Original Paper for Journal of Pest Science

Meritxell Pérez-Hedo[1*], Miquel Alonso-Valiente[1], Sandra Vacas[2], Carolina Gallego[1], Clara Pons[3], Vicente Arbona[4], José L. Rambla[4], Vicente Navarro-Llopis[2], Antonio Granell[3], Alberto Urbaneja[1]

Plant exposure to herbivore-induced plant volatiles: a sustainable approach through eliciting plant defenses

[1] Instituto Valenciano de Investigaciones Agrarias (IVIA). Centro de Protección Vegetal y Biotecnología, (IVIA), CV-315, Km 10.7, 46113 Moncada, Valencia, Spain

[2] Centro de Ecología Química Agrícola – Instituto Agroforestal del Mediterráneo. Universitat Politècnica de València. Camino de Vera s/n, 46022 Valencia, Spain

[3] Instituto de Biología Molecular y Celular de Plantas. (IBMCP), Consejo Superior de Investigaciones. Científicas, Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain

[4] Ecofisiologia i Biotecnologia, Departament de Ciències Agràries i del Medi Natural. Universitat Jaume I, Castelló de la Plana, Spain

* Corresponding author: perez_merhed@gva.es

ORCID

Meritxell Pérez-Hedo: 0000-0003-3411-0761
Miquel Alonso-Valiente: 0000-0002-2065-6125
Sandra Vacas: 0000-0001-6911-1647
Carolina Gallego: 0000-0002-3385-3072
Clara Pons: 0000-0002-8296-2937
Vicente Arbona: 0000-0003-2232-106X
José L. Rambla: 0000-0003-4704-2534
Vicente Navarro-Llopis: 0000-0003-3030-3304
Antonio Granell: 0000-0003-4266-9581
Alberto Urbaneja: 0000-0001-5986-3685

Abstract

Modern agricultural policies across the globe are committed to a significant reduction in chemical pesticide dependency; however, pest management strategies are still based on the use of synthetic pesticides. There is an urgent need to find new, sustainable and biorational tools for pest management programs. Plants communicate with each other and activate defense mechanisms against pests using Herbivore-Induced Plant Volatiles (HIPVs). The use of such HIPVs could be an ecologically sustainable alternative. However, as of now, there has been no comprehensive studies on HIPVs, from selection to practical use in industry production. Here, we describe the first case of an HIPV successfully implemented for pest control under commercial greenhouse conditions. In this research, tomato plants induced with *(Z)*-3-hexenyl propanoate [(Z)-3-HP] were less susceptible to the attack of economically important tomato pests. We designed and calibrated polymeric dispensers for the constant release of (Z)-3-HP. These dispensers maintained commercial tomato plant defenses activated for more than two months reducing herbivore pest damage without reducing plant productivity. Transcriptomic and metabolomic analyses of plants induced with (Z)-3-HP confirmed that genes involved in specialized anti-herbivore defense were upregulated, resulting in an increased production of fatty acid-derived compounds, activation of the lipoxygenase pathway and accumulation of specific defense compounds. Our work demonstrates under commercial greenhouse conditions how the release of HIPVs as elicitors of plant defenses via designed polymeric dispensers can be successfully integrated as a new biorational and sustainable tool for pest control.

Keywords: Elicitors, pest management, *Tuta absoluta*, predatory mirids, tomato

Declarations

Funding: The research leading to these results was partially funded by the Spanish Ministry of Economy and Competitiveness MINECO (AGL2014-55616-C3 and RTA2017-00073-00-00) and the Conselleria d'Agricultura, Pesca i Alimentació de la Generalitat Valenciana.

Competing interests: M.P.-H., A.U., M.A.-V., V.N.-LL., S.V., J.R. and A.G. are inventors on the requested Spanish Patent No. P202030330 entitled "Uso de propanoato de (Z)-3-hexenilo y método para proteger plantas frente a plagas" E.K.-H.C. The other authors declare no conflict of interest.

Availability of data and material: The RNA-Seq datasets analyzed during the current study are available in the in NCBI's Gene Expression Omnibus (Edgar et al. 2002) and are accessible through GEO Series accession number GSE150659 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE150659).Code availability: Not applicable'

Key Message

- Plants communicate with each other by means of warning signals when under attack.
- Plants receiving warning signals may become defensively induced against different stressors.
- In this research, we take advantage of this to develop a new sustainable method for pest management.
- We demonstrate how exposure of tomato plants to one selected volatile using polymeric dispensers enhances resistance to key tomato pests in commercial greenhouses.
- We anticipate our results to be a starting point for new biorational strategies in pest management.

Authors' contributions: M.P.-H. and A.U. conceived the idea. M.P.-H., A.G., V.N.-LL. and A.U. designed the research methodology. M.A.-V., C.G., S.V., J.R., V.A., M.P.-H. performed the experiments. M.P.-H., C.P., J.R., V.A., S.V., V.N.-LL. and A.U. analyzed the data. All the authors discussed the drafts, took part in writing the manuscript and gave final approval for publication.

Introduction

Public and private organizations, the scientific community and society have long been demanding sustainable agricultural practices which guarantee food security for a growing world human population without compromising biodiversity and the environment (UE 2009; Poore and Nemecek 2018; Pretty 2018; Mokany et al. 2020; Johnson et al. 2020). However, the reality is that most of the pest management programs are still dependent on the use of synthetic pesticides. If suitable alternatives are not found, this will lead to increased pesticide use and the inherent problems associated with its use (Nicolopoulou-Stamati et al. 2016; Calvo-Agudo et al. 2019; Tortell 2020), hence, there is a great need to identify and exploit novel mechanisms and develop new strategies for pest management.

Plants respond to herbivore attack both directly by adapting their suitability as a host plant or affecting herbivore survival and reproductive success (*direct defense*), and indirectly through other species such as natural enemies of the insect pests (*indirect defense*) (War et al. 2012). Direct defenses are mediated by plant characteristics that affect the herbivore's biology such as mechanical protection on the surface of the plants (War et al. 2012) or by the production of proteinase inhibitors (PIs) and specialized metabolites including terpenoids, phenolics and alkaloids. These compounds are often produced by plants as deterrents or toxins that may act directly on the insects as feeding inhibitors (Duffey and Stout 1996; Züst and Agrawal 2016; Block et al. 2019; Hussain et al. 2019; Perez-Fons et al. 2019). Indirect defenses are mediated by the release of a blend of Herbivore-Induced Plant Volatiles (HIPVs) that specifically attract natural enemies of the herbivores and/or by providing food and housing to enhance the effectiveness of the natural enemies (Tumlinson et al. 1999; War et al. 2012).

Plants have the capacity to communicate between themselves to warn one another of external stressors (Baldwin 1998; van Hulten et al. 2006; Heil and Silva Bueno 2007; Frost et al. 2008; Martinez-Medina et al. 2016). In the case of herbivore attacks, this communication is carried

112 out by HIPVs. The production of these volatiles is induced by herbivore injury and emitted by
113 the plant thereafter (Pare and Tumlinson 1997, 1999; Kessler 2001; Dicke and Baldwin 2010).
114 Plants which receive these volatile warning messages are capable of activating their defense
115 system and enter into a state of alert aimed at minimizing potential imminent damage
116 (Arimura et al. 2000; Frost et al. 2008). This activation can set off a wide range of defense
117 responses, such as the production of PIs, the release of volatile compounds, the production of
118 alkaloids, the formation of trichomes, and the secretion of extra floral nectar (Farag and Pare
119 2002; Choh and Takabayashi 2006; Heil and Ton 2008).

120 In previous work, we demonstrated how the phytophagous behavior of some
121 zoophytophagous predatory mirids (Hemiptera: Miridae) triggers the release of HIPVs in
122 tomatoes (Naselli et al. 2016; Zhang et al. 2018, 2019; Pérez-Hedo et al. 2018b, a; Bouagga et
123 al. 2018, 2020). Some of these volatiles were responsible for inducing defenses in adjacent
124 intact plants with no previous exposure to mirids (Pérez-Hedo et al. 2015). In order to decipher
125 which volatiles were responsible for plant communication, we exposed individual plants to
126 each of these mirid-induced volatiles [1-hexanol, *(Z)*-3-hexenol, *(Z)*-3-hexenyl acetate, *(Z)*-3-
127 hexenyl propanoate, *(Z)*-3-hexenyl butanoate, hexyl butanoate and methyl salicylate] for 24
128 hours (Pérez-Hedo et al. 2021). Methyl jasmonate, one of the most studied plant defense
129 activators, was also tested in this study. All HIPVs overexpressed defensive genes in exposed
130 tomato plants when compared to unexposed tomato plants. In a further step, (Z)-3-hexenyl
131 propanoate [(Z)-3-HP, hereafter] and methyl salicylate (MeSA, hereafter), were selected based
132 on their ability to induce the expression of the basic pathogenesis-related protein precursor
133 (*PR1*), a marker gene for the the salicylic acid (SA) signaling pathway, and two plant PI (Sl-PI-I
134 and *PIN2*) markers. Plants previously exposed to these two HIPVs were repellent to *Bemisia*
135 *tabaci* (Gennadius) (Hemiptera: Aleyrodidae), *Tuta absoluta* (Meyrick) (Lepidoptera:
136 Gelechiidae) and *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), attractive to
137 the parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), and indifferent to

138 *Tetranychus urticae* Koch (Acari: Tetranychidae) (Pérez-Hedo et al. 2021).

139 Interest in the potential applications of the use of these volatiles to induce plant defenses has
140 increased greatly in recent years (Turlings and Erb 2018). However, taking advantage of HIPVs
141 and the plants' ability to communicate for improved pest management has not yet been
142 demonstrated under commercial production. To date, practical research with HIPVs has mainly
143 focused on four directly related approaches: *i*) use of HIPVs as repellents or attractants of
144 pests and / or natural enemies (James 2003; Uefune et al. 2012), *ii*) genetic plant selection to
145 produce more HIPVs of agronomic interest (Birkett and Pickett 2014), *iii*) use of companion
146 plants that emit HIPVs of interest to the crop (Pickett et al. 2014) and *iv*) spray of HIPVs as
147 elicitors to induce defenses in the plant (Baysal et al. 2003). In this work, as a continuation of
148 the work mentioned above (Pérez-Hedo et al. 2021) where (Z)-3-hexenyl propanoate and
149 methyl salicylate were selected as HIPVs of interest for inducing tomato plant defenses, we
150 research a new approach to take advantage of HIPVs for crop protection. With the novel use of
151 designed polymeric dispensers, we show for the first time that the exposure of a plant to
152 defense elicitor HIPVs can be used as an environmentally and economically sustainable tool for
153 the protection of an important crop such as tomato (*Solanum lycopersicum* L.). Tomato is the
154 leading vegetable crop globally, with a total yield of 18 million tons cultivated in over 4.7
155 million ha in 2018 and generated a revenue of $190.4 billion in the same period (Research and
156 Markets 2020). Tomato is especially susceptible to economic injury due to pests, and in the
157 absence of control strategies yield losses can reach 100% (Pérez-Hedo et al. 2017; Biondi and
158 Desneux 2019). Here, we disentangle the behavioral, physiological and agronomical traits
159 which has allowed us to develop a pest control tool in tomato based on HIPVs and plant
160 communication. This knowledge will be useful for future application in other crops.

