Biological activity and specificity of Miridae-induced plant volatiles

Abstract The ability of zoophytophagous predators to produce defensive plant responses due to their phytophagous behavior has been recently demonstrated. In the case of tomatoes, the mirids *Nesidiocoris tenuis* and *Macrolophus pygmaeus* are able to attract or repel pests and/or natural enemies in different ways. Nevertheless, the herbivore-induced plant volatiles (HIPVs) released by the phytophagy of both mirids, which are responsible for these behaviors, are unknown. In this work, the HIPVs produced by the plant feeding of *N. tenuis* and *M. pygmaeus* were characterized. In addition, the role of each HIPV in the repellence or attraction of two tomato pests, *Bemisia tabaci* and *Tuta absoluta*, and of the natural enemy *Encarsia formosa* was evaluated. Six green leaf volatiles (GLVs) plus methyl salicylate and octyl acetate clearly stood out as major differential peaks on the chromatogram in a directed analysis. The six GLV and methyl salicylate were repellent for *B. tabaci* and attractive to *E. formosa*, whereas they showed no effect on *T. absoluta*. Octyl acetate, which was significantly present only in the *M. pygmaeus*-punctured plants, was significantly attractive to *T. absoluta*, repellent to *E. formosa* and indifferent to *B. tabaci*. Unlike the remaining HIPVs, octyl acetate was emitted directly by *M. pygmaeus* and not by the plant. Our results showed that mirid herbivory could modulate the pest and natural plant enemy locations, since tomato plants release a blend of volatiles in response to this activity. These results could serve as a basis for future development of plant protection.

Key words: plant response, herbivore-induced plant volatiles, mirid bugs.

Introduction

Within the large insect family Miridae, members of the Dicyphini tribe are generalist predators well-known for their polyphagy on herbivorous pests such as whiteflies, leafminers, aphids, thrips, mites and lepidopterans (Barnadas et al. 1998; Urbaneja et al. 2009, 2012; Abbas et al. 2014). Dicyphini are also characterized by their zoophytophagous behavior, which means that they can feed on plants and prey during the same developmental stage (Castañé el al. 2011). Zoophytophagy is a positive feature for natural enemies because these predators can survive in a crop even when prey is scarce or totally absent (Eubanks and Denno 1999; Sanchez et al. 2004; Urbaneja et al. 2005), since the plant can provide them with water (essential for predation) and nutrients (Gillespie and McGregor 2000; Sinia et al. 2004). Herbivory of some mirid species may result in injuries to the vegetative and reproductive parts of the plant, causing even yield loss (Calvo et al. 2009; Sanchez 2009; Castañé et al. 2011; Biondi et al. 2016). Nevertheless, the capacity of mirids to induce plant damage varies among species (Castañé el al. 2011). During recent decades, zoophytophagous mirid bugs received special attention due to their increasing role in the biological control of important agricultural pests (Arnó et al. 2010; Perdikis et al. 2011; Pérez-Hedo and Urbaneja 2015; Messelink et al. 2015; Naselli et al. 2016). Among them, *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae) are probably the mirid species that are most used in biological control programs since both are mass-reared and recommended to be released and/or conserved in several successful integrated pest management (IPM) programs (Calvo et al. 2012; Urbaneja et al. 2012; Zappalà et al. 2012; Zappalà et al. 2013; Perez-Hedo and Urbaneja 2015; Perez-Hedo and Urbaneja 2016; Pérez-Hedo et al. 2017; Lenteren et al. 2017).

It is widely known that the attack of a plant by herbivorous arthropods induces the release of semiochemicals called herbivore-induced plant volatiles (HIPVs) (Turlings et al. 1990; Paré

and Tumlinson 1999), most of which are terpenoids, fatty acid derivatives, phenylpropanoids and benzenoids (Dudareva et al. 2004). These volatiles are qualitatively and quantitatively different among different herbivore species (Dicke 2009), which can attract natural enemies, repel herbivores and alert neighboring plants (priming) (Sabelis et al. 1999; Paré and Tumlinson 1999; Frost 2008). Tritrophic interactions in plant defense (plants, herbivores and natural enemies) are important to understanding both the evolution of such interactions and improving biological control (Sabelis et al. 1999). Previous works demonstrated that the plant-feeding activities of *N. tenuis* and *M. pygmaeus* could differentially induce plant responses in tomato (Pérez-Hedo et al. 2015a,b; Naselli et al. 2016). While the phytophagy activity of *N. tenuis* resulted in a non-preference effect on two key tomato pests, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), plants punctured by *M. pygmaeus* did not repel *B. tabaci* and curiously resulted in an attraction to *T. absoluta*, although both mirid predators induced the attraction of the whitefly parasitoid *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) (Pérez-Hedo et al. 2015b). Pappas et al. (2015) obtained similar results for *M. pygmaeus*, which induced defensive responses in tomato plants against the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). These plants, when previously exposed to *M. pygmaeus*, reduced the performance of a subsequently infesting herbivore. Such defensive responses activated by mirid plant feeding seemed to be activated through the upregulation of the phytohormone pathways of abscisic acid (ABA) and jasmonic acid (JA) (Pérez-Hedo et al. 2015 a,b; Naselli et al. 2016). However, which volatiles are elicited through these pathways' upregulation and their roles in the repellence or attraction of herbivores or natural enemies remains unknown. The response of plants to herbivory is very specific and makes the relation between HIPV blends and specific plant-herbivore systems, more complicate.

