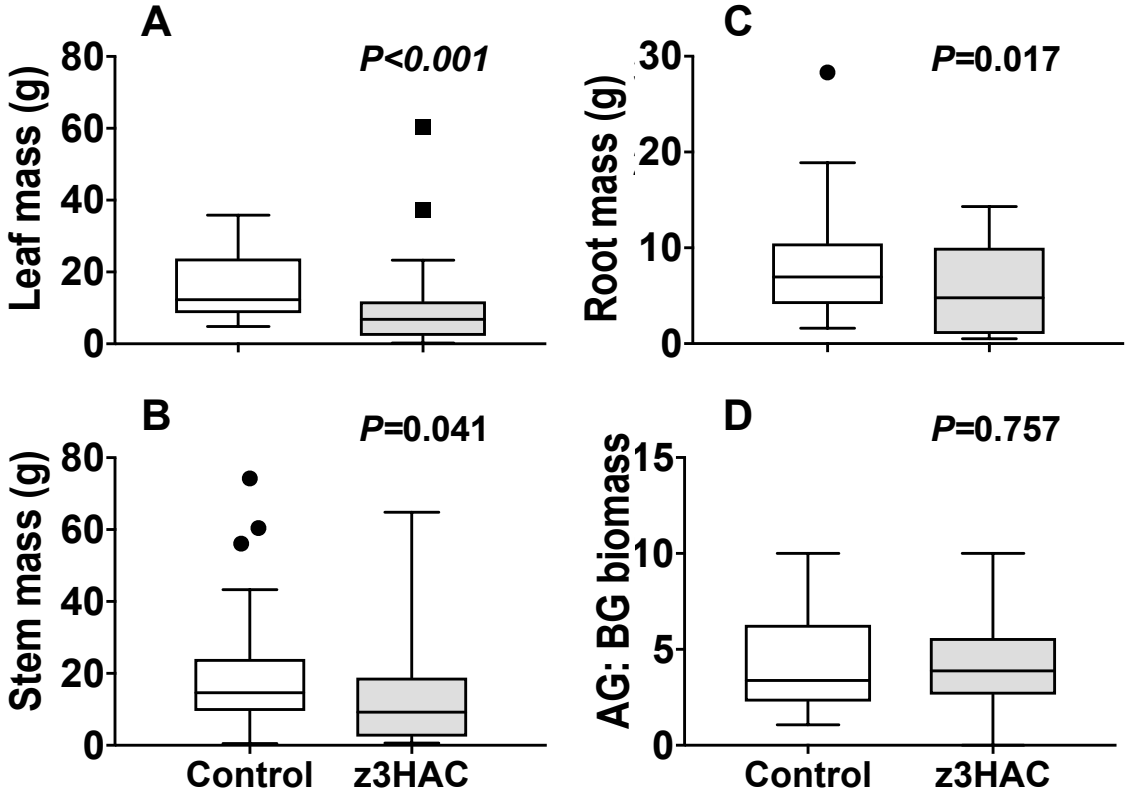

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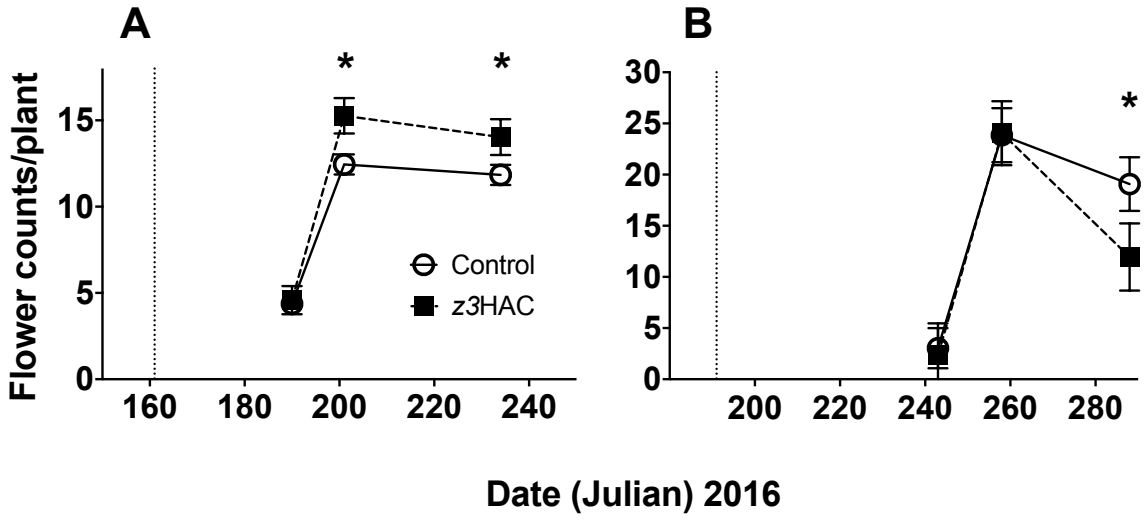