161 **Materials and Methods**

162 **Plants, insects and HIPVs**

163 The tomato *S. lycopersicum* cv. Moneymaker was used in all experiments, except for the
164 commercial greenhouses where the cv. Raf was planted. Moneymaker seeds were sown in soil
165 in seedling trays and two weeks after germination, seedlings were individually transplanted
166 into pots (8 × 8 × 8 cm). Plants were maintained undisturbed at 25 ± 2 ºC, with constant
167 relative humidity of 65% ± 5% and a photoperiod of 14:10 h (light: dark). All tomato plants
168 were pesticide-free. At four weeks of age (approximately 20 cm high), plants were used for
169 experimentation.

170 *Tetranychus urticae* adults were obtained from a culture established at Instituto Valenciano de
171 Investigaciones Agrarias (IVIA) in 2011 originally collected from the region of La Plana (Castelló,
172 Spain). Mites were maintained on tomato plants kept in a climatic chamber under the same
173 conditions described above. *Tuta absoluta* females were obtained from tomato colonies
174 maintained at IVIA in a glasshouse located at IVIA at 25 ± 4ºC, 60 ± 15% RH and under natural
175 photoperiod. Newly emerged (less than 5 days old) adult females were used in all trials.

176 All synthetic standards of the tomato volatile compounds were purchased from Sigma-Aldrich
177 (St. Louis, MO, USA), (Z)-3-HP purity > 97% and MeSA purity > 99%.

178 **Transcriptome response to HIPV exposure**

179 To provide insight into the molecular responses of the plants exposed to HIPVs, total RNA of
180 the apical part of 4 tomato plant exposed for 24h to either (Z)-3-HP or MeSA and intact plants
181 were extracted. Volatile emitters were prepared from 2 x 2 cm filter paper impregnated each
182 with 10 µl of the corresponding volatile (Pérez-Hedo et al. 2018b). The volatiles were firstly
183 diluted in methanol at 1:100 (v/v) and then further diluted in water at 1:100 (v:v; volatile
184 mix:water) so that the final test concentration was 1:10,000 (v/v). The control consisted of
185 1:100 methanol:water (v/v). Pérez-Hedo et al. (2018b) demonstrated that this volatile
186 concentration was very similar to those emitted by mirid-induced tomato plants. Two
187 impregnated volatile emitters were then placed in the bottom part of a 30 × 30 × 30 cm

188 experimental cage (BugDorm-1 insect tents; MegaView Science Co., Ltd, Taichung, Taiwan)
189 together with an intact tomato plant. Plants and HIPVs were kept undisturbed for 24 hours in
190 isolated climatic chambers to avoid any volatile interference and maintained at 25 ± 2ºC, 65 ±
191 10% RH and a 14:10 h (L:D) photoperiod. Samples from the apical part of the tomato plant
192 were immediately ground in liquid nitrogen. Each plant, either exposed or intact, was used just
193 once.

194 RNA sequencing was performed at MacroGen Inc. (www.macrogen.com) using Illumina HiSeq
195 4000 platform. Library construction and sequencing followed the TruSeq and HiSeq standard
196 sequencing protocols recommended by Illumina. Twelve sequencing libraries, four replicates
197 for plants exposed to either (Z)-3-HP or MeSA and control were constructed. 101- base Paired
198 End sequencing approach was applied. Read quality was evaluated using the FastQC suite.
199 Reads over phred score of 20 were accepted as good quality. Trimmomatic program 0.32
200 (Bolger et al. 2014) was used to remove adapter sequences and bases with Q-score lower than
201 three from the ends. In addition, using the sliding window method, bases of reads that did not
202 qualify for window size 4, and mean quality 15 were trimmed. Afterwards, reads with length
203 shorter than 36bp were dropped to produce trimmed data. Trimmed reads were mapped to
204 tomato Heinz reference genome build SL2.50 (GCF_000188115.3_SL2.50) with HISAT2 splice-
205 aware aligner version 2.0.5 (Pertea et al. 2016) through Bowtie2 2.2.6 aligner. Known genes
206 and transcripts were assembled with StringTie version 1.3.3b (Pertea et al. 2015, 2016) based
207 on reference genome model.

208 The statistics summary of transcriptome sequencing is shown in Table S1. The data set
209 supporting the results of this article has been deposited in NCBI's Gene Expression Omnibus
210 (Edgar et al. 2002) and are accessible through GEO Series accession number GSE150659
211 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE150659). As exposure to HIPVs
212 treatments was used to elicit herbivore response, DEG genes were inspected for genes with

annotations (GO and KO terms) related to plant pathogen and wounding response, and hormones with known roles in defense and wounding [jasmonic acid (JA), SA and ethylene (ET)]. An enrichment analysis for each KEGG pathway term in genes up and down regulated in plants experiencing exposure to (Z)-3-HP and MeSA was performed at $P < 0.001$. Details for the identification of DEGs and gene ontology, the defensive Gene identification and the confirmation of induced genes expression profiles by qRT-PCR can be found in the Electronic Supplementary Material.

Plant metabolome response to HIPV exposure

Plant metabolic analysis was conducted to determine the molecular responses that HIPVs induce on the plants and explain their impact on pests. Using the same methodology described above, frozen plant material from the apical part of 4 tomato plants exposed for 24h to either (Z)-3-HP or MeSA and intact plants was homogenized in liquid nitrogen with pestle and mortar, and the resulting powder stored at -80ºC until analyzed. Determination of primary metabolites was performed using hydrophilic interaction liquid chromatography (HILIC) coupled to hybrid quadrupole-time of flight mass spectrometry (QTOF-MS) as modified from Gika et al. (2012). Details about the analysis of plant metabolites are fully described in the Electronic Supplementary Material.

***Tetranychus urticae* and *T. absoluta* performance on HIPVs-exposed plants**

Given the activation of defense related marker genes in response to (Z)-3-HP and MeSA exposure exposure, we wanted to know whether this activation could influence the performance of two important phytophagous species in tomato, the two spotted spider mite (*T. urtic*ae) and the lepidopteran *T. absoluta*. Both phytophagous species were subjected to bioassays under two distinct plant exposure treatments. In one treatment the plant was pre-exposed to the volatile for 24 hours and then moved to a fresh air chamber where the

herbivores were added. In the other treatment, the plants were continuously exposed to the volatile for the whole duration of the experiment (permanent exposure).

The experiment was conducted in three growth chambers maintained at 25 ± 2 ºC, 65 ± 5% RH, 14:10 (L:D) h photoperiod. To avoid interference between volatiles, one chamber was assigned to treatments with (Z)-3-HP, another to treatments with MeSA and the last to the control treatment. The growth chambers where HIPVs were tested consisted of 12 cages (60 cm × 60 cm × 60 cm) (BugDorm-2 insect tents. MegaView Science Co., Ltd., Taichung, Taiwan), six per treatment (24 h exposure or permanent exposure). On the other hand, the chamber with the control treatment consisted of only six cages. Cages were equally distributed at a distance of 1.5 meters from each other. Each cage represented one replicate.

Two impregnated volatile emitters prepared as described above, were placed on the floor of the cages. In the permanent treatments, where volatile exposure lasted throughout the length of experiment, the volatile emitters were replaced every two days. Eight tomato plants (cv. Moneymaker) were introduced into each cage. The plants were individually isolated without touching each other or the cage walls in order to avoid spider mite movement from plant to plant. Additionally, plants were placed on top of a small brick inside a plastic tray full of water; all pots were painted with a band of glue. Plants were artificially infested with *T. urticae* from the previously described laboratory population. Twenty *T. urticae* females were released per plant; they were distributed equally throughout the leaves with the aid of a fine brush. Sampling was conducted seven, 14, and 21 days after releasing *T. urticae*. Sampling involved counting the total number of *T. urticae* females on each plant. This was done with the naked eye, *in situ*, without removing leaves from the plant.

To evaluate the effect of exposure to both of the volatiles on *T. absoluta*, two consecutive experiments were performed. In the first, the effect on oviposition was studied. Selected eggs, which had been laid in the first experiment, were subsequently used in the second experiment

to study the mortality of immature *T. absoluta* raised on plants exposed to the HIPVs. The same five treatments described above for *T. urticae* were used in the case of *T. absoluta*. Oviposition of *T. absoluta* was evaluated on 8 tomato plants (cv. Moneymaker) per treatment. Each plant was isolated inside a plastic cage (60 x 60 x 60 cm) (BugDorm-2 insect tents) maintained in a growth chamber at 25 ± 2 ºC, 65 ± 5% RH, 14:10 (L:D) h photoperiod following the same treatment distribution described for *T. urticae*. Inside each cage (replicate), 4 adult *T. absoluta* (2 males and 2 females) were released and left undisturbed for 72 hours. After this time, *T. absoluta* adults were removed and the number of eggs was counted.

To study the mortality of *T. absoluta* in plants exposed to the 5 treatments described above, 6 *T. absoluta* eggs per plant were distributed equally throughout the leaves with the aid of a fine brush. The eggs used in each treatment came from the corresponding treatment of the first experiment. Mortality of *T. absoluta* was evaluated on 8 tomato plants (cv. Moneymaker) per treatment. Each plant was isolated inside a plastic cage (60 x 60 x 60 cm) (BugDorm-2 insect tents) maintained in a growth chamber at 25 ± 2 ºC, 65 ± 5% RH, 14:10 (L:D) h photoperiod following the same treatment distribution described above. Plants were left undisturbed until *T. absoluta* adults emerged. Each day, newly emerging adults were counted and removed from the cages.

Suitability of HIPV dispensers and tomato plant productivity

In light of the results obtained with (Z)-3-HP, we decided to formulate this volatile into controlled release dispensers which allow to emit (Z)-3-HP at constant rates for long periods. We chose (Z)-3-HP because it had the greatest effects on the performance of the pests. To determine the emission rate of (Z)-3-HP that was needed to achieve plant activation, we prepared three formulations with permeable polymer vial dispensers which provided three different emission rates for (Z)-3-HP: 3.4, 0.8 and 0.08 mg/day (Electronic Supplementary Material; Fig. S1). Then, we selected the dispensers that provided the highest (low density polymer, LD) and the lowest (high density polymer, HD) emission rates to check in a

288 preliminary glasshouse trial the level of plant activation (gene expression). Accordingly, we
289 selected the polymeric dispenser LD, as it provided significant plant activation via the study of
290 the PIN2 gene (Electronic Supplementary Material; Fig. S2).

