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Eliciting sweet pepper plant resistance to *Aulacorthum solani* and attractiveness on *Aphelinus abdominalis* by exposure to (Z)-3-hexenyl propanoate

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Eliciting sweet pepper plant resistance to *Aulacorthum solani* and attractiveness on *Aphelinus abdominalis* by exposure to (Z)-3-hexenyl propanoate / Depalo, Laura; Urbaneja, Alberto; Gallego, Carolina; Fournarakos, Alexander; Alonso, Miquel; Pérez-Hedo, Meritxell. - In: ENTOMOLOGIA GENERALIS. - ISSN 0171-8177. - ELETTRONICO. - 42:5(2022), pp. 743-749. [10.1127/entomologia/2022/1595]

Availability:

This version is available at: <https://hdl.handle.net/11585/897185> since: 2022-10-25

Published:

DOI: <http://doi.org/10.1127/entomologia/2022/1595>

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Depalo, Laura and Urbaneja, Alberto and Gallego, Carolina and Fournarakos, Alexander and Alonso, Miquel and Pérez-Hedo, Meritxell, Eliciting sweet pepper plant resistance to *Aulacorthum solani* and attractiveness on *Aphelinus abdominalis* by exposure to (Z)-3-hexenyl propanoate, *Entomologia Generalis*, 2022, Vol.42, Issue 5, 743-749

The final published version is available online at:

<https://doi.org/10.1127/entomologia/2022/1595>

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1 **Eliciting sweet pepper plant resistance to *Aulacorthum solani* and attractiveness on**
2 ***Aphelinus abdominalis* by exposure to (Z)-3-hexenyl propanoate**

3 **Short title: Eliciting plant resistance by exposure to (Z)-3-HP**

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11 **Keywords:** HIPVs, plant response, plant defense, biological control, biopesticides, IPM

12 **Abstract**

13 It is widely documented that plants respond to herbivory through the release of volatile
14 compounds mediated by phytohormone signaling pathways. Herbivore-Induced Plant Volatiles
15 (HIPVs), among which are the green leaf volatiles, can repel herbivores and attract their natural
16 enemies, as well as warn neighboring plants of herbivore attacks. Plants that received these
17 warning signals activate defense mechanisms and therefore become more resistant against pests
18 and diseases. In this work, we tested whether plants activated by exposure to the green leaf
19 volatile (Z)-3-hexenyl propanoate [(Z)-3-HP] can enhance management of one of the most
20 important pests of sweet peppers, the aphid *Aulacorthum solani* (Kalt.) (Homoptera:
21 Aphididae). Here, we show that sweet pepper plants exposed to (Z)-3-HP induce plant defenses
22 which repel *A. solani* winged adults, and attracted females of *Aphelinus abdominalis* (Dalman)
23 (Hymenoptera: Aphelinidae), an aphid parasitoid used to control a plethora of aphid pests,
24 including *A. solani*. Additionally, (Z)-3-HP-exposed plants were less infested by *A. solani*
25 compared to their non-exposed counterparts under greenhouse conditions. Significant

26 transcriptional differences were obtained when studying the temporal gene expression pattern
27 of three defense-related genes, *ASR1*, *PIN2*, and *AMP1*, markers of abscisic acid, jasmonic acid
28 and salicylic acid respectively, during the duration of the greenhouse experiment. Our results
29 demonstrate how the use of volatiles as plant defense inducers can play a role in the
30 management of *A. solani* in sweet pepper and opens the door to exploring this technique on
31 other aphid pests in other crops.