788 **Figure 3**



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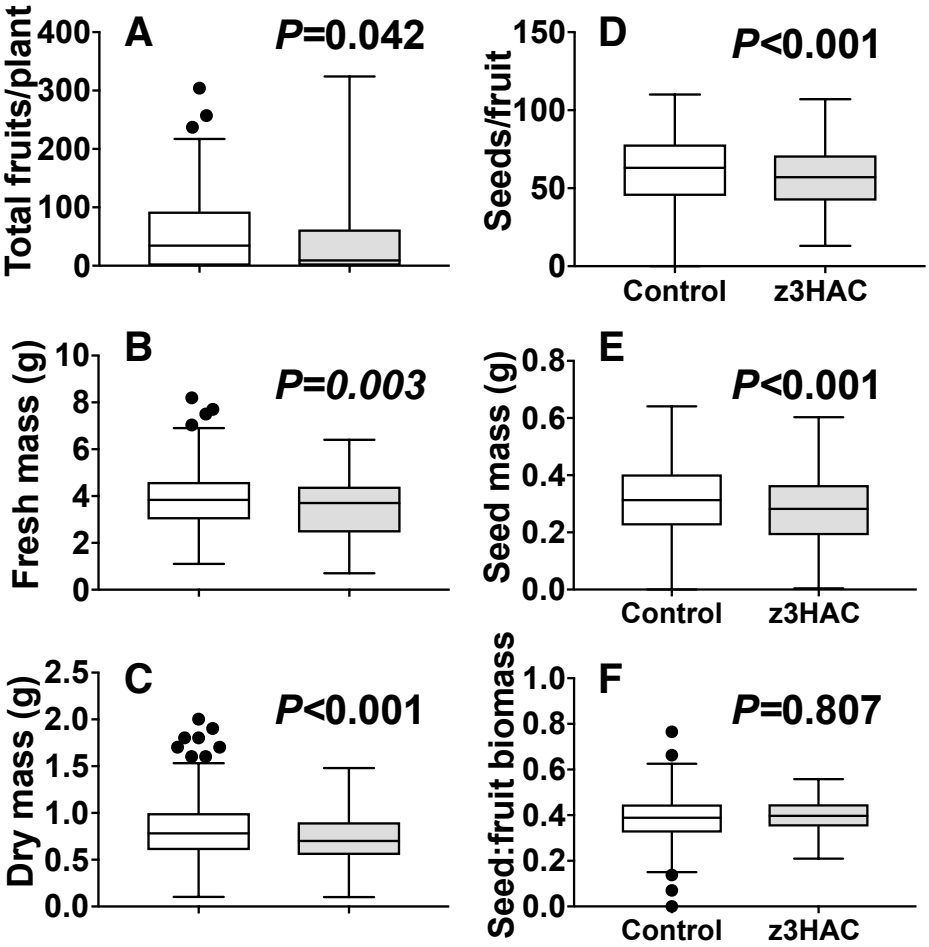
807 **Figure 4**