291 To test whether or not the use of these volatiles could activate the defenses of tomato during
292 a continuous period of time and whether the activation would have some type of trade-off in
293 fruit producing plants, a glasshouse experiment was conducted. The selected LD dispensers
294 were placed inside 25 m^2 isolated test chambers containing 60 plants within a glasshouse at
295 25°C ±1°C and RH 65% ± 10% and natural photoperiod (aprox. 14:10, L:D). Tomato plants were
296 transplanted into individual polyethylene 20-liter pots filled with a mixture of sand and peat
297 (1:2 w:w) medium in the greenhouse in twelve rows of five plants each (2 plants/m^2). Crop
298 cultivation techniques typical of tomato greenhouse cultivation in Spain were followed: a
299 trellis of one wire-guide for each plant, to which the main stem was trained tied with green
300 polyethylene string, weekly pruning of secondary shoots, application of a standard nutrient
301 solution for tomato by means of an automated-irrigation system with an irrigation frequency
302 adjusted to the environmental conditions and an irrigation time of 15 min. On November 15,
303 2019, in one of the test chambers, one LD dispenser loaded with 1 ml (Z)-3-HP was hung in the
304 center of the chamber, while an empty LD dispenser was employed in the control chamber.
305 The dispenser was hung in the middle of each chamber at 50 cm above the plants; as plants
306 grew, the dispenser was moved upwards to maintain a standardized distance.

307 Each week, the height of 14 plants from each chamber was measured and their yield in fruit
308 was weighed. Samples from the apical part of the 6 tomato plants were ground in liquid
309 nitrogen and the expression of *PR1*, *PIN2* and *Sl*-PI-I markers was quantified 24 hours and 1, 4
310 and 8 weeks after the dispensers were installed. Portions of these samples were then used for
311 RNA extraction. Total RNA (1.5 µg) was extracted using a Plant RNA Kit (Omega Bio-Tek Inc.,
312 Doraville, GA, USA) and was treated with RNase-free DNase (Promega Corporation, Madison,
313 Wisconsin, USA) to eliminate genomic DNA contamination. The RT reaction and the PCR SYBR

314 reaction were performed as described by Pérez-Hedo et al. (2015a). Quantitative PCR was
315 performed using the Smart Cycler II (Cepheid, Sunnyvale, CA, USA) sequence detector with
316 standard PCR conditions. Expression of EF1 was used for normalization as a standard control
317 gene. The nucleotide sequences of the gene specific primers are described in Table S2.

318 Additional dispensers were aged under the same trial conditions and were weighed weekly in
319 on a precision balance (0.0001 g). The weight differences over the duration of the experiment
320 were considered the amount of (Z)-3-HP released from the dispenser.

321 **Commercial greenhouse experiment**

322 To test the effect of the HIPV dispenser treatment under commercial greenhouse conditions,
323 four greenhouses were selected from a single farm located in Xilxes (Castellón, Spain) (Fig. S3).
324 Each greenhouse was considered a block in a replicated complete block design with 2
325 treatments and 4 replicates. The two plot treatments within each replicate block were
326 adjacent (Fig. S3*)*.

327 The tomato plant variety ‘‘Raf’’ were transplanted on September 4, 2018 to soil to give an
328 overall density of 1.2 plant m^2 (25,450 plants in 21,200 m^2). Common cultivation techniques
329 were followed: the plant’s main stem was trained with plastic rings supported by strings
330 attached to an overhead wire; secondary shoots and senescent leaves were pruned weekly
331 and a standard nutrient solution for tomato was applied weekly by means of an automated
332 drip irrigation system. Transplanted plants were already inoculated with the predator
333 *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) (Urbaneja-Bernat et al. 2015). A dose of 1 *N.*
334 *tenuis* per plant was released in the nursery in addition to *E. kuehniella* eggs as an alternative
335 prey. This pre-plant strategy with *N. tenuis* results in very efficient control of the two key
336 tomato pests, *B. tabaci* and *T. absoluta* in tomato greenhouses (Pérez-Hedo et al., 2020).
337 Indeed, from the date of transplanting (September 4, 2018) until the day when the dispensers

were hung (February 22, 2019), none of the greenhouses received any chemical treatment and pest regulation depended only on the predator *N. tenuis*.

In these greenhouses, as is typical in other long cycle tomato production in Spain where *N. tenuis* pre-release strategy is used, populations of *N. tenuis* decrease as winter approaches and remain at very low levels until spring and temperatures begin to rise. During this winter/early spring period, *T. absoluta* populations increase under the relative absence of *N. tenuis*, as was occurring in the selected greenhouses. Under these conditions, producers typically apply chemical treatments to stop the attack of *T. absoluta* in spring. These greenhouses were therefore selected, to investigate whether the use of the dispensers could activate tomato plant defenses and control of *T. absoluta* during the end of winter and spring period.

Twenty randomly chosen plants selected from the central part of each replicate were sampled weekly for 11 weeks, beginning on January 30, 2019. First, the number of leaflets infested per plant were counted. Then *N. tenuis* (adults and nymphs) were counted in the whole apical third of the plant (leaves, flowers and shoots). On February 22, 2019, polymeric LD dispensers loaded with 4 ml of (Z)-3-HP were hung at a dose of 1 per 20 m^2 (Fig. S3). Dispensers were distributed every 4 m within crop lines and every 5 m between crop lines. Each dispenser was hung at 50 cm above the plants. As plants grew, dispensers were moved upwards to maintain a standardized distance. The apical part of 6 tomato plant samples per treatment per replicate were ground in liquid nitrogen and the expression of *PR1*, *PIN2* and *Sl-PI-I* genes was quantified as described above one day before dispensers were hung and 4 and 8 weeks after the dispensers were installed.

Additional dispensers were aged under the same experimental conditions and were weighed weekly on a precision balance (0.0001 g). The weight differences over the duration of the experiment were considered the amount of (Z)-3-HP released from the dispenser.

Data analyses

The results of the transcriptional responses with markers, the number of eggs per plant, and the % of egg-adult of *T. absoluta* mortality were subjected to one-way analysis of variance, and the Tukey test was used for mean separation at $P < 0.05$. Two-tailed Student's t-test ($P < 0.05$) was performed to compare the yield in the trade-offs study and the quantified expression of defense genes between control and exposed plants in both experimental and commercial greenhouses for each single date. Measurements of height in the trade-off experiment and number of mites per leaf, number of *T. absoluta* per plant and number of infested leaflets per plant on the different sample dates in both the experimental and commercial greenhouse were analyzed using a generalized linear mixed model (GLMM) with repeated measures. Treatment was considered as a fixed factor. The GLMM used a normal distribution with the identity as the link function. The results are expressed as the means ± SE.

Results

Transcriptome response to HIPV exposure

After trimming and filtering, low-quality reads were removed (Table S1 and S3*)* and a total of 19,293 genes were found to be expressed (reads per kilobase per million mapped reads [RPKM] > 0) in all twelve samples (Table S4). MDS analysis of the four biological replicates in each group ((Z)-3-HP, MeSA and control) indicated that groups were well separated, with biological replicates clustering together (Fig. S4). A total of 849 transcripts were differentially expressed (DEG;|fc|≥2; P <0 .05) in HIPV-elicited plants when compared to non-exposed plants: 715 and 577 genes in plants exposed to (Z)-3-HP and MeSA, respectively (Fig. 1; Table S5). Of the 715 DEG identified in plants exposed to (Z)-3-HP, 492 transcripts were up-regulated while 233 were down-regulated (Fig. 1A). Of the 577 DEG in response to MeSA treatment, 371 were up-regulated and 206 were down-regulated (Fig. 1A). The Venn diagram (Fig. 1B, Table S5) indicated that 310 and 133 were up and down-regulated by both exposure treatments,

388 respectively. No DEGs (|fc|≥2; $P < 0.05$) were up or down regulated in opposite ways by the
389 treatments. This indicates that despite (Z)-3-HP having a higher effect in the transcriptome of
390 plants, both HIPV treatments induced similar responses in tomato plants; although with some
391 specific molecular changes associated to each compound.

392 Out of the 849 DEG only 67 had GO and KO terms related to defense, wounding or JA, SA and
393 ET (Table S6). This corresponds to a 5.7% of genes related to defense, 1.5 % to wounding and
394 2.2% to JA, SA and/or ET (Table S7). Out of these genes, approximately 40% of them were up-
395 regulated by both compounds, (Z)-3-HP and MeSA, and 20-35% only when plants were treated
396 with (Z)-3-HP (Table S8, S9 and S10). Only 12 were down regulated by the volatile exposure
397 treatments.

398 Among the 29 common up-regulated genes related to defense, wounding or biotic stress
399 hormones (Table S6) we found 12 genes encoding proteinases and peptidases, a fatty acid
400 hydroperoxide lyase (HPL, Solyc07g049690.3) involved in JA and green leaf VOC production,
401 an ACC oxidase (Solyc02g036350.3) involved in ethylene production, two genes related to
402 strigolactone biosynthesis and signalling (Solyc04g077860.3, Solyc02g064770.3) and six genes
403 encoding transcription factors and signalling elements mediating JA, ET and/or SA signalling
404 and expression regulation (Table S5) such as jasmonate-induced oxygenase like
405 (Solyc03g096050.3) and ethylene response factors (Solyc05g051200.1, Solyc05g052030.1,
406 Solyc05g052040.1 and Solyc12g056980.1).

407 Regarding gene expression, (Z)-3-HP seems more effective in activating defense responses
408 than MeSA as only three genes related to defense, wounding or biotic stress hormones were
409 specifically up-regulated by MeSA (a monoterpene alcohol dehydrogenase, a wound-induced
410 protein 1 and a DAHP synthase 1 precursor; Table S6). In contrast, 22 genes were up-regulated
411 specifically by (Z)-3-HP. Among DEG up-regulated specifically by (Z)-3-HP we found three
412 additional proteases/peptidases (Solyc09g010220.3, Solyc09g083120.3, and

413 Solyc03g059260.3), another gene encoding for a JA biosynthesis (Solyc12g094520.2, encoding
414 OPC-8:0 CoA ligase1), two PR1 encoding genes (Solyc09g007020.2 and Solyc07g006710.2), two
415 chitinases (Solyc10g055800.2 and gene24759), three genes involved in the metabolism of
416 monoterpenoids, the secondary metabolite glycosylation and polyamine biosynthesis
417 (Solyc01g099560.3, Solyc03g078780.2 and Solyc10g054440.2, respectively) and a gene
418 involved in JA signal transduction (JAR1-like; Solyc07g054580.3). In addition, two WRKY
419 transcription factors transcription, one described in response to JA (WRKY31/*SlWRKY33A*;
420 Solyc06g066370.3) and the other in response to SA (WRKY1/WRKY40; Solyc06g068460.3),
421 were also detected.