In this work, we characterized the volatile emissions from intact and *M. pygmaeus*- and *N. tenuis*-punctured tomato plants using a solid phase microextraction technique combined with gas chromatography-mass spectrometry, assuming that the volatile blend released is specific for a particular insect-plant system. When identified, the role of each HIPV in the repellence and/or attraction of two key tomato pests, *B. tabaci* and *T. absoluta*, and to one parasitoid, *E. formosa*, were evaluated. Because one of the identified compounds (octyl acetate) could not be assigned as HIPV, whether this compound was emitted directly by both mirid species was also studied.

Materials and Methods

Plants and insects

Solanum lycopersicum (cv. Optima) plants were germinated in soil, and 2 weeks after germination, the seedlings were individually transferred to pots and maintained at 25 ± 2ºC with high relative humidity (>60%) and a 16:8 h L:D photoperiod. Six-week-old plants with seven to eight fully expanded leaves were used for the experiments.

To induce the plants, we enclosed one tomato plant exposed to 20 *N. tenuis* or *M. pygmaeus* adults in a plastic cage of 60 x 60 x 60 cm (BugDorm-2; MegaView Science Co., Ltd.; Taichung, Taiwan) for 24 h prior to the assay. All mirid individuals were removed from the punctured plants before volatile collection. The intact plants were left undisturbed and isolated from arthropods until use.

B. tabaci, *E. formosa*, *N. tenuis* and *M. pygmaeus* individuals were obtained directly from the mass rearings of Koppert Biological Systems, S.L. (Águilas, Murcia, Spain), and *T. absoluta* individuals were obtained from colonies maintained at IVIA (Abbas et al., 2014). Once received at IVIA, the two mirids and the whitefly remained on tomato plants for 24 hours before their use. The parasitoids used in the assays had no previous contact with any plant or

host and were referred to as naïve. Newly emerged adult females of the six species of insects (1–5 days old) were used in all experiments. All females were presumably mated except the parasitoid *E. formosa* which is an uniparental species.

Headspace collection of volatiles

The HIPVs of the tomato plants involved in the responses to the two zoophytophagous plant bugs were collected using an olfactometer, described below, in a static condition for 3 h. Thus, the volatile compounds were captured by means of solid phase microextraction (SPME) and were separated and detected by means of gas chromatography coupled to mass spectrometry (GC/MS). Volatile compounds were adsorbed in a 65-µm PDMS/DVB SPME fiber (polydimethylsiloxane/divinylbenzene; Supelco, Bellefonte, PA, USA). The adsorbent-coated fiber was mounted on an SPME fiber holder and injected through the first septum of the sample container (glass jars of 5 l volume). Agitation of the atmosphere inside the container was achieved by pumping at a rate of 5 ml/min using an injecting syringe through the second septum of the sample container. In total, 11 biological replicates were sampled per treatment (intact plants, *M. pygmaeus*-punctured plants and *N. tenuis*-punctured plants), each replicate consisting in one plant.

Desorption was performed using a CombiPAL autosampler (CTC Analytics) at 250°C over 1 min in splitless mode in the injection port of a 6890N gas chromatograph coupled to a 5975B mass spectrometer (Agilent Technologies). To prevent cross-contamination, fibers were cleaned after desorption in an SPME fiber conditioning station (CTC Analytics) at 250°C for 5 min under helium flow. Chromatography was performed on a DB-5 ms (60 m, 0.25 mm, 1.00 µm) column with helium as carrier gas at a constant flow of 1.2 ml/min. The GC interface and MS source temperatures were 260°C and 230°C, respectively. The oven programming conditions were 40°C for 2 min, 5°C/min ramp to 250°C, and a final hold at 250°C for 6 min. Data were recorded in the 35-300 m/z range at 5 scans/s, with the electronic

impact ionization set at 70 eV. Untargeted analysis of the chromatograms was performed using MetAlign software (WUR, http://www.metalign.nl).

Kovats retention indexes (KIs) were calculated for all the compounds. Differentially emitted compounds were first tentatively identified based on the comparison of their mass spectra with those in the NIST 05 Mass Spectral Library. When available, identity was confirmed by co-elution with pure standards (Sigma-Aldrich). For relative quantitation of the selected compounds, one specific ion was selected for each, and the corresponding peak area from the extracted ion chromatogram was integrated by means of the ChemStation E.02.02 software (Agilent Technologies). The criteria for ion selection were the highest signal-to-noise ratio and sufficient specificity in that particular region of the chromatogram to provide good peak integration.

Olfactory Response to HIPV

Once the HIPVs involved in the plant response were identified, the olfactory responses to them were evaluated on two herbivore pests (*B. tabaci* and *T. absoluta*) and a parasitoid (*E. formosa*) in a Y-tube olfactometer (Analytical Research Systems, Gainesville, FL) consisting of a Y shaped glass tube connected via plastic tubes to two identical 5 L glass jars, each of which contained a tested odor source and was connected to an air pump that produced a unidirectional airflow. The environmental conditions in the Y-tube experiments were 23 ± 2ºC and 60 ± 10% RH (Pérez-Hedo and Urbaneja 2015). Each female was observed until she had walked at least 3 cm up one of the side arms or until 15 min had elapsed. Females that had not walked up one of the side arms after 15 min were considered to be 'non-responders' and were excluded from the subsequent data analysis. There was no Y-tube experiment in which the number of non-responder was higher than 6. Each individual was tested only once. After testing 5 individuals, odor sources were interchanged to avoid any spatial effect on

choices. All synthetic standards of the tomato volatile compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The volatiles released into the jar consisted of a piece of paper with 10 µl to 1:10000 methanol/water (control) or 1:10000 with the volatile to test. The dilutions of 1:10000 of pure compounds were also collected on SPME fibers and analyzed using the GC/MS; the peak areas in the chromatograms were very similar to those observed in the biological samples, they did not differ from those emitted by tomato plants, and in all cases, they were of the same order of magnitude.