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36 **Introduction**

37 Pressure is increasing for the use of new alternatives to synthetic pesticides in modern
38 agriculture which drives the research, development and implementation of new sustainable
39 techniques (European Union, 2009; Pretty, 2018; Mokany et al., 2020). In this sense, one of the
40 fields still to explore is the natural immune system of the plants (War et al., 2012). Plants exhibit
41 a wide variety of natural defense mechanisms against herbivory, including constitutive
42 resistance and induced resistance. A very important distinction between the two types of
43 resistances is that constitutive resistance concerns traits that are always expressed by the plant
44 regardless of external stimuli, such as wax, trichomes and spines, whereas induced resistance
45 concerns the production of bioactive compounds of the plant in response to herbivory (Arimura
46 et al., 2005). Induced resistance includes both direct and indirect defenses. Direct induced
47 defenses concern physical or chemical changes to the plants, namely silica deposition,
48 lignification, and biosynthesis of herbivore-induced plant volatiles (HIPVs), including
49 terpenoids, fatty acid derivatives, phenylpropanoids and benzenoids (Paré and Tumlinson,
50 1999; Dudareva et al., 2004; Heil, 2008), which are produced by the leaves, flowers, fruits, and
51 roots of plants. Indirect induced defenses concern the interactions between plants and
52 organisms of higher trophic levels through the production of HIPVs (Dicke et al., 1990). HIPVs
53 are able to repel or attract herbivores and their natural enemies, as well as transmit the message
54 of warning to neighboring plants, which in turn activates the same defensive systems (Frost et
55 al., 2008; Martinez-Medina et al., 2016). HIPVs stimulation is a promising application in
56 agriculture to improve plant defense and resistance against herbivorous pests (Pérez-Hedo et
57 al., 2021a,b).

58 Plants activate their immune system to counteract attack by pathogens or herbivorous insects
59 triggered by a diverse suite of plant hormones, acting as central players in the plant defense
60 signaling network. Salicylic acid (SA), jasmonic acid (JA) with its derivatives (collectively

61 called jasmonates), and abscisic acid (ABA) are recognized as the major defense hormones
62 (Pieterse et al., 2012). Jasmonic acid has a very important role in inducing the defenses of the
63 plants against herbivorous insects, it stimulates the production of protease inhibitors in plants,
64 which decrease infestation of herbivorous insects and reduces physical damage sustained by
65 the plant (Fouad et al., 2016). Salicylic acid, on the other hand, is responsible for inducing the
66 production of several defensive metabolites that act as deterrents against pests (Pasteels and
67 Rowell-Rahier, 1992). Abscisic acid is an important modulator of the plant immune signaling
68 network and has a role in development and adaptation to abiotic stress, in particular drought
69 and salinity stress (Pieterse et al., 2012).

70 Following this idea, Pérez-Hedo et al. (2021b) proved that tomato plants previously exposed to
71 different HIPVs [1-hexanol, (Z)-3-hexenol, (Z)-3-hexenyl acetate, (Z)-3-hexenyl propanoate
72 ((Z)-3-HP), (Z)-3-hexenyl butanoate, hexyl butanoate, methyl jasmonate and methyl salicylate]
73 were capable of activating defensive response in tomato plants, upregulating the expression of
74 the defense-related genes; proteinase Inhibitor II (PIN2), pathogenesis-related protein precursor
75 (PR1) and SI-PI-I marker genes for the jasmonic acid (JA), salicylic acid (SA) and plant
76 Proteinase Inhibitor I signaling pathway, respectively. In addition, tomato plants exposed to
77 two HIPVs selected from that study [(Z)-3-hexenyl propanoate and methyl salicylate] were
78 repellent to *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), *Frankliniella occidentalis*
79 (Pergande) (Thysanoptera: Thripidae) and *Bemisia tabaci* (Gennadius) (Hemiptera:
80 Aleyrodidae) whereas were attractive to the whitefly parasitoid *Encarsia formosa* (Gahan)
81 (Hymenoptera: Aphelinidae) (Pérez-Hedo et al., 2021b). In a further step, polymeric dispensers
82 releasing a constant rate of (Z)-3-HP in commercial tomato greenhouses, plant defenses (JA
83 and SA pathways were upregulated) were activated and maintained for more than two months,
84 which reduced *T. absoluta* damage in 60% without diminishing plant productivity (Pérez-Hedo
85 et al., 2021a). More recently, Riahi et al. (2022) demonstrated that the exposure of sweet pepper