422 In general, genes up and down regulated by both treatments were enriched in genes encoding
423 for biosynthesis of secondary metabolites (p-value $<10^{-8}$-10^{-25}) (Table 1). In addition, genes
424 related to carbon metabolism were enriched among down regulated genes. More specifically,
425 enrichment analysis indicated that both (Z)-3-HP and MeSA up-regulated genes were rich in
426 genes related to phenylpropanoid biosynthesis and diterpenoid biosynthesis. Our enrichment
427 analyses highlighted differences in the functional categories enriched in response to the two
428 elicitor compounds. (Z)-3-HP up-regulated genes were rich in genes related to pathogen-plant
429 interactions, MAPK signaling pathways as well as with genes related to steroid and carotene
430 biosynthesis. MeSA specific up regulated genes, on the other hand, were rich in genes related
431 to protein processing in endoplasmic reticulum. In the case of down-regulated genes, DEG
432 down-regulated by (Z)-3-HP were enriched in ribosomal structural genes and ribosome
433 biogenesis. MeSA down regulated genes, however, were rich in genes related with flavonoid
434 biosynthesis. The genes in these functional categories are in Table S11 and S12.

435 **Metabolome response to HIPV exposure**

436 Non-targeted LC-MS metabolite analyses (Table S4) revealed relatively modest (50% to 75%
437 increase) but significant differences in the metabolite complement of the leaves of elicited

plants. LC-MS confirmed the slight but consistent activation of a number of defense compounds including phenylpropanoids and glycoalkaloids (including α-tomatine) (see highlighted compounds in Table S13).

HIPV exposure reduces subsequent phytophagous infestation

By 21 days after the *T. urticae* addition to the plants, under both 24 hour pre-exposure and permanent exposure treatment, a reduction in the number of spider mites per plant was recorded when compared to the control but only when plants were exposed to (Z)-3-HP volatiles (Fig. 2A). A population reduction of 50.3 ± 6.3% was observed in the 24 hour pre-exposure treatment and of 83.9 ± 5.0% in the permanently exposed treatment ($F_{4,85}$ = 4.437; P = 0.003) (Fig. 2A) (Table S14).

As for *T. absoluta*, the 24 h pre-exposure treatment did not result in significant differences in oviposition nor in mortality when compared to the control (Fig. 2B, D). However, in the experiment with permanent exposure to (Z)-3-HP the number of eggs laid was reduced by 67.2 ± 15.0% when compared to the control ($F_{2,23}$ = 3.746; P = 0.041) (Fig. 2B). Furthermore, the mortality of *T. absoluta* from egg to adult was significantly higher than in the control ($F_{2,23}$ = 6.944; P = 0.005) in the experiments with plants permanently exposed to (Z)-3-HP, where 81.2% mortality was observed (Fig. 2D). On the other hand, when plants were exposed to MeSA, there were no significant differences in either number of eggs laid by *T. absoluta* ($F_{2,23}$ = 1.147; P = 0.337) (Fig. 2C) or in the mortality of immature of *T. absoluta* when compare with the control ($F_{2,23}$ = 0.588; P = 0.564) (Fig. 2E).

HIPV dispensers do not reduce plant productivity

In plants exposed to (Z)-3-HP, expression of marker genes for the SA and JA pathway were significantly upregulated with respect to control plants during the whole duration of the experiment (8 weeks) (Fig. 3 A, B, C). Plant height (n=14) was measured weekly, with no

462 significant differences between HIPV exposed and control treatment plants ($F_{1, 278}$ = 0.137; P =
463 0.712) (Fig. 3D). Furthermore, throughout the experiment, fruits were harvested and weighed
464 with no significant differences in yield between the two treatments ($t_{1, 26}$ = 0.023; P = 0.974)
465 (Fig. 3E). Gravimetric release studies of the LD dispenser gave a mean emission rate of 9.6
466 mg/day (Fig. 3F) under glasshouse conditions.

467 **HIPV dispensers reduce *T. absoluta* infestation under commercial greenhouses**

468 Before the dispensers were hung, the expression of both marker genes was similar in plants
469 from the (Z)-3-HP treatment and control plots. However, at 30 and 60 days the expression of
470 both genes was significantly greater in the plants from the (Z)-3-HP treatment plots than those
471 from the control plots (Table S15) (Fig. 4 A, B).

472 The population of the mirid, *N. tenuis*, was similar in both treatments ($F_{1, 86}$ = 2.112; P = 0.150)
473 (Fig. 4C), however, the level of *T. absoluta* infestation was significantly lower (approx. 58%) in
474 the treatment with the (Z)-3-HP ($F_{1, 86}$ = 11.375; P < 0.001) (Fig. 4D). The volatile (Z)-3-HP was
475 emitted at a constant rate of 12.2 mg/day during the period of the study (Fig. 4E).

476 **Discussion**

477 This research demonstrates for the first time how the exposure of tomato plants to HIPVs can
478 activate the defense mechanism and enhances resistance against pest infestation under
479 commercial production. In addition, the results presented here indicate that there is a plant
480 reprograming after (Z)-3-HP and MeSA exposure involving production of secondary
481 metabolites and a plethora of protein inhibitors, especially by (Z)-3-HP, indicating that a
482 defensive response is stimulated by the exposition to this HIPV. This activation of direct
483 defenses explains the lower performance of the important tomato pests tested in this work, *T.*
484 *urticae* and *T. absoluta,* in plants exposed to (Z)-3-HP.

Various studies have used HIPVs to reduce the impact of pests or increase the attraction of natural enemies [see review (Turlings and Erb 2018)]. However, to the best of our knowledge, this study is the first that uses a dispenser that continuously releases Z-(3)-HP, which functions as an elicitor of the plant defense mechanism, under practical and commercial application. Previous laboratory studies in corn have demonstrated the potential of green leaf volatiles (GLVs) for priming undamaged plants. The exposure of corn plants to a pure synthetic GLV chemical such as (Z)-3-hexenal, (Z)-3-hexen-1-ol and (Z)-3-hexenyl acetate was reported to induce priming against two generalist carterpillars, *Spodoptera exigua* (Hübner) and *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) upon activating the JA pathway (Engelberth et al. 2004; Ton et al. 2006). Our work goes one step further, with the use of polymeric diffusers for the continuous release of (Z)-3-HP, we have maintained the commercial tomato crop defenses activated throughout the duration of the whole experiment.

(Z)-3-HP and MeSA induce subsets of anti-herbivore defenses

Our results showed that there is a clear reprogramming of the tomato plant after exposure to (Z)-3-HP and MeSA. Though both volatiles induced defensive responses, (Z)-3-HP elicited further the production of secondary metabolites and a large number of protein inhibitors. However, less than 6% of genes up-regulated by both volatiles had GO terms related to defense (mediated by JA, SA and ET). This result suggests that only some of the relevant aspects of herbivore defense in tomato plants are elicited by (Z)-3-HP and MeSA and full plant induction might depend on the interplay between different HIPVs.

Changes in the metabolome as assessed by non-targeted LC-MS revealed a discrete but consistent activation of specialized metabolism in response to plant exposure to HIPVs. This includes the main classes of metabolites involved in plant-insect interactions (Douglas 2018). Both HIPV treatments resulted in a common metabolite signature but also in the specific activation of other metabolites. Pérez-Hedo et al. (2021) studied the volatile compounds on

tomato plants exposed to (Z)-3-HP and MeSA by means of headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography/mass spectrometry (GC-MS) and showed that both HIPVs activate the HPL branch of the lipoxygenase (LOX) pathway (including the cascade amplification of several C5 and C6 derived volatiles). Transcriptomic analysis also confirmed HPL branch activation by HIPVs. Both (Z)-3-HP and MeSA up-regulated the expression of the HPL gene. It should be noted that, despite our metabolomic analysis indicating that the LOX pathway was active upon plant exposure to HIPVs (Table S4), no significant induction of LOX genes was detected by RNAseq. This result is not surprising since up-regulation of LOX genes seems to be more dependent on wounding rather than HIPVs (Howe et al. 2000; Erb et al. 2015). The fact that exposure to both HIPVs activate HPL pathway derivates, suggest that defensive GLV emission in tomato is mediated by JA pool, but not by JA-Ile. LOX and JA-Ile pathways regulate specific aspects of herbivore resistance (Van Poecke and Dicke 2003; Wang et al. 2008; Schuman et al. 2018; Ye et al. 2019).

Our transcriptome analysis indicates that both (Z)-3-HP and Me-JA up-regulated genes were rich in genes related to phenylpropanoid biosynthesis. These could function as preformed and inducible antimicrobial compounds, as well as signal molecules, in plant–microbe interactions (Naoumkina et al. 2010) and diterpenoid biosynthesis genes, among which a gene encoding trimethyltridecatetraene (TMTT)/dimethylnonatriene (DMNT) synthase was up-regulated. TMTT and DMNT are among the most widespread volatiles produced in angiosperm plant tissues when under herbivore attack (Tholl et al. 2011) and are involved in direct aphid repellence (Bruce et al. 2008) as in attracting parasitoids and predators (Van Poecke and Dicke 2003; De Boer et al. 2004). Furthermore, increased levels of the apocarotenoid β-ionone was found on (Z)-3-HP or MeSA-exposed tomato plants (Pérez-Hedo et al. 2021). This volatile has been shown to significantly inhibit *B. tabaci* egg production and repel both *T. urticae* and the crucifer flea beetle *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae) (Cáceres et al. 2016). The accumulation of β-ionone was consistent with the up-regulation of Carotenoid

Cleavage Dioxygenase 4B (SlCCD4b, *Solyc08g075490*) in response to (Z)-3-HP or MeSA. In addition, steroidal alkaloid derivatives of α-tomatine, which have been reported as active against insect pests in Solanaceae (Weissenberg et al. 1998), were also found activated by both HIPVs. Transcriptome analysis confirms glycoalkaloids as part of the defensive response induced by (Z)-3-HP, several genes in the steroid pathway as well as Sl SSR2(Solyc02g069490), a key enzyme in the biosynthesis of toxic SGAs derived from cholesterol (Sawai et al. 2014), were up-regulated by (Z)-3-HP.

MeSA induces and extensive down regulation of the flavonoid pathway, including the first enzyme of the pathway, the chalcone synthase (CHS), which indicates a redirection of the metabolic flux from lignin pathway. In strawberry, reduced levels of *CHS* mRNA and enzymatic activity precursors of the flavonoid pathway were diverted to the phenylpropanoid pathway leading to a large increase in levels of (hydroxy) cinnamoyl glucose esters (Lunkenbein et al. 2006; Hoffmann et al. 2006), as well the production of phenylpropene volatiles such as eugenol (Hoffmann et al. 2011).