Octyl acetate volatile

The octyl acetate volatile was emitted only by *M. pygmaeus*-punctured plants, and traces were not detected either on intact or *N. tenuis*-punctured plants. For this reason, we wondered whether this compound was released by the tomato plant as a response to *M. pygmaeus* activity or by the mirid itself. To unveil this question, the octyl acetate was collected in SPME fibers using an olfactometer in static condition for 3 h and analyzed using the GC/MS as explained above in the following treatments: 10 couples of *M. pygmaeus*, 10 couples of *N. tenuis*, tomato plant with the presence of 10 couples of either *N. tenuis* or *M. pygmaeus* and two controls, one of them consisting of an empty jar (5 l in volume) and the other of an intact tomato plant. For the treatments with plant and mirid, the mirids were put in contact with the plant 24 h before the volatile collection was done. To analyze the octyl acetate volatile, three biological replicates were conducted when the volatile was collected on both mirids without plant, whereas four replicates were conducted for the rest of the treatments.

Data analysis

Differences in volatile compounds were subjected to one way analysis of variance, and the Tukey test was used for mean separation at $P<0.05$. The results are expressed as the means ± SE. The data for the octyl acetate volatile were analyzed using the one-tailed Student's *t*-

test (P<0.05). χ^2-Tests were used to test the hypothesis that the distribution of side-arm choices between pairs of odors deviated from the null model of odor sources being chosen with equal frequency.

Results

Composition of volatile blends

When identifying the HIPVs involved in the tomato plant responses induced by *M. pygmaeus* and *N. tenuis*, six green leaf volatiles (GLV), methyl salicylate and octyl acetate clearly stood out as major differential peaks on the chromatogram in a directed analysis (Fig. 1). In general, *N. tenuis*-punctured plants emitted more volatiles than *M. pygmaeus*-punctured plants, and the latter emitted more volatiles than did the intact plants. When analyzing the relative peak areas of the eight representative compounds, four of the volatiles were significantly emitted in the largest amount by *N. tenuis*-punctured plants, compared to *M. pygmaeus*-punctured or intact tomato plants: (Z)-3-hexenol ($F_{2,32}$= 4.57; P= 0.0184), (Z)-3-hexenyl propanoate ($F_{2,32}$= 4.059; P= 0.0269), (Z)-3-hexenyl butanoate ($F_{2,32}$= 4.022; P= 0.0277) and methyl salicylate ($F_{2,32}$= 10.17; P= 0.0004). Surprisingly, octyl acetate was present only in the *M. pygmaeus*-punctured plants, qualitatively. The amounts of 1-hexanol ($F_{2,32}$= 0.2552; P= 0.7764), (Z)-3-hexenyl acetate ($F_{2,32}$= 2.103; P= 0.1397) and hexyl butanoate ($F_{2,32}$= 0.7096; P= 0.4999) were not significantly different among treatments.

Biological activities of HIPVs induced by zoophytophagous predatory mirids

The response of the whitefly *B. tabaci* in a Y-tube olfactometer when exposed to the control and the eight synthetic HIPVs identified in the previous section is shown in Fig. 2. The whitefly showed a strong preference for the control over the jars with the volatiles 1-hexanol (χ^2_1= 20.0, P< 0.0001), (Z)-3-hexenol (χ^2_1= 57.8, P< 0.0001), (Z)-3-hexenyl acetate (χ^2_1= 9.8,

198*P*= 0.0017), (Z)-3-hexenyl propanoate (χ^2_1= 16.2, *P*< 0.0001), (Z)-3-hexenyl butanoate (χ^2_1=
1999.8, *P*= 0.0017), hexyl butanoate (χ^2_1= 51.2, *P*< 0.0001) and methyl salicylate (χ^2_1= 12.8, *P*=
2000.0003). No preference was observed between the control and octyl acetate volatile, which
201was detected only in plants punctured by *M. pygmaeus*.

202The parasitoid *E. formosa* behaved contrary to the whitefly (Fig. 3). Octyl acetate had a clear
203repellent effect to the parasitoid (χ^2_1= 33.8, *P*< 0.0001), whereas the remaining compounds, 1-
204hexanol (χ^2_1= 33.8, *P*< 0.0001), (Z)-3-hexenol (χ^2_1= 7.2, *P*= 0.0073), (Z)-3-hexenyl acetate
205(χ^2_1= 20.0, *P*< 0.0001), (Z)-3-hexenyl propanoate (χ^2_1= 39.2, *P*< 0.0001), (Z)-3-hexenyl
206butanoate (χ^2_1= 16.2, P< 0.0001), hexyl butanoate (χ^2_1= 20.0, *P*< 0.0001) and methyl
207salicylate (χ^2_1= 12.8, *P*= 0.0003) significantly attracted this parasitoid.