86 plants to the same eight HIPVs mentioned above [1-hexanol, (Z)-3-hexenol, (Z)-3-hexenyl
87 acetate, (Z)-3-hexenyl propanoate ((Z)-3-HP), (Z)-3-hexenyl butanoate, hexyl butanoate,
88 methyl jasmonate and methyl salicylate], unless 1-hexanol, were also capable of activating the
89 sweet pepper immune system. In sweet pepper, all those tested HIPVs induced plant defenses
90 by upregulating the JA and SA signalling pathway. Furthermore, exposing sweet peppers plants
91 to (Z)-3-HP and methyl salicylate repelled *F. occidentalis* while the predator *Orius laevigatus*
92 (Fieber) (Hemiptera: Anthocoridae) showed a strong preference to plants exposed to (Z)-3-
93 hexenol, (Z)-3-HP, (Z)-3-hexenyl butanoate, methyl salicylate and methyl jasmonate.

94 Following the results obtained with (Z)-3-HP exposed tomato and sweet pepper plants by Pérez-
95 Hedo et al. (2021a) and by Riahi et al. (2022), respectively, we decided to go one step further
96 and evaluate its effect on one of the most threatening pest for sweet pepper, the foxglove aphid
97 *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae). *Aulacorthum solani* is an important
98 aphid pest of greenhouse peppers due to high toxicity of the salivary secretion which causes
99 deformation and discoloration of leaves, leading to complete plant defoliation and at high
100 densities, deformed fruit (Sanchez et al., 2007). Moreover, this pest was recently reclassified
101 from occasional to serious pest of vegetables and ornamental plants in greenhouses of North
102 America and the UK (Whittaker, 2020). In this work, we used Y-tube olfactometry to evaluate
103 the olfactory response of winged female *A. solani* and females of its parasitoid *Aphelinus*
104 *abdominalis* (Dalman) (Hymenoptera: Aphelinidae) to sweet pepper plants previously exposed
105 to (Z)-3-HP and to unexposed sweet pepper plants. Secondly, a greenhouse experiment was
106 conducted to evaluate whether sweet pepper plant defenses induced by (Z)-3-HP had an effect
107 on *A. solani*. Finally, gene expression analysis was used to assess whether sweet pepper plants
108 exposed to (Z)-3-HP activated the immune signalling response throughout the duration of the
109 experiment.

110 **Materials and methods**

111 *Plants, insects and volatile*

112 Pesticide-free *Capsicum annum* (Solanaceae) cv. (Lipari) (Dulce Italiano, Mascarell Semillas
113 S.L., Valencia, Spain) plants were used in all the experiments. Two weeks after germination
114 plants were individually transplanted into plastic pots (8 x 8 x 8 cm) and maintained in a climatic
115 chamber at Instituto Valenciano de Investigaciones Agrarias (IVIA) at 25 ± 2 °C, a relative
116 humidity RH of $65 \pm 10\%$ and a photoperiod of 14:10 h (L:D). Plants with six fully developed
117 leaves (approximately 20 cm height) were used for the experiments.

118 *Aulacorthum solani* individuals were obtained from a culture established at IVIA in 2020
119 originally provided from Gerben Messelink laboratory (Wageningen Plant Research, The
120 Netherlands) and reared on *C. annum* plants maintained in chambers at 25 ± 2 °C, with a
121 constant relative humidity of $65\% \pm 5\%$ and a photoperiod of 14:10 h (light: dark). *Aphelinus*
122 *abdominalis* pupae were provided by Koppert Biological Systems, S.L. (Águilas, Murcia,
123 Spain) and upon reception were enclosed in a Petri dish (9 cm in diameter) with a small drop
124 of honey provided as food, where they were allowed to emerge under ambient laboratory
125 conditions (25 ± 2 °C). *Aphelinus abdominalis* were starved for 24 h before use. Individuals of
126 both species tested in the Y-tube experiments were always less than five days-old. The synthetic
127 standard of the volatile compound (Z)-3-HP (purity > 97%) was purchased from Sigma-Aldrich
128 (St. Louis, MO, USA).