(Z)-3-HP activates the JA and JA-ile biosynthetic pathways

Changes in gene expression assessed by RNAseq and RT-PCR support the functional role of (Z)-3-HP and MeSA in anti-herbivore defense in tomato, although (Z)-3-HP seems more effective in eliciting defense responses than MeSA. Both (Z)-3-HP and MeSA induced the expression of JA and SA signaling marker genes, but plant exposure to (Z)-3-HP resulted in higher expression levels of the PR1 and PIN2 genes (Pérez-Hedo et al. 2021). Furthermore, RNAseq analysis indicated that, after 24 hours of exposure to HIPVs, a jasmonate-induced oxygenase-like (JOX-like) gene was up-regulated by both HIPVs. JOX genes are indicative of an active JA pathway, since they are involved in the JA-pathway negative feedback system that play an important role in removing the excess of JA and determine the amplitude and duration of JA responses to balance the growth–defense trade-off (Caarls et al. 2017). However, (Z)-3-HP exposure

561 additionally caused the up-regulation of the JA biosynthetic enzyme (OPCL1: OPC-8:0 CoA
562 ligase 1) and the jasmonyl-isoleucine (JAR1-like) conjugating enzyme, that releases the most
563 known JA active form (Suza and Staswick 2008; Fonseca et al. 2009). This confirms that (Z)-3-
564 HP induced both JA and Ile-JA biosynthesis in tomato more effectively than MeSA. (Z)-3-HP,
565 therefore, elicited a more effective applicable defense response in tomato than MeSA.

566 The activation of the plant defense mechanism through the JA pathway may also affect the
567 infection and multiplication of plant diseases. Recently our group examined how the
568 phytophagous behavior of mirids activates plant defense mechanisms and, through the
569 activation of the JA and SA pathways, can decrease multiplication rates of the Tomato Spotted
570 Wilt Virus (TSWV), an economically important virus in horticultural crops (Bouagga et al. 2020).
571 Furthermore, the exposure of tomato to (Z)-3-HP and (Z)-3-hexenyl butanoate induces
572 stomatal closure. This stomatal closure can significantly reduce infection rates by the bacteria
573 *Pseudomonas syringe* cv. *tomato* (López-Gresa et al. 2018). Though the effect of (Z)-3-HP
574 exposure on stomatal closure was not evaluated in our study, we did verify that the other
575 mechanisms involved in defense mechanism activation, as shown in the transcriptome results,
576 reduce the performance of economically important tomato pests, such as *T. urticae* and *T.*
577 *absoluta*.

578 **(Z)-3-HP does not incur metabolic costs**

579 In our research, no metabolic trade-offs were found with the activation of the plant defense
580 mechanism throughout the two-month duration of the bioassay. Neither growth nor weight of
581 fruit was affected by maintained activation of the plant defense mechanism during this
582 extended period (two months). Additionally, no negative effect in any agronomic parameter
583 was observed in the commercial greenhouse experiment. However, a recent study conducted
584 in tomato found a significant trade-off between defense priming and constitutive defense
585 (Zhang et al. 2020). We hypothesize that in our study, any potential physiological costs of

maintaining plant defenses activated was mitigated by the optimal growth conditions of the tomato plants both under experimental and commercial greenhouse conditions. The plants were cultivated under optimal fertilization, irrigation and photosynthetic light; therefore, no growth or developmental limitations were expected.

The added value of the combined use of a polymeric dispenser and (Z)-3-HP

The polymeric dispenser employed in the commercial greenhouse trial provided mean release rate of 12.2 mg/day of (Z)-3-HP. The polymeric dispenser, therefore, remains functional for an entire crop cycle without the need for replacement. Semiochemicals (including pheromones and allelochemicals) are increasingly being used as biorational and sustainable alternatives in Integrated Pest Management programs and part of their success relies on using the appropriate dispensing technology (Muñoz-Pallares et al. 2001). Long-life controlled release dispensers are commonly developed to apply semiochemicals, such as insect sex pheromones in mating disruption treatments (Anfora et al. 2008; Vacas et al. 2010; Knight et al. 2012). In general, the practical application of the mating disruption treatment technique requires the release of sufficient amounts of pheromone over extended periods (Witzgall et al. 2008) to treat wide areas and avoiding the regular replacement of the dispensers at high labor costs. Field testing of HIPVs has been documented (James 2005), such as the controlled release of MeSA using plastic sachets for the recruitment of beneficial insects in grapes and hops (James and Price 2004; Gadino et al. 2012). Rowen et al. (2017) showed that MeSA emitting lures could also elicit plant defenses in tomato plants. These authors demonstrated that MeSA lures could inadvertently protect against pathogens, but did not show a clear effect on herbivore pests. Here, the combined use of a polymeric dispenser and (Z)-3-HP, provides reliable and consistent results and may form the basis to develop further systems using different HIPVs in other crops.

Conclusion

This research demonstrates how the practical use of volatiles as inducers of plant defense mechanism can be a valuable tool in pest management in tomatoes. The combined use of continuously released (Z)-3-HP with a polymer dispenser has proven to be an effective and environmentally sustainable method to keep an economically important crop such as tomato defensively activated for more than 2 months. The use of volatiles as inducers of plant defenses is yet to be exploited for the management of crop pests. Our work provides new potential for sustainable pest management using plant communication networks and the manipulation of defense mechanisms in crops.

Acknowledgments

The research leading to these results was partially funded by the Spanish Ministry of Economy and Competitiveness MINECO (AGL2014-55616-C3 and RTA2017-00073-00-00) and the Conselleria d'Agricultura, Pesca i Alimentació de la Generalitat Valenciana. The authors thank Dr. Alejandro Tena (IVIA) and Alice Mockford (University of Worcester) for helpful comments on earlier versions of the manuscript and Julio Marco for letting us conduct this research in his greenhouse and for his selfless support throughout project.

628 **References**

629 Anfora G, Baldessari M, De Cristofaro A, et al (2008) Control of *Lobesia botrana* (Lepidoptera:
630 Tortricidae) by biodegradable ecodian sex pheromone dispensers. J Econ Entomol
631 101:444–450. https://doi.org/10.1093/jee/101.2.444
632 Arimura G, Ozawa R, Shimoda T, et al (2000) Herbivory-induced volatiles elicit defence genes in
633 lima bean leaves. Nature 406:512–515. https://doi.org/10.1038/35020072
634 Baldwin IT (1998) Jasmonate-induced responses are costly but benefit plants under attack in
635 native populations. Proc Natl Acad Sci U S A 95:8113–8118.
636 https://doi.org/10.1073/pnas.95.14.8113
637 Baysal O, Soylu EM, Soylu S (2003) Induction of defence-related enzymes and resistance by the
638 plant activator acibenzolar-S-methyl in tomato seedlings against bacterial canker caused
639 by *Clavibacter michiganensis* ssp. *michiganensis*. Plant Pathol 52:747–753.
640 https://doi.org/10.1111/j.1365-3059.2003.00936.x
641 Biondi A, Desneux N (2019) Special issue on *Tuta absoluta*: recent advances in management
642 methods against the background of an ongoing worldwide invasion. J Pest Sci 92:1313–
643 1315. https://doi.org/10.1007/s10340-019-01132-6
644 Birkett MA, Pickett JA (2014) Prospects of genetic engineering for robust insect resistance. Curr
645 Opin Plant Biol 19:59–67. https://doi.org/10.1016/j.pbi.2014.03.009
646 Block AK, Vaughan MM, Schmelz EA, Christensen SA (2019) Biosynthesis and function of
647 terpenoid defense compounds in maize (*Zea mays*). Planta 249:21–30.
648 https://doi.org/10.1007/s00425-018-2999-2
649 Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence
650 data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170
651 Bouagga S, Urbaneja A, Depalo L, et al (2020) Zoophytophagous predator-induced defences
652 restrict accumulation of the tomato spotted wilt virus. Pest Manag Sci 76:561–567.
653 https://doi.org/10.1002/ps.5547
654 Bouagga S, Urbaneja A, Rambla JL, et al (2018) Zoophytophagous mirids provide pest control
655 by inducing direct defences, antixenosis and attraction to parasitoids in sweet pepper
656 plants. Pest Manag Sci 74:1286–1296. https://doi.org/10.1002/ps.4838
657 Bruce TJA, Matthes MC, Chamberlain K, et al (2008) cis-Jasmone induces *Arabidopsis* genes
658 that affect the chemical ecology of multitrophic interactions with aphids and their
659 parasitoids. Proc Natl Acad Sci U S A 105:4553–4558.
660 https://doi.org/10.1073/pnas.0710305105
661 Caarls L, Elberse J, Awwanah M, et al (2017) *Arabidopsis* Jasmonate-induced oxygenases down-

662 regulate plant immunity by hydroxylation and inactivation of the hormone jasmonic acid.
663 Proc Natl Acad Sci U S A 114:6388–6393. https://doi.org/10.1073/pnas.1701101114
664 Cáceres LA, Lakshminarayan S, Yeung KKC, et al (2016) Repellent and Attractive Effects of α-, β-
665 , and Dihydro-β- Ionone to Generalist and Specialist Herbivores. J Chem Ecol 42:107–117.
666 https://doi.org/10.1007/s10886-016-0669-z
667 Calvo-Agudo M, González-Cabrera J, Picó Y, et al (2019) Neonicotinoids in excretion product of
668 phloem-feeding insects kill beneficial insects. Proc Natl Acad Sci U S A 116:16817–16822.
669 https://doi.org/10.1073/pnas.1904298116
670 Choh Y, Takabayashi J (2006) Herbivore-induced extrafloral nectar production in lima bean
671 plants enhanced by previous exposure to volatiles from infested conspecifics. J Chem Ecol
672 32:2073–2077. https://doi.org/DOI 10.1007/s10886-006-9130-z
673 De Boer JG, Posthumus MA, Dicke M (2004) Identification of volatiles that are used in
674 discrimination between plants infested with prey or nonprey herbivores by a predatory
675 mite. J Chem Ecol 30:2215–2230. https://doi.org/10.1023/B:JOEC.0000048784.79031.5e
676 Dicke M, Baldwin IT (2010) The evolutionary context for herbivore-induced plant volatiles:
677 beyond the "cry for help." Trends Plant Sci 15:167–175.
678 https://doi.org/10.1016/j.tplants.2009.12.002
679 Douglas AE (2018) Strategies for enhanced crop resistance to insect pests. Annu Rev Plant Biol
680 69:637–660. https://doi.org/10.1146/annurev-arplant-042817-040248
681 Duffey SS, Stout MJ (1996) Antinutritive and toxic components of plant defense against insects.
682 Arch Insect Biochem Physiol 32:3–37. https://doi.org/10.1002
683 Engelberth J, Alborn HT, Schmelz EA, Tumlinson JH (2004) Airborne signals prime plants against
684 insect herbivore attack. Proc Natl Acad Sci U S A 101:1781–1785.
685 https://doi.org/10.1073/pnas.0308037100
686 Erb M, Veyrat N, Robert CAM, et al (2015) Indole is an essential herbivore-induced volatile
687 priming signal in maize. Nat Commun 6:6273. https://doi.org/10.1038/ncomms7273
688 Farag MA, Pare PW (2002) C6-green leaf volatiles trigger local and systemic VOC emissions in
689 tomato. Phytochemistry 61:545–554. https://doi.org/10.1016/S0031-9422(02)00240-6
690 Fonseca S, Chini A, Hamberg M, et al (2009) (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous
691 bioactive jasmonate. Nat Chem Biol 5:344–350. https://doi.org/10.1038/nchembio.161
692 Frost CJ, Mescher MC, Carlson JE, De Moraes CM (2008) Plant defense priming against
693 herbivores: Getting ready for a different battle. Plant Physiol 146:818–824.
694 https://doi.org/10.1104/pp.107.113027
695 Gadino AN, Walton VM, Lee Jc (2012) Evaluation of methyl salicylate lures on populations of
696 *Typhlodromus pyri* (Acari: Phytoseiidae) and other natural enemies in western Oregon