208The lepidopteran *T. absoluta* was significantly more attracted by octyl acetate over the control
209jar in the y-tube olfactometer (χ^2_1= 16.69, *P*< 0.0001), whereas a lack of preference was
210observed for the remaining volatiles tested, 1-hexanol (χ^2_1=0.2667, *P*= 0.6056), (Z)-3-hexenol
211(χ^2_1= 0.00, *P*= 1.00), (Z)-3-hexenyl acetate (χ^2_1=3.200, *P*= 0.0736), (Z)-3-hexenyl propanoate
212(χ^2_1= 0.2667, *P*= 0.6056), (Z)-3-hexenyl butanoate (χ^2_1= 0.00, P= 1.00), hexyl butanoate (χ^2_1=
2132.632, *P*= 0.1080) and methyl salicylate (χ^2_1= 1.800, *P*= 0.1797) (Fig. 4).

214

215*Octyl acetate volatile released by* M. pygmaeus

216As shown above, the octyl acetate volatile was emitted only by *M. pygmaeus*-punctured
217plants. To know whether this compound was released by the tomato plant as a response to *M.*
218*pygmaeus* activity or by the mirid itself, the octyl acetate emitted by the tomato plant with or
219without both mirid species or by an empty jar with or without the mirids was collected and
220analyzed. A strong significant difference was observed for the emission of octyl acetate
221between *M. pygmaeus* and *N. tenuis* individuals (t_7= 5.935, *P*= 0.020), and was 77 times
222higher for *M. pygmaeus* than for *N. tenuis* (Fig. 5a). When octyl acetate was collected and

analyzed in the treatments that had both tomato plant and mirid, this compound could be detected only in the treatment with *M. pygmaeus* (Fig. 5b).

Discussion

Plants have different strategies to protect themselves against insect attack, ranging from production of physical and chemical defenses to changes in the plant's primary and/or specialized metabolism (War et al. 2012; Zhou et al. 2015). In this complex metabolic network that determines specific responses, HIPVs play an important role in tritrophic interactions, which are crucial to understand the evolution of plant-predator mutualisms. We identified six green leaf volatiles and methyl salicylate involved in the tomato plant responses induced by *M. pygmaeus* and *N. tenuis*. The herbivory of insects always induce the production of plant volatiles (Paré and Tumlinson 1999; Leitner et al. 2005; Shiojiri 2006; Dicke 2009). One of the novelties of our work is that the insects, which are considered beneficial and widely used and are released as biocontrol agents in many agricultural crops, can also be responsible to induce these HIPVs.

Our results confirm that HIPVs induced by zoophytophagous predators are species-dependent and have a differential response on pest and natural enemies, although this fact had previously been reported for different herbivores on the same plant species (Turlings et al. 1998; Delphia et al. 2007). In particular, when analyzing the emission levels of the eight dominant compounds in a directed analysis, we observed that five compounds [(Z)-3-hexenol, (Z)-3-hexenyl propanoate, (Z)-3-hexenyl butanoate, methyl salicylate and octyl acetate] were the discriminating compounds that resulted in the difference in the volatile profile between the intact tomato plants and *N. tenuis*- and *M. pygmaeus*-punctured plants. Our work also confirms that both herbivores and natural enemies can perceive a wide range of plant volatiles that are herbivore-induced (Ardanuy et al. 2016), in our case by the plant feeding of two

zoophytophagous mirid bugs and that a single synthetic HIPV can have an effect of attraction or repellence by itself on pests and/or natural enemies (James 2005; Rodriguez-Saona et al. 2011; Ozawa et al. 2008; Giunti et al. 2017).

Attraction of insect parasitoids by volatiles emitted from damaged plants has been well documented, as plants can defend themselves against herbivores by attracting natural enemies of the herbivores (Bukovinszky 2005; Ozawa et al. 2008). However, little is known about the effects of these chemicals on the herbivores as an alternative function of HIPV, which could repel herbivores (Bernasconi et al. 1998; Kessler and Baldwin 2001; Ulland et al. 2008). Our results showed that *E. formosa* may use methyl salicylate and the six GLVs tested as an olfactory cue for host location, showing a clear attraction for the parasitoid. Contrarily, the biological assays conducted in the Y-tube olfactometer demonstrated that these HIPVs, which were attractive for *E. formosa,* had a repellent effect on the whitefly *B. tabaci*. These results should be seen in the context of different selection pressures on a plant's emission of volatiles in a multitrophic context. Understanding these selection pressures will provide insight into the roles of induced volatiles in the biology of plants (Dicke and Baldwin 2010).

Interestingly, the lepidopteran *T. absoluta* did not respond to any of the abovementioned HIPVs. However, *T. absoluta* was attracted to octyl acetate, a compound significantly identified only in *M. pygmaeus* punctured plants and in *M. pygmaeus* alone treatment. Taking these results together with those obtained by Pérez-Hedo et al. (2015b) who showed that *T. absoluta* was also attracted by tomato plants previously infested by *M. pygmaeus*, prompted the hypothesis that octyl acetate is a volatile emitted directly by *M. pygmaeus* and not by the *M. pygmaeus*-punctured tomato plant, which results in attraction due to the traces that *M. pygmaeus* leaves on the plant. Octyl acetate is known as a specific compound of some species of the Miridae family as some *Phytocoris* spp. (Millar and Rice 1998; Millar et al. 1997; Zhang and Aldrich 2008). Octyl acetate was also detected as a pheromone alert in both males

and females of the hemipteran *Leptocorisa chinensis* Dallas (Hemiptera: Alydidae), although in significantly higher amounts in females (Yamashita et al. 2016), and in the composition of the natural sting alarm pheromone components in bees (Wager and Breed 2000; Wang et al. 2016), which cause other bees to behave defensively when alarm pheromones are released at the moment that a bee stings another animal. Interestingly this compound was also detected as part of the sex pheromone of the moth, *Batrachedra amydraula* Meyrick (Lepidoptera: Batrachedridae) (Levi-Zada et al. 2013). Thus, to study ecological functions of the octyl acetate volatile, we investigated its responses on two key tomato pests, *T. absoluta* and *B. tabaci*, and on the parasitoid *E. formosa* in the olfactometer. The lack of response to this volatile by the whitefly and the parasitoid and the strong attraction of the lepidopteran to this volatile could be attributed to the specificity of this compound for *T. absoluta*. We could hypothesize from these results that this compound or one very close could be or be part of a pheromone for this lepidopteran. To date the (3E,8Z,11Z)-3,8,11-tetradecatrienyl acetate has been identified as the major sex pheromone component of *T. absoluta* (Attygalle et al. 1996). Therefore, further experiments should be conducted to clarify the role of this potential compound on *T. absoluta* management.