129 *Y-tube olfactometer bioassays*

130 The behavioral responses of *A. solani* winged females and females of the parasitoid *A.*
131 *abdominalis* to pre-exposed plants to (Z)-3-HP were investigated in a Y-tube olfactometer
132 (Analytical Research Systems, Gainesville, FL). Plants were prepared in groups of 4 plants and
133 were exposed to (Z)-3-HP using a polymeric low-density dispenser which guaranteed a
134 constant release rate of 9.6 mg/day (Pérez-Hedo et al., 2021a). The dispenser was filled with

135 cotton wool soaked with 1 ml of (Z)-3-HP and then placed in 60 x 60 x 60 plastic cage
136 (BugDorm-2 insect tents; MegaView Science Co., Ltd., Taichung, Taiwan).

137 Plants and (Z)-3-HP were kept undisturbed in isolated climatic chambers to avoid any volatile
138 interference and maintained at 25 ± 2 °C, $65 \pm 10\%$ RH and a 14:10 h (L:D) photoperiod.
139 Control plants were kept in a second isolated chamber at the same conditions but were not
140 exposed to the volatile emitter.

141 The olfactometer consisted of a 2.4-cm-diameter Y-shaped glass tube with a 13.5-cm-long base
142 and two 5.75-cm-long arms. The base of the Y-tube was connected to an air pump that produced
143 a unidirectional airflow at 150 ml/min from the arms to the base of the tube. The arms were
144 connected via plastic tubes to two identical glass jars (5-l volume), each of which contained an
145 exposed plant or a control plant. Each jar was connected to a flow meter and a water filter. Four
146 60-cm-long fluorescent tubes (OSRAM, L18 W/765, OSRAM GmbH, Germany) were
147 positioned 40 cm above the arms. The light intensity over the Y-tube was measured with a
148 ceptometer (LP-80 AccuPAR, Decagon Devices, Inc., Pullman, WA) at 2,516 lux. The
149 environmental conditions in the Y-tube experiments were 23 ± 2 °C and $60 \pm 10\%$ RH (Pérez-
150 Hedo and Urbaneja 2015).

151 Aphids and parasitoids were starved for at least 3 h before the tests. Each adult was observed
152 until it had walked at least 3 cm up one of the side arms or until 10 min had elapsed. Adults that
153 did not choose a side arm within 10 min were considered to be ‘non-responders’ and were not
154 included in the subsequent data analysis. A total of 80 aphid and 40 parasitoid responsive
155 individuals were tested and each individual was used only once. After five individuals had been
156 tested, the olfactometer arms were flipped around (180°) to minimize the spatial effect on arm
157 choice and the plant was replaced with a new one. After ten adults had been tested, the
158 olfactometer setup was rinsed with soap, water and acetone, and then dried air.

159 *Greenhouse experiment and plant gene expression*

160 The influence of plants continuously exposed to (Z)-3-HP on the performance of *A. solani* was
161 evaluated under greenhouse conditions, 25 °C ± 1 °C, 65% ± 10% RH and natural photoperiod
162 (approx. 14:10, L:D). For each experimental treatment [(Z)-3-HP-exposed plants and intact
163 control plants], four sweet pepper plants per cage (60 x 60 x 60 plastic cage; BugDorm-2 insect
164 tents), and six replicates (cages) per treatment were prepared as previously explained for Y-
165 tube olfactometer bioassays. To avoid volatile interference between both treatments, one
166 greenhouse chamber was assigned to the treatment with (Z)-3-HP and a second one to the
167 control treatment. Within each greenhouse chamber, cages were equally distributed at a distance
168 of 1.5 m from each other. Plants were artificially infested with second and third nymphal stages
169 of *A. solani*, collected from the previously described laboratory colony. Ten nymphs were
170 released per plant, and they were distributed equally throughout the leaves with the aid of a fine
171 brush. Plants were individually isolated without touching each other or the cage walls in order
172 to limit insect movement from plant to plant. The total number of aphids per plant was counted
173 every 7 days after release for 8 weeks.