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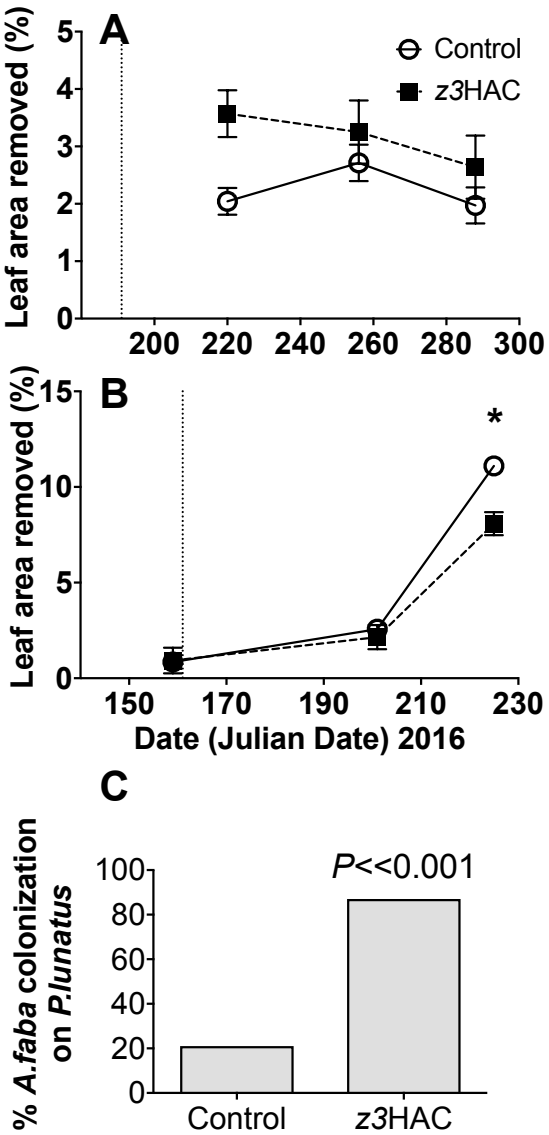
819 **Figure 5**



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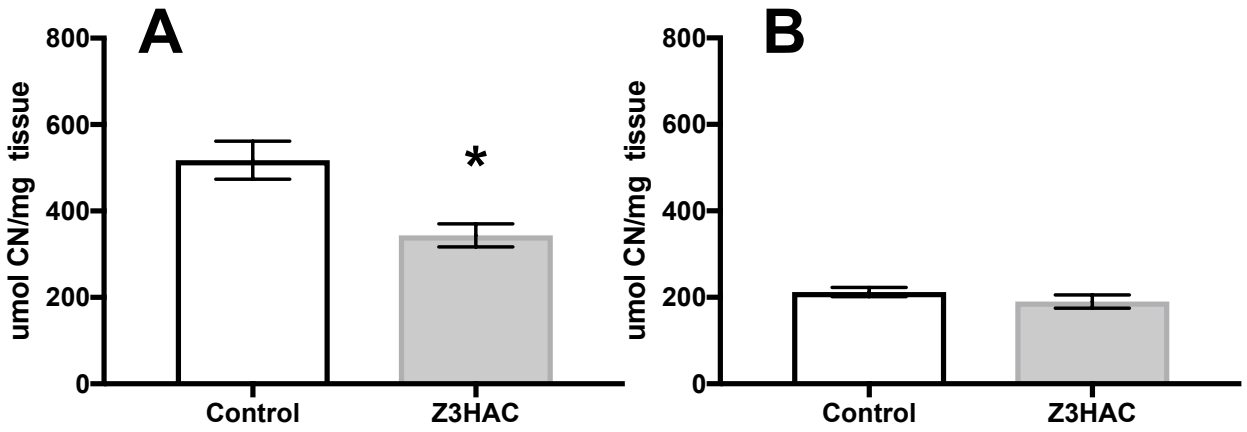
828 **Figure 6**



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