697 vineyards. Biol Control 63:48-55. https://doi.org/10.1016/j.biocontrol.2012.06.006
698 Gika HG, Theodoridis GA, Vrhovsek U, Mattivi F (2012) Quantitative profiling of polar primary
699 metabolites using hydrophilic interaction ultrahigh performance liquid chromatography–
700 tandem mass spectrometry. J Chromatogr A 1259:121–127.
701 https://doi.org/10.1016/j.chroma.2012.02.010
702 Heil M, Silva Bueno JC (2007) Within-plant signaling by volatiles leads to induction and priming
703 of an indirect plant defense in nature. Proc Natl Acad Sci U S A 104:5467–5472.
704 https://doi.org/10.1073/pnas.0610266104
705 Heil M, Ton J (2008) Long-distance signalling in plant defence. Trends Plant Sci 13:264–272.
706 https://doi.org/10.1016/j.tplants.2008.03.005
707 Hoffmann T, Kalinowski G, Schwab W (2006) RNAi-induced silencing of gene expression in
708 strawberry fruit (*Fragaria* × *ananassa*) by agroinfiltration: a rapid assay for gene function
709 analysis. Plant J 48:818–826. https://doi.org/10.1111/j.1365-313X.2006.02913.x
710 Hoffmann T, Kurtzer R, Skowranek K, et al (2011) Metabolic engineering in strawberry fruit
711 uncovers a dormant biosynthetic pathway. Metab Eng 13:527–531.
712 https://doi.org/10.1016/j.ymben.2011.06.002
713 Howe GA, Lee GI, Itoh A, et al (2000) Cytochrome P450-dependent metabolism of Oxylipins in
714 tomato. cloning and expression of allene oxide synthase and fatty acid hydroperoxide
715 lyase. Plant Physiol 123:711–724. https://doi.org/10.1104/pp.123.2.711
716 Hussain M, Debnath B, Qasim M, et al (2019) Role of saponins in plant defense against
717 specialist herbivores. Molecules 24:2067. https://doi.org/10.3390/molecules24112067
718 James DG (2003) Synthetic Herbivore-Induced Plant Volatiles as Field Attractants for Beneficial
719 Insects. Environ Entomol 32:977–982. https://doi.org/10.1603/0046-225X-32.5.977
720 James DG (2005) Further Field Evaluation Of Synthetic Herbivore-Induced Plan Volatiles As
721 Attractants For Beneficial Insects. J Chem Ecol 31:481-495.
722 https://doi.org/10.1007/s10886-005-2020-y
723 James DG, Price TS (2004) Field-testing of methyl salicylate for recruitment and retention of
724 beneficial insects in grapes and hops. J Chem Ecol 30:1613–1628.
725 https://doi.org/10.1023/B:JOEC.0000042072.18151.6f
726 Johnson AC, Jin X, Nakada N, Sumpter JP (2020) Learning from the past and considering the
727 future of chemicals in the environment. Science 367:384–387.
728 https://doi.org/10.1126/science.aay6637
729 Kessler A (2001) Defensive Function of Herbivore-Induced Plant Volatile Emissions in Nature.
730 Science 291:2141–2144. https://doi.org/10.1126/science.291.5511.2141
731 Knight A, Light D, Chebny V (2012) Evaluating dispensers loaded with codlemone and pear

732 ester for disruption of codling moth (Lepidoptera: Tortricidae). Environ Entomol 41:399–
733 406. https://doi.org/10.1603/EN11309
734 López-Gresa MP, Payá C, Ozáez M, et al (2018) A new role for green leaf volatile esters in
735 tomato stomatal defense against *Pseudomonas syringe pv. tomato*. Front Plant Sci 9:1–
736 12. https://doi.org/10.3389/fpls.2018.01855
737 Lunkenbein S, Coiner H, De Vos CHR, et al (2006) Molecular characterization of a stable
738 antisense chalcone synthase phenotype in strawberry (*Fragaria* x *ananassa*). J Agric Food
739 Chem 54:2145–2153. https://doi.org/10.1021/jf052574z
740 Martinez-Medina A, Flors V, Heil M, et al (2016) Recognizing Plant Defense Priming. Trends
741 Plant Sci 21:818–822. https://doi.org/10.1016/j.tplants.2016.07.009
742 Mokany K, Ferrier S, Harwood TD, et al (2020) Reconciling global priorities for conserving
743 biodiversity habitat. Proc Natl Acad Sci U S A 117:9906–9911.
744 https://doi.org/10.1073/pnas.1918373117
745 Muñoz-Pallares J, Corma A, Primo J, Primo-Yufera E (2001) Zeolites as pheromone dispensers. J
746 Agric Food Chem 49:4801–4807. https://doi.org/10.1021/jf010223o
747 Naoumkina MA, Zhao Q, Gallego-Giraldo L, et al (2010) Genome-wide analysis of
748 phenylpropanoid defence pathways. Mol Plant Pathol 11:829–846.
749 https://doi.org/10.1111/j.1364-3703.2010.00648.x
750 Naselli M, Urbaneja A, Siscaro G, et al (2016) Stage-related defense response induction in
751 tomato plants by *Nesidiocoris tenuis*. Int J Mol Sci 17:1210.
752 https://doi.org/10.3390/ijms17081210
753 Nicolopoulou-Stamati P, Maipas S, Kotampasi C, et al (2016) Chemical pesticides and human
754 health: the urgent need for a new concept in agriculture. Front Public Heal 4:1–8.
755 https://doi.org/10.3389/fpubh.2016.00148
756 Pare PW, Tumlinson JH (1997) Induced synthesis of plant volatiles. Nature 385:30–31.
757 https://doi.org/Doi 10.1038/385030a0
758 Pare PW, Tumlinson JH (1999) Plant Volatiles as a Defense against Insect Herbivores by
759 releasing greater amounts of a variety. Plant Physiol 121:325–331
760 Perez-Fons L, Bohorquez-Chaux A, Irigoyen ML, et al (2019) A metabolomics characterisation of
761 natural variation in the resistance of cassava to whitefly. BMC Plant Biol 19:518.
762 https://doi.org/10.1186/s12870-019-2107-1
763 Pérez-Hedo M, Alonso-Valiente M, Vacas S, et al (2021) Eliciting tomato plant defenses by
764 exposure to HIPVs. Entomol Gen Under review
765 Pérez-Hedo M, Arias-Sanguino ÁM, Urbaneja A (2018a) Induced tomato plant resistance
766 against *Tetranychus urticae* triggered by the phytophagy of *Nesidiocoris tenuis*. Front

767 Plant Sci 9:1419. https://doi.org/10.3389/fpls.2018.01419
768 Pérez-Hedo M, Rambla JL, Granell A, Urbaneja A (2018b) Biological activity and specificity of
769 Miridae-induced plant volatiles. BioControl 63:203–213. https://doi.org/10.1007/s10526-
770 017-9854-4
771 Pérez-Hedo M, Riahi C, Urbaneja A (2020) Use of zoophytophagous mirid bugs in horticultural
772 crops: current challenges and future perspectives. Pest Manag Sci, In press: ps.6043.
773 https://doi.org/10.1002/ps.6043
774 Pérez-Hedo M, Suay R, Alonso M, et al (2017) Resilience and robustness of IPM in protected
775 horticulture in the face of potential invasive pests. Crop Prot 97:119–127.
776 https://doi.org/10.1016/j.cropro.2016.11.001
777 Pérez-Hedo M, Urbaneja-Bernat P, Jaques JA, et al (2015) Defensive plant responses induced
778 by *Nesidiocoris tenuis* (Hemiptera: Miridae) on tomato plants. J Pest Sci 88:543–554.
779 https://doi.org/10.1007/s10340-014-0640-0
780 Pertea M, Kim D, Pertea GM, et al (2016) RNA-seq experiments with HISAT, StringTie and
781 Ballgown. Nat Protoc 11:1650–1667. https://doi.org/10.1038/nprot.2016-095
782 Pertea M, Pertea GM, Antonescu CM, et al (2015) StringTie enables improved reconstruction
783 of a transcriptome from RNA-seq reads. Nat Biotechnol 33:290–295.
784 https://doi.org/10.1038/nbt.3122
785 Pickett JA, Woodcock CM, Midega CAO, Khan ZR (2014) Push–pull farming systems. Curr Opin
786 Biotechnol 26:125–132. https://doi.org/10.1016/j.copbio.2013.12.006
787 Poore J, Nemecek T (2018) Reducing food's environmental impacts through producers and
788 consumers. Science 360:987–992. https://doi.org/10.1126/science.aaq0216
789 Pretty J (2018) Intensification for redesigned and sustainable agricultural systems. Science
790 362:eaav0294. https://doi.org/10.1126/science.aav0294
791 Research and Markets (2020) World - Tomato - Market Analysis, Forecast, Size, Trends and
792 Insights. URL: https://www.researchandmarkets.com/reports/4701312/world-tomato-
793 market-analysis-forecast-size.
794 https://www.researchandmarkets.com/reports/4701312/world-tomato-market-analysis-
795 forecast-size. Accessed 20 September 2020
796 Rowen E, Gutensohn M, Dudareva N, Kaplan I (2017) Carnivore Attractant or Plant Elicitor?
797 Multifunctional Roles of Methyl Salicylate Lures in Tomato Defense. J Chem Ecol 43:573–
798 585. https://doi.org/10.1007/s10886-017-0856-6
799 Sawai S, Ohyama K, Yasumoto S, et al (2014) Sterol side chain reductase 2 is a key enzyme in
800 the biosynthesis of cholesterol, the common precursor of toxic steroidal glycoalkaloids in
801 potato. Plant Cell 26:3763–3774. https://doi.org/10.1105/tpc.114.130096