The obtained results might open the door to the exploitation of these volatiles in new strategies for pest management. As an example, using new mesoporous dispensers, which could emit regular concentrations of one or a mix of these volatiles, could result in saturated repellent and attractant environments for *B. tabaci* and *E. formosa*, respectively. Plant breeding programs could also be focused on obtaining plants with higher rates of emission of one of the most active HIPVs, as in the work of Kappers et al. (2005), who manipulated HIPVs through genetic engineering, which resulted in attracting the phytoseiid predator *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). Further research will address the potential benefits of inducing plant volatile emissions under field conditions as a potential

tool for enhancing populations of beneficial insects as a component of conservation biological control (James 2005; Rodriguez-Saona et al. 2011). In this respect, the possible tradeoffs that the continuous exposure to these volatiles could induce on plants might be evaluated.

In summary, our results suggest that the effectiveness attributed to predatory mirids in pest management is due not only to their zoophagy but also to their herbivory which, as demonstrated in our work, could modulate pest and natural plant enemy locations, since tomato plants release a blend of volatile compounds in response to their activity. These results could partially explain the great success achieved by the predatory mirids in recent years in tomato crops.

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Figure Legends

Fig. 1 Total emission of volatiles (mean peak area of all mass fragments + SE) emitted by intact plants (X), *M. pygmaeus*-punctured plants and *N. tenuis*-punctured plants. Different letters over the bars indicate significant differences (ANOVA and Tukey comparisons; $P<0.05$) which must be read for each graph separately.

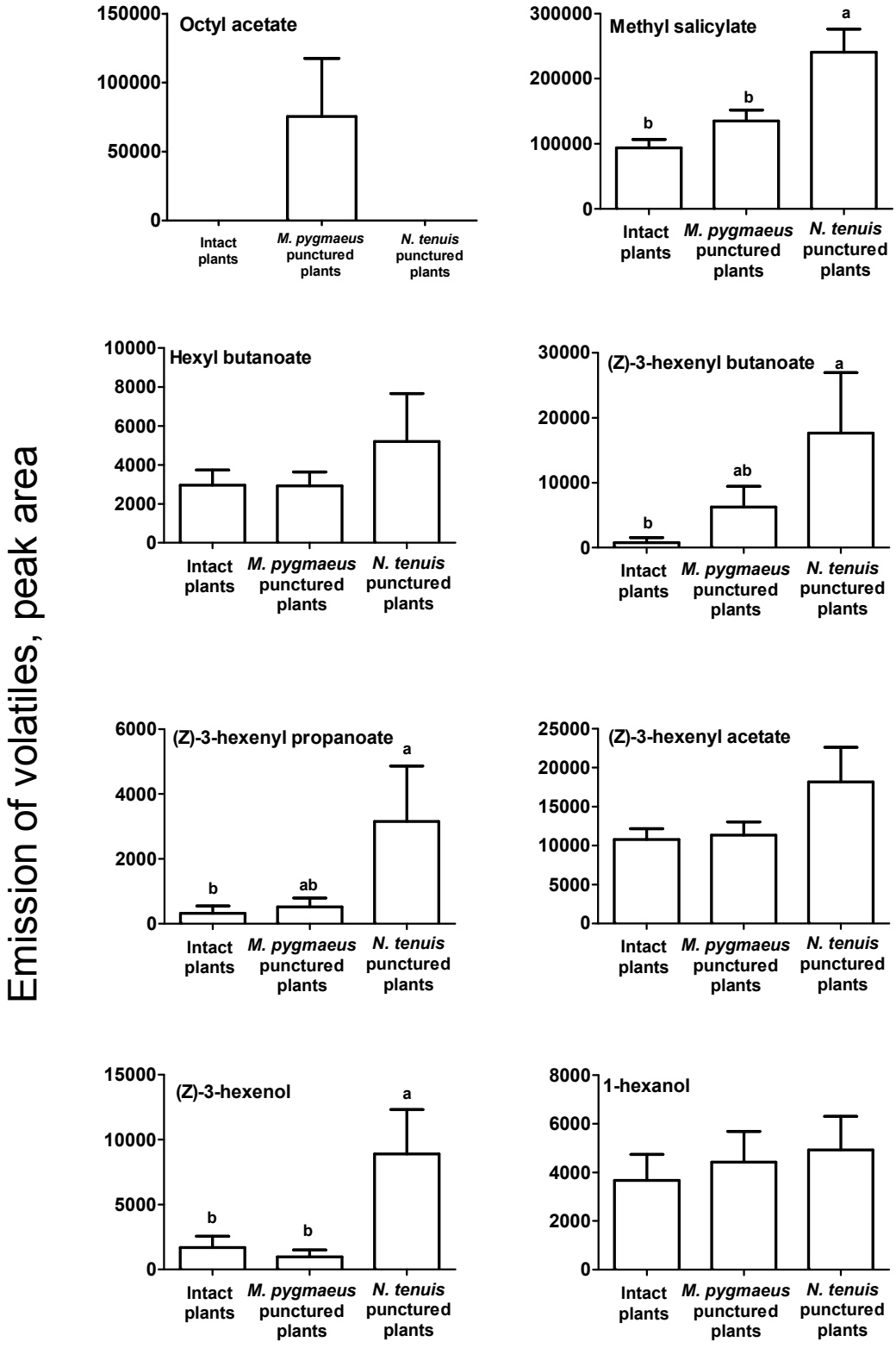
Fig. 2 Response (% + SE) of *B. tabaci* females in a Y-tube olfactometer when exposed to control (1:10000 methanol/water) and the eight synthetic HIPVs identified (1:10000 with the volatile to test). Asterisks indicate significant differences in the distribution of side-arm choices (χ^2_1-tests; $P < 0.05$).

