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Archivio istituzionale della ricerca

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

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03 May 2024

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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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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Introduction

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

Plants activate their immune system to counteract attack by pathogens or herbivorous insects triggered by a diverse suite of plant hormones, acting as central players in the plant defense 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 Sl-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 ºC, 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 Neatherlands) 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 ± 2ºC). *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 ± 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) *ASR1* (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 *ASR1, 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, *ASR1*, *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 *ASR1*, 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: Icheneumonidae)] 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**

316 **Funding**

322 **Acknowledgments**

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441

442

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

Primers	Forward	Reverse
PIN2	*5'-CTTGCCCCAAGAATTGTGAT-3'*	*5'-GCCCTAGCGTATTACGGAGA-3'*
AMP1	*5'-TCCCTGCAACAACGAGTACC-3'*	*5'-CCTAAGTCTGTGATCCCCGC-3'*
ASR1	*5'-TGTGCAATTTGTCTTGTGGAA-3'*	*5'-CGGACATGACGAGTTCGATA-3'*
EF1	*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