174 *Plant gene expression*

175 To confirm that sweet pepper plant defenses were activated, six additional cages each
176 containing four sweet pepper plants were used in parallel per treatment. The relative expression
177 of three marker genes, which are often used as robust markers for ABA, JA and SA-signaling
178 pathway activation, was estimated: (i) *ASRI* (abscisic acid stress ripening protein 1), (ii) *PIN2*
179 (wound-induced proteinase inhibitor II precursor), and (iii) *AMP1* (antimicrobial peptid 1),
180 respectively. Distribution of plastic cages within both greenhouse chambers, sweet pepper plant
181 arrangement within each cage, and infestation by *A. solani* nymphs were the same as previously
182 described for the performance experiment.

183 Samples of the apical part of volatile-exposed and unexposed sweet pepper plants were
184 collected at 7, 14 and 21 days after the dispensers were installed and grounded in liquid nitrogen

185 for NZYol (NZYTech, Lisboa, Portugal) based RNA extraction. 1µg of each RNA sample was
186 treated with TURBO DNA-free™ Kit (Ambion®, Life Technologies, CA, USA) to remove
187 contaminating DNA. Reverse transcription RT was executed, and cDNA was synthesized using
188 Prime Script™ RT Reagent Kit (TAKARA Bio, CA, USA). Real-time PCR amplification was
189 performed in LightCycler® 480 System (Roche Molecular Systems, Inc., Switzerland),
190 using NZYSpeedy qPCR Green Master Mix (2x) (NZYTech, Lisboa, Portugal) as described by
191 Bouagga et al., (2018). Primers sequences of defensive genes *ASRI*, *PIN2*, *AMP1* and the
192 housekeeping gene *Ef1* (Elongator factor 1) used as standard control gene for normalization
193 are represented in Table 1.

194 *Statistical analyses*

195 All statistical analyses were conducted in RStudio (RStudio Team, 2021) Version 1.1.463 for
196 R version 4.0.5 (R Core Team, 2021). A Chi-square test was used to compare differences in Y-
197 tube olfactometer choice bioassays at $P < 0.05$. A generalized linear mixed model (GLMM)
198 analysis was carried out using package “lme4” (Bates et al., 2015) to compare the number of
199 *A. solani* per plant on the different sample dates in both treatments. In this analysis, the number
200 of individuals per plant was regressed against treatment, sampling dates were considered as
201 repeated measures and replicate as random factor. The data were fitted by maximum likelihood
202 (Laplace Approximation) to a negative binomial generalized linear mixed model (GLMM) with
203 a log link function. Two-tailed Student’s t test ($P < 0.05$) was performed to compare the
204 quantified expression of defense genes between exposed and control plants.

205 **Results**

206 (Z)-3-HP-exposed plants alter *A. solani* and *A. abdominalis* plant selection.

207 A repellent effect of plants pre-exposed to (Z)-3-HP on *A. solani* winged adults was detected
208 in Y-tube olfactometer bioassays ($\chi^2 = 5.0$, $P = 0.0253$) (Fig. 1); 62.5 % of responding
209 individuals chose the intact plant, compared with 37.5 % choosing the pre-exposed activated

210 plant. A total amount of 80 *A. solani* individuals responded to the stimuli out of 110
211 individuals tested. Contrarily, when testing the parasitoid, 74 % of *A. abdominalis* females
212 were attracted towards (Z)-3-HP exposed plants ($\chi^2 = 11.52$, $P = 0.0007$). All the female
213 parasitoids tested ($n = 50$) responded to one of the two stimuli in the Y-tube.
214 (Z)-3-HP- exposed plants reduce *A. solani* performance. The continuous exposure of plants to
215 (Z)-3-HP significantly influenced the number of *A. solani* infesting sweet pepper plant ($F =$
216 33.894 ; $df = 1$, $P < 0.0001$) (Table 2). The abundance of aphids per plant increased over time
217 as expected in both treatment ($F = 344.429$; $df = 1$, $P < 0.0001$), but significant differences
218 between (Z)-3-HP-exposed sweet pepper plants and control plants were detected from the
219 fourth sampling date until the end of the experiment, with higher infestation in control plants
220 (Fig. 2). At day 56, the number of *A. solani* per plant was significantly reduced by 62% on
221 (Z)-3-HP-exposed plants compared to the control plants.