Fig. 3 Response (% + SE) of *T. absoluta* females in a Y-tube olfactometer when exposed to control (1:10000 methanol/water) and the eight synthetic HIPVs identified (1:10000 with the volatile to test). Asterisks indicate significant differences in the distribution of side-arm choices (χ^2_1-tests; $P < 0.05$).

Fig. 4 Response (% + SE) of *E. formosa* females in a Y-tube olfactometer when exposed to control (1:10000 methanol/water) and the eight synthetic HIPVs identified (1:10000 with the volatile to test). Asterisks indicate significant differences in the distribution of side-arm choices (χ^2_1-tests; $P < 0.05$).

Fig. 5 Total emission of volatiles (mean peak area of all mass fragments + SE) emitted by a) control (empty jar), 10 pairs of *M. pygmaeus*, and 10 pairs of *N. tenuis* and b) tomato intact plant, tomato plant with the presence of 10 couples of either *N. tenuis* or *M. pygmaeus* that were put in contact with the plant 24 h before. Asterisk indicate significant differences for the total emission of octyl acetate between *M. pygmeus* and *N. tenuis* when placed alone into the jars (*t*-test; $P < 0.05$).

Fig. 1



497
498**Fig. 2**

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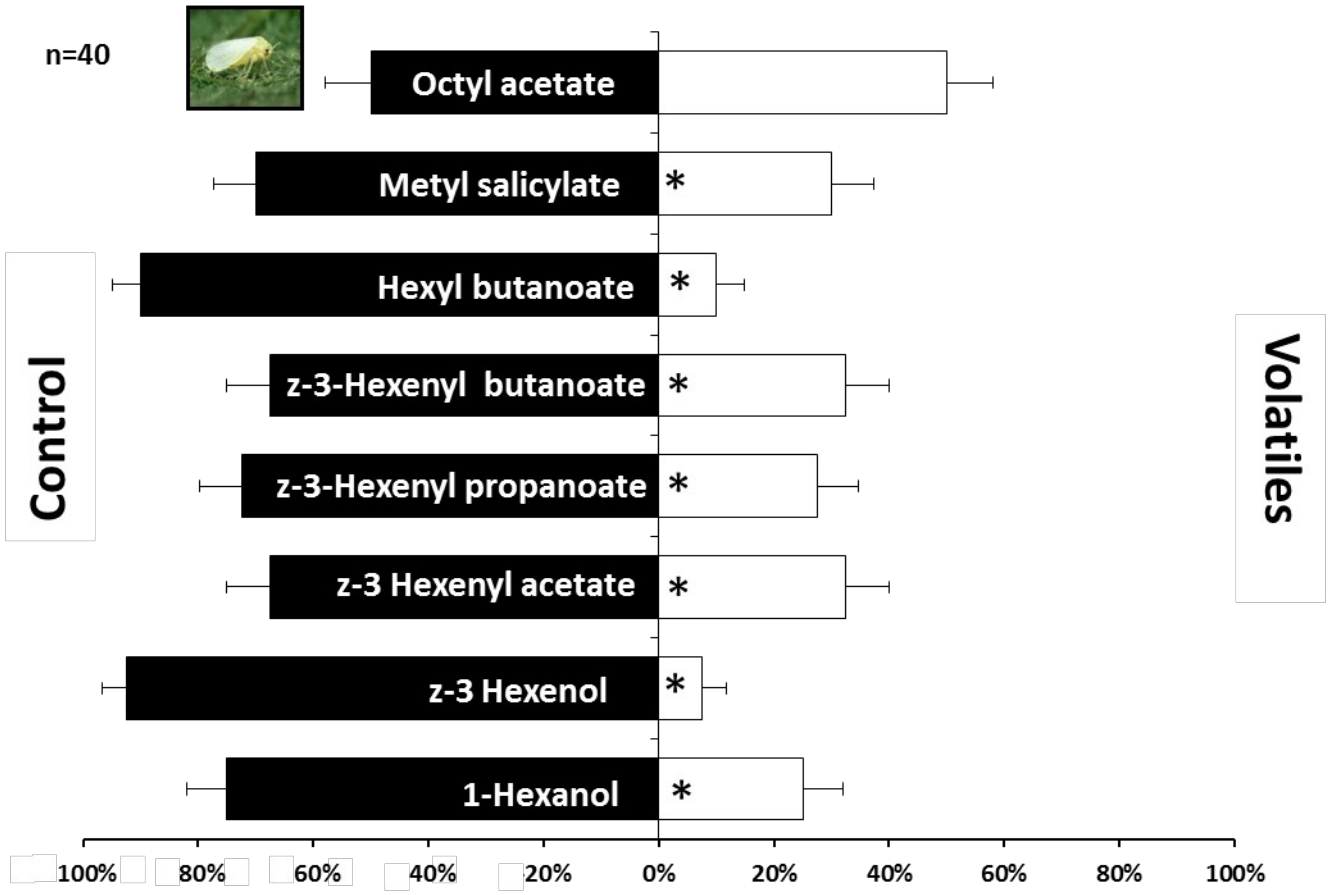

