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

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

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

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Abstract

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

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

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

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

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

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

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

Materials and methods

Plants, insects and volatile

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

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

Y-tube olfactometer bioassays

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

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

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

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

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

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

for NZYol (NZYTech, Lisboa, Portugal) based RNA extraction. 1µg of each RNA sample was treated with TURBO DNA-*free*™ Kit (Ambion®, Life Technologies, CA, USA) to remove contaminating DNA. Reverse transcription RT was executed, and cDNA was synthesized using Prime Script™ RT Reagent Kit (TAKARA Bio, CA, USA). Real-time PCR amplification was performed in LightCycler® 480 System (Roche Molecular Systems, Inc., Switzerland), using NZYSpeedy qPCR Green Master Mix (2x) (NZYTech, Lisboa, Portugal) as described by Bouagga et al., (2018). Primers sequences of defensive genes *ASR1, PIN2, AMP1* and the housekeeping gene *EF1* (Elongator factor 1) used as standard control gene for normalization are represented in Table 1.

Statistical analyses

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

Results

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

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

plant. A total amount of 80 *A. solani* individuals responded to the stimuli out of 110 individuals tested. Contrarily, when testing the parasitoid, 74 % of *A. abdominalis* females were attracted towards (Z)-3-HP exposed plants (χ^2 = 11.52, *P* = 0.0007). All the female parasitoids tested (n = 50) responded to one of the two stimuli in the Y-tube.

(Z)-3-HP- exposed plants reduce *A. solani* performance. The continuous exposure of plants to (Z)-3-HP significantly influenced the number of *A. solani* infesting sweet pepper plant (*F* = 33.894; df = 1, *P* < 0.0001) (Table 2). The abundance of aphids per plant increased over time as expected in both treatment (*F* = 344.429; df = 1, *P* < 0.0001), but significant differences between (Z)-3-HP-exposed sweet pepper plants and control plants were detected from the fourth sampling date until the end of the experiment, with higher infestation in control plants (Fig. 2). At day 56, the number of *A. solani* per plant was significantly reduced by 62% on (Z)-3-HP-exposed plants compared to the control plants.

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

Discussion

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

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

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

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

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

For the duration of the experiment, temporal gene expression pattern of three phytohormone-responsive plant immunity marker genes, *ASR1*, *PIN2* and *AMP1* was evaluated at 7, 14 and 21 days of treatment. The *AMP1* gene related to the SA signaling pathway was up regulated for the duration of the study, by almost 2-fold increase in gene expression in all the temporal points evaluated, whereas *PIN2* gene involved in the JA signaling pathway, was overexpressed at 14 days with 3-fold change in gene expression and continuing this pattern after 21 days of treatment with 2-fold increase (Figure 3). Our analyses suggest an early SA-dependent response and background role in induce resistance signaling, while late JA-dependent response can play a major role in plant defense. As observed in the work of Beyer et al. (2021), SA-responsive genes were typically activated earlier than those responding to JA in the stress responses of soybean plants. This observation shows a mechanism to prioritize one pathway over the other maybe dependent on the sequence and type of attackers, as it could be a response to the aphid infestation and the subsequent release of plant volatiles; a widely known induced plant defense mediated by JA in response to herbivore attack (Bosch et al., 2014). In the same direction, previous studies have shown that application of HIPVs can increase JA levels as well as induce the transcription of JA regulated defense-related genes in different plants (Naselli et al., 2016; Pérez-Hedo et al., 2021b; Silva et al., 2021). On the other hand, both the control and the activated plants exhibited similar levels of expression of the *ASR1*, the marker gene for ABA signaling pathway, however, it is speculated that the expression of this gene is mostly attributed to reduced water availability resulting from the feeding of the aphids, rather than the herbivory itself.

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

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

Conflict of Interest

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

Author Contributions

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

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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'*

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Figure captions

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

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

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