222 Analysis of the relative expression of genes *ASR1*, *PIN2*, and *AMP1* showed transcriptional
223 differences (Fig. 3). The *ASR1* gene was significantly up-regulated in (Z)-3-HP-exposed plants
224 21 day after the start of the experiment when compared to control plants ($t_{10} = 3.842$, $P =$
225 0.0086). (Z)-3-HP-exposed sweet pepper plants showed an increase of the expression of the
226 gene *PIN2* at day 14 ($t_{10} = 3.354$, $P = 0.0153$) and at day 21 ($t_{10} = 2.727$, $P = 0.0343$). The
227 expression of *AMP1* gene in plants exposed to (Z)-3-HP significantly increased in comparison
228 with control plants during the whole duration of the experiment (Day 7: $t_{10} = 4.797$, $P = 0.0030$;
229 day 14: $t_{10} = 4.647$, $P = 0.0035$ and day 21 $t_{10} = 7.626$, $P = 0.0003$).

230 **Discussion**

231 Our study confirms that the exposure of sweet pepper plants to the synthetic volatile (Z)-3-HP
232 elicits the resistance of plants against pest infestation and that this activation can ameliorate the
233 pest infestation rates pressure of the aphid *A. solani*. The fatty acid derivate group, commonly
234 called green leaf volatiles (GLVs), is a well-studied group of compounds released by plants

235 immediately after mechanical damage, herbivore or zoophytophagous feeding (Bouagga et al.,
236 2018; Pérez-Hedo et al., 2018; Turlings and Erb, 2018). Therefore, GLVs are important
237 components of a blend of volatiles, which rapidly provide information about the exact location
238 of a feeding herbivore (Yu et al., 2008). Previous studies already proved the potential of HIPVs
239 to manage agricultural pests or attract natural enemies (Turlings and Erb, 2018; Zhang et al.,
240 2019; Pérez-Hedo et al., 2021c; Silva et al., 2021). The application of HIPVs as plant elicitors
241 can enhance the biological control of crop pests by inducing plant defense responses, playing
242 an important role in the chemical communication between plants and pests. The exposure of
243 tomato plants to Z-3-hexenol for example negatively influenced the performance of the whitefly
244 *B. tabaci* thus reducing the transmission of plant viruses (Su et al., 2020) while increasing the
245 attraction of the parasitoid *E. formosa* (Yang et al., 2020).

246 However, to our knowledge only two previous studies had used a polymeric dispenser to release
247 a volatile that would activate the plant's defenses (Pérez-Hedo et al., 2021a; Riahi et al., 2022).
248 In the first study, the continuous release of (Z)-3-HP upregulated JA and SA pathways in
249 commercial tomato plants, which resulted in a decrease in the impact of the South American
250 pinworm *T. absoluta*. Riahi et al. (2022) demonstrated that the exposure of sweet pepper to (Z)-
251 3-HP was repellent to *F. occidentalis* whereas *O. laevigatus* showed a strong preference to the
252 activated plants. Our results showed that the exposure of sweet pepper plants to (Z)-3-HP using
253 the same polymeric dispensers under greenhouse conditions induced repellence to the aphid *A.*
254 *solani*, attract the parasitoid *A. abdominalis* and reduced aphid attack.

255 Plant selection is a very important factor for establishing aphid populations on host plants.
256 Aphids may either find the plant unsuitable for colonization or settle on the plant and begin
257 feeding. Plants exhibit antixenosis and antibiosis as natural defense mechanisms against aphid
258 pests. Antixenosis affects aphid behavior, such as host-seeking behavior as well as feeding and
259 oviposition, effectively rendering the plant not appropriate for establishing a colony (Nalam et

260 al., 2019). Antibiosis influences aphid growth, survival, and reproductive prowess. One such
261 example is the plants of the Brassicaceae family, which produce metabolites that are toxic
262 against aphids feeding from the plants (Kim et al., 2008).

