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[Silva, D. B., Urbaneja, A., & Pérez-Hedo, M. (2021). Response of mirid predators to synthetic herbivore-induced plant volatiles. *Entomologia Experimentalis et Applicata*, 169, 125–132.]

**ivia**  
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The final publication is available at

[\[https://doi.org/10.1111/eea.12970\]](https://doi.org/10.1111/eea.12970)

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1Special Issue-IEIC 6

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4Response of mirid predators to synthetic herbivore-induced plant volatiles

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18

19**Short title:** Miridae response to HIPVs

20

21Keywords: plant defense; behavior; *Nesidiocoris tenuis*; *Macrolophus pygmaeus*;

22*Dicyphus bolivari*

23

24

## 25Abstract

26Zoophytophagous plant bugs feed on plant tissue as a source of water and nutrients.  
27By phytophagy mirid predators activate tomato plant defensive responses through  
28different pathways resulting in the release of herbivore-induced plant volatiles  
29(HIPVs). The synthetic versions of those volatile compounds repel herbivores and  
30attract parasitoids and predators. Nevertheless, their influence on mirid plant  
31selection is still unknown. Thus, with Y-tube olfactometer trials we evaluated the  
32responses of *Nesidiocoris tenuis*, *Macrolophus pygmaeus* and *Dicyphus bolivari*  
33(Hemiptera: Miridae) to ten synthetics HIPVs. *Nesidiocoris tenuis* responded to five  
34out of ten HIPVs; while *M. pygmaeus* and *D. bolivari* responded to four out ten. Two  
35green leaf volatiles (GLVs) (*Z*)-3-hexenyl propanoate and (*Z*)-3-hexenyl acetate and  
36the ester methyl salicylate were attractive for all three mirid predators. Our results  
37demonstrate that the volatiles released by tomato plants activated by *N. tenuis* and  
38*M. pygmaeus* phytophagy are attractive to their conspecifics and also to *D. bolivari*.  
39Further studies should evaluate the potential of those compounds to attract  
40predatory mirids in the field.

41

## 42Introduction

43

44 Upon herbivore attack, plants produce and emit volatile organic compounds,  
45commonly known as herbivore-induced plant volatiles (HIPV). They have an  
46important role in tritrophic interactions, between plants, herbivores, and their natural  
47enemies. Herbivore-induced plant volatiles have already been demonstrated to  
48provide important foraging cues for natural enemies of the herbivores (Turlings &

49Erb, 2018). The use of these HIPVs increases predator and parasitoid capabilities;  
50efficiency is increased by reducing search time and thus increasing predation and  
51parasitism rates (Vet & Dicke, 1992, Allison & Hare, 2009; Bouagga et al., 2018a).

52        Among those natural enemies, hemipteran predators from the family Miridae  
53are being used to control greenhouse pests including the tomato borer *Tuta absoluta*  
54(Meyrick) (Lepidoptera: Gelechiidae), and the phloem-sucking whitefly *Bemisia*  
55*tabaci* (Gennadius) (Hemiptera: Aleyrodidae) which are considered to be the key  
56pests of tomato crops (Urbaneja et al., 2012; Calvo et al., 2012a; Van Lenteren,  
572012; Mollá et al., 2014). Mirid predators such as *Nesidiocoris tenuis* (Reuter),  
58*Macrolophus pygmaeus* (Rambur), *Dicyphus bolivari* (Lindberg) [= *D. maroccanus*  
59(Wagner)] can persist on tomato crops even when the density of the target pest is  
60low; this is due to their ability to feed on other insect pests such as thrips or aphids  
61(Castañé et al., 2011; Calvo et al., 2012a; Pérez-Hedo & Urbaneja, 2016; Salas-  
62Gervasio et al., 2017). In addition, their zoophytophagous behavior allows these  
63generalist predators to remain on the crop prior to and between infestations which  
64may enhance their fitness when they also feed on prey (Coll & Guershon, 2002;  
65Calvo et al., 2012a; 2012b; Calvo et al., 2016; Silva et al., 2016).

66        Recent studies suggest that mirid predators use HIPVs to locate their prey  
67(Moayeri et al., 2007; Lins Jr. et al., 2014; Silva et al., 2018). The blend of  
68compounds consists of plant semiochemicals frequently found in tomato-pest  
69interactions (Fang et al., 2013; Errard et al., 2015; Silva et al., 2017, 2018).  
70Moreover, phytophagy by predators mirids can also induce tomato plant defenses,  
71leading to the emission of HIPVs; also called zoophytophagous-induced plant  
72volatiles (ZIPVs) (Pappas et al., 2015; Pérez-Hedo et al., 2015a; Pérez-Hedo et al.,  
732018). This blend of volatiles plays an important role in pest management. For

74instance, tomato plants punctured by *N. tenuis* are attractive to conspecific *N. tenuis*  
75females, and to the parasitoid *Encarsia formosa* (Gahan) (Hymenoptera:  
76Aphelinidae), and repels some herbivorous insects such as *T. absoluta* and *B. tabaci*  
77(Lins Jr. et al., 2014; Pérez-Hedo et al., 2015b).