802 Schuman MC, Meldau S, Gaquerel E, et al (2018) The active Jasmonate JA-Ile regulates a
803 specific subset of plant Jasmonate-mediated resistance to herbivores in nature. Front
804 Plant Sci 9:787. https://doi.org/10.3389/fpls.2018.00787
805 Suza WP, Staswick PE (2008) The role of JAR1 in Jasmonoyl-l-isoleucine production during
806 *Arabidopsis* wound response. Planta 227:1221–1232. https://doi.org/10.1007/s00425-
807 008-0694-4
808 Tholl D, Sohrabi R, Huh J-H, Lee S (2011) The biochemistry of homoterpenes – Common
809 constituents of floral and herbivore-induced plant volatile bouquets. Phytochemistry
810 72:1635–1646. https://doi.org/10.1016/j.phytochem.2011.01.019
811 Ton J, D'Alessandro M, Jourdie V, et al (2006) Priming by airborne signals boosts direct and
812 indirect resistance in maize. Plant J 49:16–26. https://doi.org/10.1111/j.1365-
813 313X.2006.02935.x
814 Tortell PD (2020) Earth 2020: Science, society, and sustainability in the Anthropocene. Proc
815 Natl Acad Sci U S A 117:8683–8691. https://doi.org/10.1073/pnas.2001919117
816 Tumlinson JH, Pare PW, Lewis WJ (1999) Plant production of volatile semiochemicals in
817 response to insect-derived elicitors. In: Novartis Foundation Symposium. Wiley Online
818 Library, pp 95–104
819 Turlings TCJ, Erb M (2018) Tritrophic Interactions Mediated by Herbivore-Induced Plant
820 Volatiles: Mechanisms, Ecological Relevance, and Application Potential. Annu Rev
821 Entomol 63:433–452. https://doi.org/10.1146/annurev-ento-020117-043507
822 UE (2009) Directive 2009/128/EC of the European Parliament and of the Council of 21 October
823 2009 establishing a framework for Community action to achieve the sustainable use of
824 pesticides. Off J Eur Union L309:71–86
825 Uefune M, Kugimiya S, Sano K, Takabayashi J (2012) Herbivore-induced plant volatiles enhance
826 the ability of parasitic wasps to find hosts on a plant. J Appl Entomol 136:133–138.
827 https://doi.org/10.1111/j.1439-0418.2011.01621.x
828 Urbaneja-Bernat P, Mollá O, Alonso M, et al (2015) Sugars as complementary alternative food
829 for the establishment of *Nesidiocoris tenuis* in greenhouse tomato. J Appl Entomol
830 139:161–167. https://doi.org/10.1111/jen.12151
831 Vacas S, Alfaro C, Navarro-Llopis V, Primo J (2010) Mating disruption of California red scale,
832 *Aonidiella aurantii* Maskell (Homoptera: Diaspididae), using biodegradable mesoporous
833 pheromone dispensers. Pest Manag Sci 66:745–751. https://doi.org/10.1002/ps.1937
834 van Hulten M, Pelser M, van Loon LC, et al (2006) Costs and benefits of priming for defense in
835 *Arabidopsis*. Proc Natl Acad Sci U S A 103:5602–5607.
836 https://doi.org/10.1073/pnas.0510213103

Van Poecke RMP, Dicke M (2003) Signal transduction downstream of salicylic and jasmonic acid in herbivory-induced parasitoid attraction by *Arabidopsis* is independent of JAR1 and NPR1. Plant, Cell Environ 26:1541–1548. https://doi.org/10.1046/j.1365-3040.2003.01078.x

Wang L, Allmann S, Wu J, Baldwin IT (2008) Comparisons of Lipoxygenase3- and Jasmonate-resistant4/6-silenced plants reveal that jasmonic acid and jasmonic acid-amino acid conjugates play different roles in herbivore resistance of *Nicotiana attenuata*. Plant Physiol 146:904–915. https://doi.org/10.1104/pp.107.109264

War AR, Paulraj MG, Ahmad T, et al (2012) Mechanisms of plant defense against insect herbivores. Plant Signal Behav 7:1306–1320. https://doi.org/10.4161/psb.21663

Weissenberg M, Levy A, Svoboda JA, Ishaaya I (1998) The effect of some *Solanum* steroidal alkaloids and glycoalkaloids on larvae of the red flour beetle, *Tribolium castaneum*, and the tobacco hornworm, *Manduca sexta*. Phytochemistry 47:203–209. https://doi.org/10.1016/S0031-9422(97)00565-7

Witzgall P, Stelinski L, Gut L, Thomson D (2008) Codling moth management and chemical ecology. Annu Rev Entomol 53:503–522. https://doi.org/10.1146/annurev.ento.53.103106.093323

Ye M, Glauser G, Lou Y, et al (2019) Molecular dissection of early defense signaling underlying volatile-mediated defense regulation and herbivore resistance in rice. Plant Cell 31:687–698. https://doi.org/10.1105/tpc.18.00569

Zhang NX, Messelink GJ, Alba JM, et al (2018) Phytophagy of omnivorous predator *Macrolophus pygmaeus* affects performance of herbivores through induced plant defences. Oecologia 186:101–113. https://doi.org/10.1007/s00442-017-4000-7

Zhang NX, van Wieringen D, Messelink GJ, Janssen A (2019) Herbivores avoid host plants previously exposed to their omnivorous predator *Macrolophus pygmaeus* . J Pest Sci 92:737–745. https://doi.org/10.1007/s10340-018-1036-3

Zhang P, Zhao C, Ye Z, Yu X (2020) Trade-off between defense priming by herbivore-induced plant volatiles and constitutive defense in tomato. Pest Manag Sci 76:1893–1901. https://doi.org/10.1002/ps.5720

Züst T, Agrawal AA (2016) Mechanisms and evolution of plant resistance to aphids. Nat Plants 2:15206. https://doi.org/10.1038/nplants.2015.206

Table 1. Heatmap showing the results of the enrichment analysis for each KEGG pathway term in genes up and down regulated in plants experiencing exposure to (Z)-3-hexeny propanoate [(Z)-3-HP] and methyl salicylate (MeSA). The gradient legend shows the level of enrichment raw p-value from the modified fisher's exact test to determine the enrichment of each gene from the gene set. The raw p-value lower than 0.001 means that the pathway has been significantly enriched.

Pathway map name	MapName	Pvalue (Z)-3-HP UP	Pvalue MeSA UP	Pvalue (Z)-3-HP DOWN	Pvalue MeSA DOWN
General maps	Metabolic pathways	1.03E-27	1.53E-22	4.52E-12	7.22E-19
	Biosynthesis of secondary metabolites	1.20E-25	1.21E-24	6.58E-08	3.79E-15
	Carbon metabolism			7.04E-06	6.57E-05
Biosynthesis of other secondary metabolite	Flavonoid biosynthesis				4.37E-08
	Phenylpropanoid biosynthesis	2.07E-04	3.55E-04		
	Photosynthesis - antenna proteins	2.04E-16	6.15E-12		
Lipid metabolism	Steroid biosynthesis	4.50E-07			
Metabolism of cofactors and vitamins	Porphyrin and chlorophyll metabolism	4.72E-05	1.29E-05		
Metabolism of terpenoids and polyketides	Carotenoid biosynthesis	4.56E-04			
	Diterpenoid biosynthesis	3.33E-04	1.26E-04		
Folding, sorting and degradation	Protein processing in endoplasmic reticulum		1.81E-04		
Translation	Ribosome			2.82E-05	
	Ribosome biogenesis in eukaryotes			4.45E-04	
Signal transduction	MAPK signaling pathway - plant	6.39E-04			
	Plant hormone signal transduction	2.28E-09	**1.81E-04**		
Environmental adaptation	Circadian rhythm - plant				4.94E-05
	Plant-pathogen interaction	8.12E-04			

878 **Figure Legends**

879 **Fig. 1** Tomato transcriptome in response to HIPVs exposure. (A) Differentially expressed genes
880 in response to (Z)-3-hexeny propanoate [(Z)-3-HP] and methyl salycilate (MeSA). Bars depicted
881 the number of up and down regulated genes based on fold change and p-value of comparison
882 pair. (B) Veen diagram indicating common and specific regulated genes.

883

884 **Fig. 2** Number (mean ± SE) of *Tetranychus urticae* females per tomato plant when comparing
885 the mite development on tomato plants exposed to (Z)-3-hexenyl propanoate [(Z)-3-HP] and
886 methyl salicylate [MeSA] compared to untreated tomato plants (Control). Both HIPVs were
887 tested in two types of bioassays, (24 h) and (Per): in the first type the plants were only exposed
888 for 24 hours to the volatile prior to *T. urticae* release (24 h); in the other type of trial the plants
889 were permanently exposed to the volatile throughout the entire length of the experiment
890 (Per). Bars with different letters are significantly different (GLMM, repeated measures $\alpha < 0.05$)
891 (2**A**). Number of eggs (mean ± SE) laid by 2 *Tuta absoluta* females during 72 hours in plants
892 exposed to (Z)-3-hexenyl propanoate [(Z)-3-HP] (2**B**) and methyl salicylate [MeSA] (2**C**) in
893 comparison to unexposed tomato plants (Control). Bars with different letters are significantly
894 different (ANOVA, Tukey $\alpha < 0.05$). Percentage mortality (mean ± SE) of *T. absoluta* from egg to
895 adult when raised on tomato plants exposed to (Z)-3-hexenyl propanoate [(Z)-3-HP] (2**D**) and
896 methyl salicylate [MeSA] (2**E**) in comparison to untreated tomato plants (Control). Bars with
897 different letters are significantly different (ANOVA, Tukey $\alpha < 0.05$).

898

899 **Fig. 3** Transcriptional response of the defensive genes PR1 (a marker gene for the SA signaling
900 pathway) (3**A**), PIN2 (a marker gene for the JA signaling pathway) (3**B**) and *Sl*-PI-I (a marker for
901 plant Proteinase Inhibitor) (3**C**) in tomato plants exposed to (Z)-3-hexenyl propanoate [(Z)-3-
902 HP] released by a polymeric dispenser and control plants at 24 hours before and 1, 4 and 8

903 weeks after the establishment of the dispensers. Asterisks indicate significant differences
904 between both treatments ($P < 0.05$). Height (cm) of the plants exposed to both treatments
905 throughout the duration of the experiment (3**D**) and total harvest (g) obtained per plant in the
906 trade-offs experiment (3**E**). Release profile of the dispenser employed fitted the linear
907 regression model depicted ($R^2 = 0.996$) (3**F**).