263 For the duration of the experiment, temporal gene expression pattern of three phytohormone-
264 responsive plant immunity marker genes, *ASRI*, *PIN2* and *AMP1* was evaluated at 7, 14 and 21
265 days of treatment. The *AMP1* gene related to the SA signaling pathway was up regulated for
266 the duration of the study, by almost 2-fold increase in gene expression in all the temporal points
267 evaluated, whereas *PIN2* gene involved in the JA signaling pathway, was overexpressed at 14
268 days with 3-fold change in gene expression and continuing this pattern after 21 days of
269 treatment with 2-fold increase (Figure 3). Our analyses suggest an early SA-dependent response
270 and background role in induce resistance signaling, while late JA-dependent response can play
271 a major role in plant defense. As observed in the work of Beyer et al. (2021), SA-responsive
272 genes were typically activated earlier than those responding to JA in the stress responses of
273 soybean plants. This observation shows a mechanism to prioritize one pathway over the other
274 maybe dependent on the sequence and type of attackers, as it could be a response to the aphid
275 infestation and the subsequent release of plant volatiles; a widely known induced plant defense
276 mediated by JA in response to herbivore attack (Bosch et al., 2014). In the same direction,
277 previous studies have shown that application of HIPVs can increase JA levels as well as induce
278 the transcription of JA regulated defense-related genes in different plants (Naselli et al., 2016;
279 Pérez-Hedo et al., 2021b; Silva et al., 2021). On the other hand, both the control and the
280 activated plants exhibited similar levels of expression of the *ASRI*, the marker gene for ABA
281 signaling pathway, however, it is speculated that the expression of this gene is mostly attributed
282 to reduced water availability resulting from the feeding of the aphids, rather than the herbivory
283 itself.

284 All the females of *A. abdominalis* responded to one of the two stimuli during the olfactometer
285 bioassays, with the majority opting for the plants exposed to (Z)-3-HP. These observations are
286 consistent between various parasitoids. In several previous studies from our group, we have
287 observed how the whitefly parasitoid *E. formosa* is attracted to plant activated by phytophagy
288 or plant exposed to HIPV instead control (Pérez-Hedo et al., 2015; Naselli et al., 2016; Bouagga
289 et al., 2018; Pérez-Hedo et al., 2018; Pérez-Hedo et al., 2021b). Similar studies have
290 documented that, parasitoids show preference to JA-induced plants. *Anagyrus nilaparvatae*
291 Pang and Wang (Hymenoptera: Encyrtidae), a rice brown planthopper parasitoid, showed
292 preference to JA-treated plants when compared to control plants (Lou et al., 2005). Similarly,
293 three parasitoid species [*Cotesia glomerata* (L.), *C. rubecula* (Marshall) (Hymenoptera:
294 Braconidae), *Diadegma semiclausum* (Hellén) (Hymenoptera: Ichneumonidae)] of the
295 Brussels sprouts caterpillars *Pieris rapae* (L.) and *Plutella xylostella* (L.) (Lepidoptera:
296 Plutellidae) also showed preference to JA-induced plants (Bruinsma et al., 2009), however, it
297 is of note, that these parasitoids also preferred the plants that were induced by herbivory, as
298 opposed to the JA-treated plants when given the choice. This could indicate that the quality of
299 the produced volatiles blend is also a factor that affects plant choice by parasitoids.

300 In summary, our results demonstrated that the use of a polymer dispenser continuously releasing
301 (Z)-3-HP is a sustainable pest management tool to enhance biological control strategies. Indeed,
302 sweet pepper plants exposed to this volatile are repellent to *A. solani*, an important pest of this
303 crop, are able to limit *A. solani* infestation over time, and can also attract economically
304 important natural enemies. Further research should explore plant defense activation against
305 other pests but also how these responses influence natural enemies' performance.