n=40
Octyl acetate
Metyl salicylate
Hexyl butanoate
z-3-Hexenyl butanoate
z-3-Hexenyl propanoate
z-3 Hexenyl acetate
z-3 Hexenol
1-Hexanol
*
Control
Volatiles
100%
80%
60%
40%
20%
0%
20%
40%
60%
80%
100%

Fig. 3

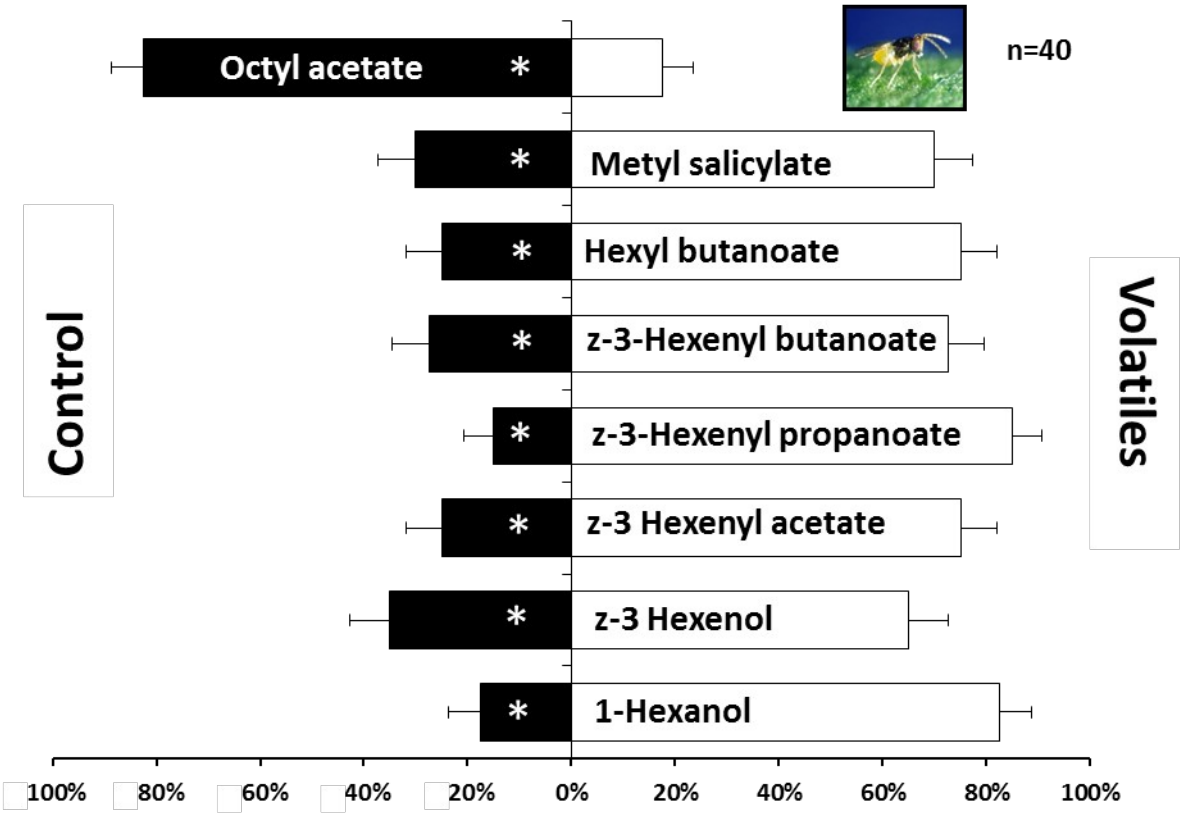
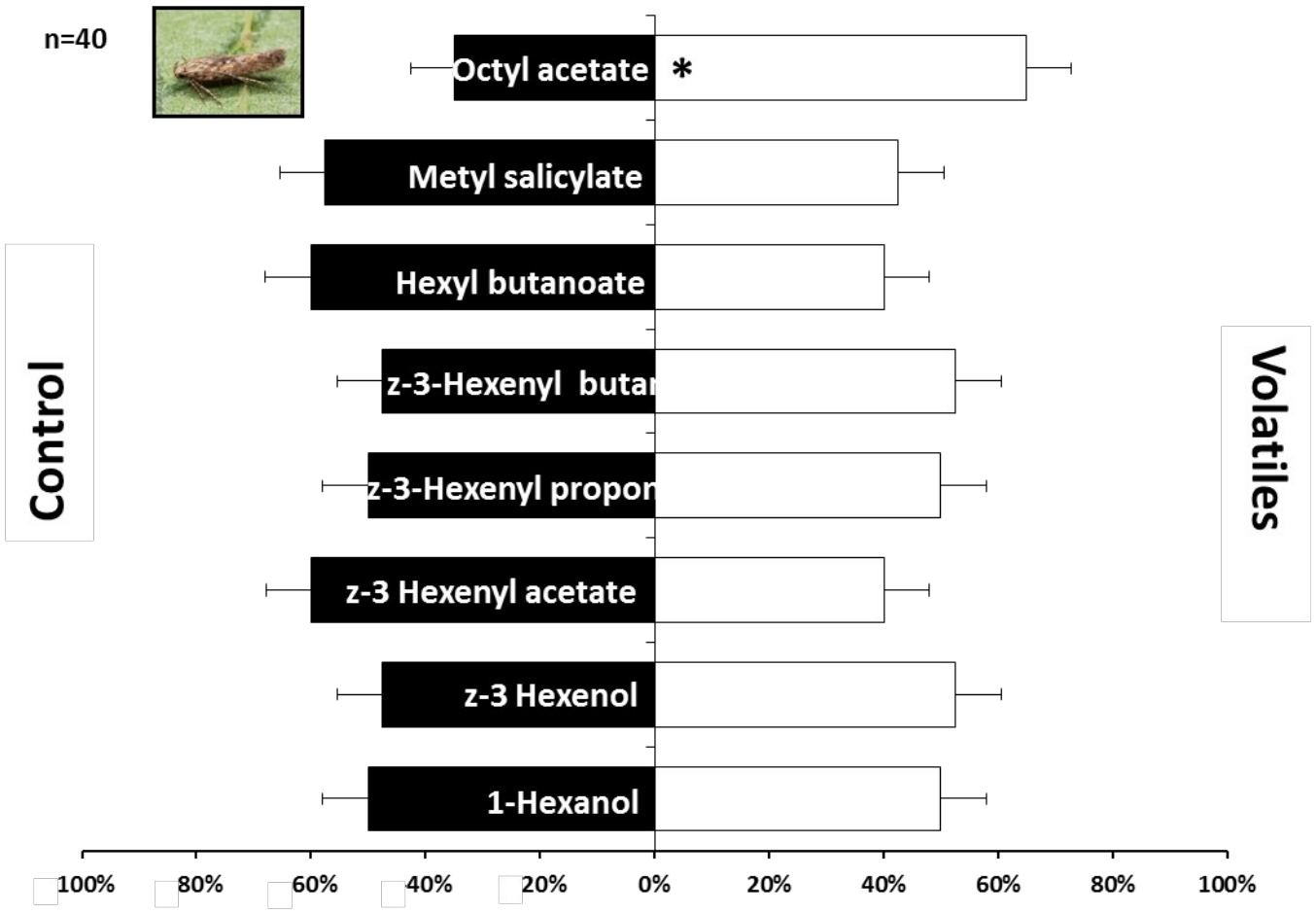