908

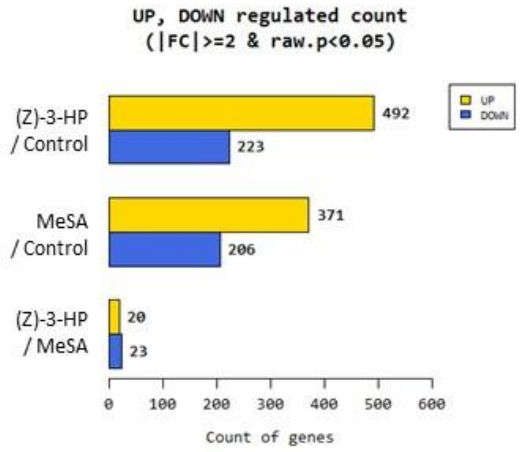
909 **Fig. 4** Transcriptional response of the defensive genes *PR1* (a marker gene for the SA signaling
910 pathway) (4**A**) and *PIN2* (a marker gene for the JA signaling pathway) (4**B**) of tomato plants
911 exposed to (Z)-3-hexenyl propanoate [(Z)-3-HP] released by polymeric dispensers and control
912 plants, 24 hours before and 4 and 8 weeks after the establishment of the dispensers in the
913 greenhouses. Asterisks indicate significant differences between both treatments ($P < 0.05$).
914 Number of *Nesidiocoris tenuis* per plant (mean ± SE) (4**C**) and number of infested leaflets per
915 plant (4**C**) (mean ± SE) in the greenhouse (GLMM, $P < 0.05$). Release profile of the dispenser
916 employed fitted the linear regression model depicted ($R^2 = 0.993$) (4**E**).

917

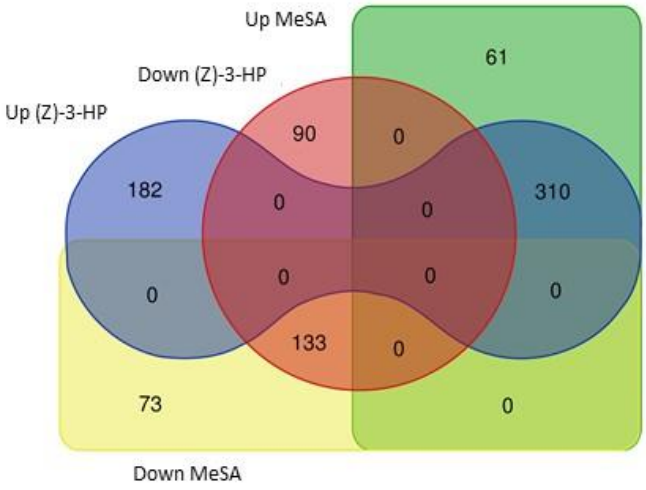
918

919 **Fig. 1**

A

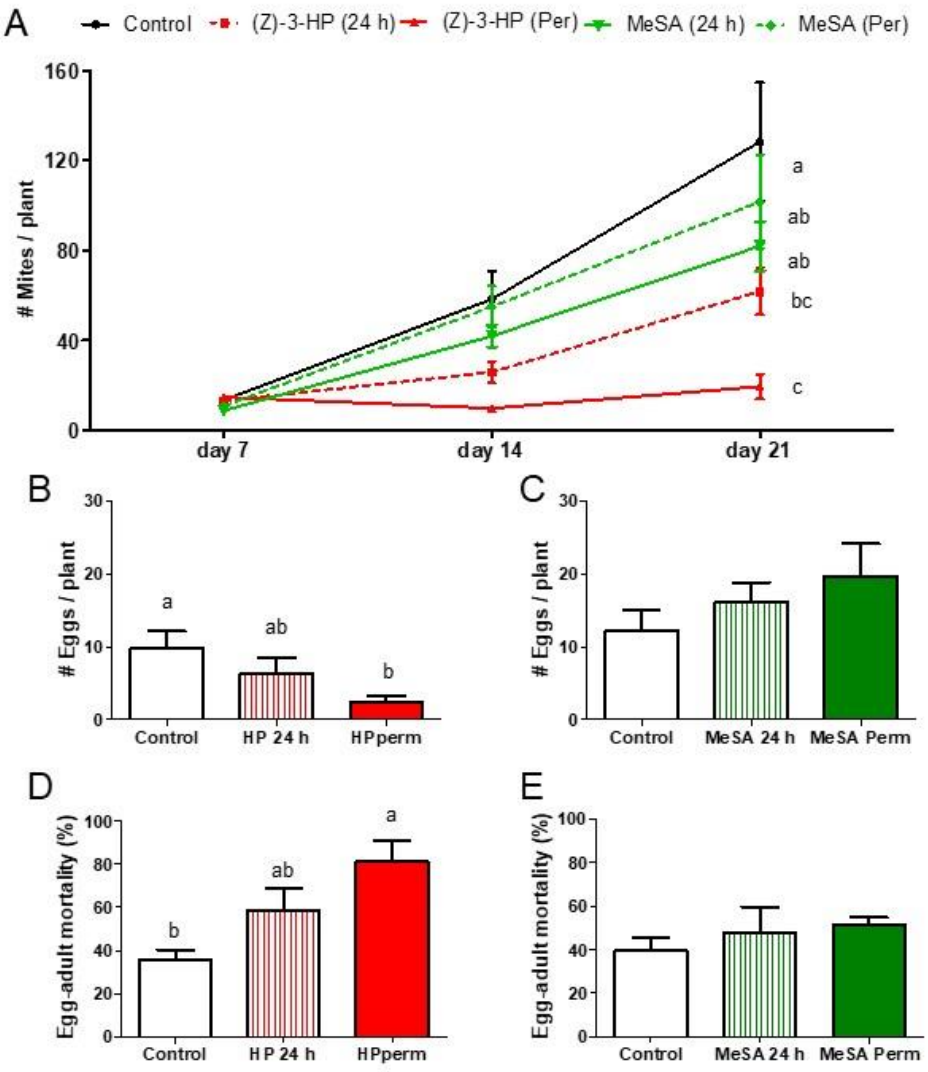


B



920

921 **Fig. 2**

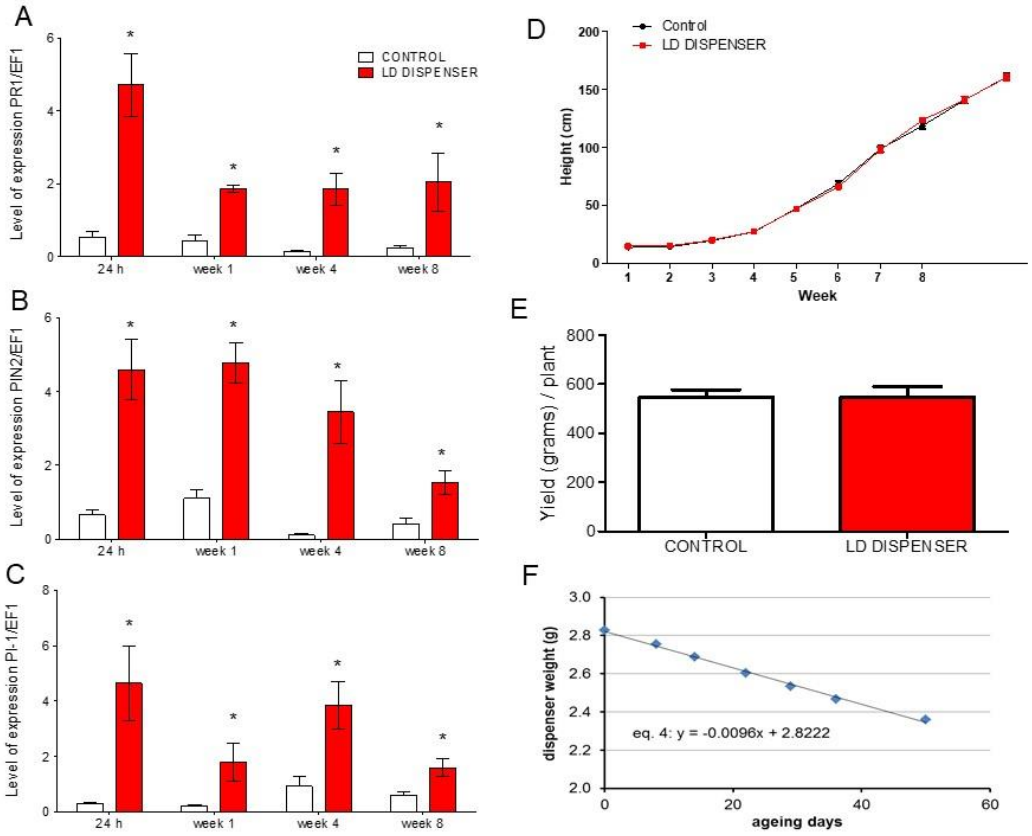


922

923

924

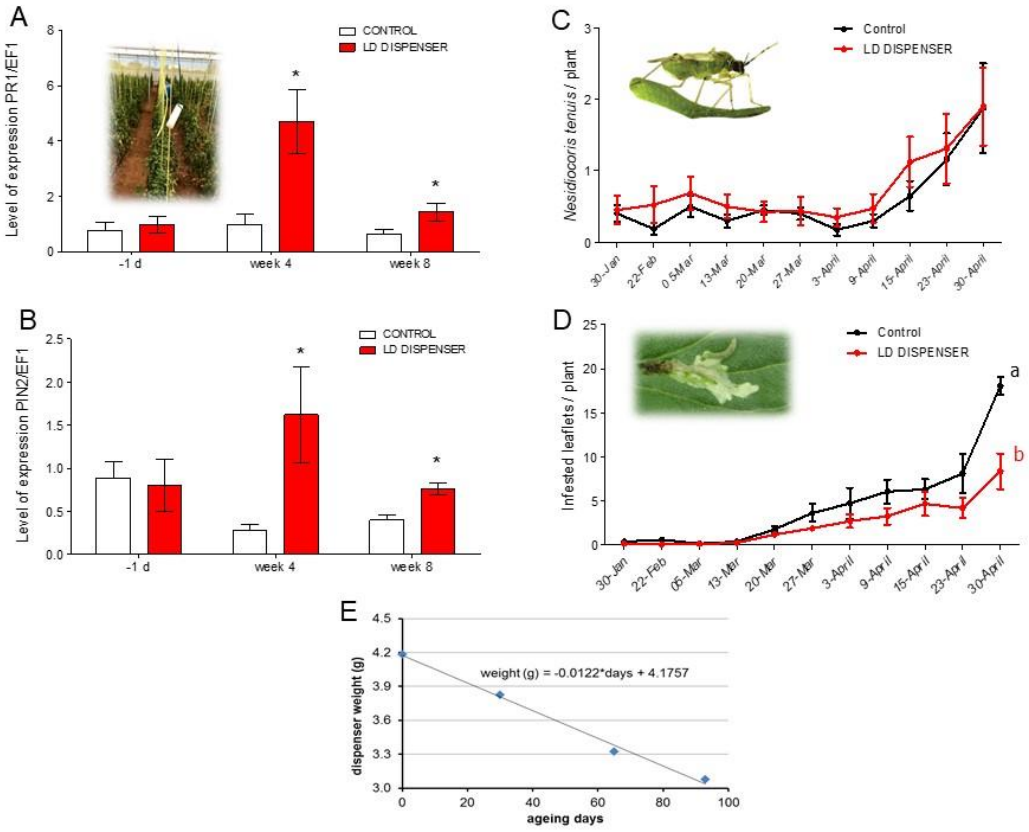
925 **Fig. 3**



926

927

928 **Fig. 4**



929

930

931

932