306

307 **Conflict of Interest**

308 M.P.-H., M.A.-V. and A.U. are inventors on the Spanish Patent No. P202030330 titled “Uso
309 de ésteres de (Z)-3-hexenilo y método para proteger plantas frente a plagas”. The other authors
310 declare no conflict of interest.

311 **Author Contributions**

312 L.D., A.U. and M.P.-H. conceived the idea. L.D., A.U., M.A.-V. and M.P.-H. designed the
313 research methodology. L.D., C.G. and A.F. performed the experiments. L.D. and M.P.-H.
314 analysed the data. All the authors discussed the drafts, took part in writing the manuscript and
315 gave final approval for publication.

316 **Funding**

317 The research leading to these results was partially funded by the Spanish Ministry of Economy
318 and Competitiveness MINECO (RTA2017-00073-00-00 and PID2020-113234RR-I00) and the
319 Conselleria d’Agricultura, Pesca i Alimentació de la Generalitat Valenciana. A. F. was
320 supported by Erasmus+ whereas L.D. was supported by fellowship awarded by OECD Co-
321 operative Research Programme.

322 **Acknowledgments**

323 The authors thank Alice Mockford (University of Worcester) for helpful comments on earlier
324 versions of the manuscript, to Dr Gerben Meselink (Wageningen Plant Research) for supplying
325 initial *Aulocorthum solani* individuals to start the colony and to Chaymaa Riahi for technical
326 support. Laura Depalo acknowledges the receipt of a fellowship from the OECD Co-operative
327 Research Programme: Biological Resource Management for Sustainable Agricultural Systems
328 in 2020.

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441

442

443 **Table 1** Forward and reverse primers used in quantification of gene expression.

Primers	Forward	Reverse
<i>PIN2</i>	5'-CTTGCCCAAGAATTGTGAT-3'	5'-GCCCTAGCGTATTACGGAGA-3'
<i>AMP1</i>	5'-TCCCTGCAACAACGAGTACC-3'	5'-CCTAAGTCTGTGATCCCCGC-3'
<i>ASR1</i>	5'-TGTGCAATTTGTCTTGTGGAA-3'	5'-CGGACATGACGAGTTCGATA-3'
<i>EF1</i>	5'-CCTGGACAGATTGGAAATGG-3'	5'-GACCACCTGTCGATCTTGGT-3'

444

445

446 **Figure captions**

447 **Figure 1.** Response (%) of female *Aulacorthum solani* (A.s.) and *Aphelinus abdominalis* (A.a.)
448 in a Y-tube olfactometer when exposed to intact sweet pepper plants and the (Z)-3-hexenyl
449 propanoate [(Z)-3-HP] sweet pepper exposed plants. “nc” indicates the number of tested
450 females that did not make a choice. A total of 80 aphid and 40 parasitoid responsive individuals
451 were tested. Asterisks indicate significant differences in the distribution of side-arm choices (χ^2
452 tests; $P < 0.05$).

453

454 **Figure 2.** Number of *Aulacorthum solani* individuals (mean \pm SE; n=6) per sweet pepper plant
455 in a glasshouse experiment comparing the aphid development on continuously exposed (Z)-3-
456 HP sweet pepper plants and intact sweet pepper plants (Control). Asterisks indicate significant
457 differences within each sampling date as detected by the generalized linear mixed model
458 (GLMM, repeated measures; $P < 0.05$).

459

460 **Figure 3.** Transcriptional response of the defensive-related genes *ASR1*, *PIN2* and *AMP1* in
461 sweet pepper plants exposed to (Z)-3-HP in comparison to sweet pepper intact plants (Control).
462 Data is presented as the mean of six independent analyses of transcript expression relative to
463 the constitutive EF1 gene \pm SE (n = 6). Significant differences using a two-tailed *t*-test are
464 marked with p < 0.05 * p < 0.01 ** p < 0.001 ***.

465

466