Octyl acetate
Metyl salicylate
Hexyl butanoate
z-3-Hexenyl butanoate
z-3-Hexenyl propanoate
z-3 Hexenyl acetate
z-3 Hexenol
1-Hexanol
*
n=40
Control
Volatiles
100%
80%
60%
40%
20%
0%
20%
40%
60%
80%
100%

Fig. 4



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550**Fig. 5**

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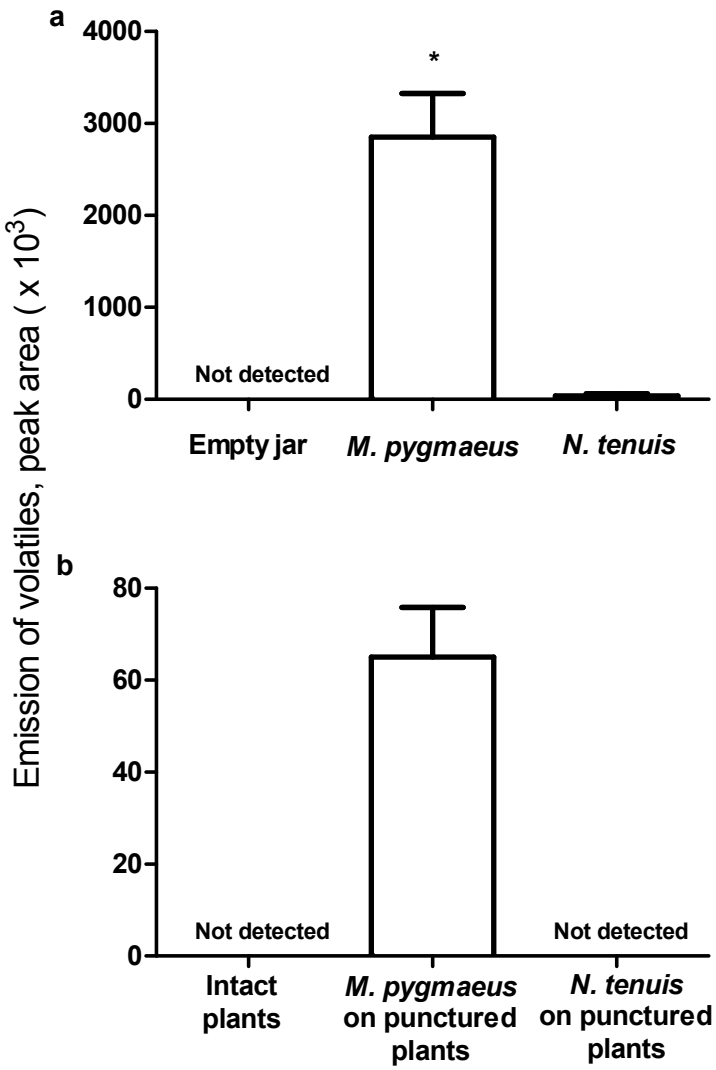

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