78        Distinct (Z)HIPVs vary both quantitatively and qualitatively, depending upon  
79the plant species (Bouagga et al., 2018a; Pérez-Hedo et al., 2018), as well as on the  
80herbivore and zoophytophagous predator species (Silva et al., 2017; Pérez-Hedo et  
81al., 2018). This distinction in the constituents of HIPVs has been demonstrated to  
82enable mirids to recognize infested and uninfested tomato plants, but information on  
83mirid synthetic-mediated behavioral mechanisms is limited (De Backer et al., 2017;  
84Pérez-Hedo et al., 2018).

85        Evidence of predators' attraction to synthetic compounds has been  
86demonstrated for at least 20 years, both in the laboratory and field (Zhu et al., 1999;  
87Ninkovic et al., 2001; James, 2003a,b; Francis et al., 2004; Mumm & Dicke, 2010;  
88James & Price, 2014; Aljbory et al., 2018). Synthetic compounds tested include  
89green leaf volatiles from the fatty acid/lipoxygenase (LOX) pathway, mono and  
90sesquiterpenes from the isoprenoid pathway and aromatic metabolites of the  
91shikimate pathway (Pare & Turmlinson, 1996; Shen et al., 2014). A number of  
92studies have reported methyl salicylate (MeSA) and GLVs such as (Z)-3-hexen-1-ol,  
93and (Z)-3-hexenyl acetate as a couple of the most attractive compounds for a variety  
94species of predators (James 2003a,b; Woods et al., 2011; Uefune et al., 2012;  
95Salamanca et al., 2017). In addition, terpenoids have also been used to attract  
96predators. Liu et al. (2018) reported attraction of *Cyrtorhinus lividipennis* Reuter  
97(Hemiptera: Miridae), an important predator of rice plant hoppers, to the  
98monoterpene, limonene. In a related study, (*E*)-  $\beta$ -ocimene and  $\beta$ -caryophyllene

99were found to be attractive for the ladybird *Adalia bipunctata* L. (Coleoptera:  
100Coccinellidae) and the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera:  
101Chrysopidae) (Laznik & Trdan, 2018).

102 In biological control strategies, compound(s) selection is crucial; it affects the  
103insect species attracted and the intensity of that attraction (Kaplan, 2012). The  
104prospect of using synthetic HIPVs which mimic those emitted by plants colonized by  
105mirids, is particularly appealing. In a recent study, Pérez-Hedo et al. (2018),  
106characterized the HIPVs which were induced by two mirid predators, *N. tenuis* and  
107*M. pygmaeus*. There were qualitative and quantitative differences among those  
108compounds induced in the tomato plants; being mainly composed of the above-  
109mentioned GLVs, aromatic compounds, and terpenes. In addition, the synthetic  
110version of those compounds is highly attractive for parasitic wasps. Thus, to increase  
111our knowledge of the use of synthetic HIPVs to manipulate the predator behavior, we  
112evaluated the responses of three mirid species, *N. tenuis*, *M. pygmaeus* and *D.*  
113*bolivari* to ten selected compounds. We, therefore, tested the hypotheses that all  
114three mirid species can detect and are attracted to the synthetic compounds.

115

## 116Materials and Methods

117

### 118Insects

119 A stock colony of *D. bolivari* originating from a colony at the University of  
120Lleida (UdL) was subsequently established at the IVIA and maintained under the  
121laboratory conditions of  $25 \pm 1$  °C,  $70 \pm 10\%$  RH and 14:10 (L:D) photoperiod. To  
122produce cohorts of experimental individuals (females), previously mated adult  
123females (progenitors) were maintained in plastic cages (30 × 30 × 30 cm) containing

124 *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs for *ad libitum* feeding.  
125 Green bean pods, as oviposition substrate, and a water source were also provided.  
126 After seven days, the green bean pods containing mirid eggs to be used in the trials  
127 were transferred to new cages where nymphs hatched and fed on *E. kuehniella* eggs  
128 until reaching the adult stage. Newly emerged adult females and males were  
129 transferred to a 40 ml glass tube with a moisture cotton and *E. kuehniella* eggs  
130 inside and for mating. After observation of copula, the females were starved for 24 h  
131 in a new 40 ml glass tube which contained a moisture cotton inside and sealed with  
132 cotton.

133 Newly emerged adult *N. tenuis* and *M. pygmaeus* were obtained directly from  
134 the mass rearing of Koppert Biological Systems, S.L. (Águilas, Murcia, Spain). Once  
135 received at IVIA, individuals of the two mirid species were kept in plastic cages (30 ×  
136 30 × 30 cm) containing *E. kuehniella* eggs for *ad libitum* feeding, green bean pods as  
137 oviposition substrate, and a water source. Same above-mentioned procedure was  
138 done for both newly emerged females' species. For all the three mirid species,  
139 individuals less than 7 days old were used in behavioral assays.

140

#### 141 Olfactometer assays

142 Responses of female mirids to synthetic HIPVs were assessed in a glass Y-  
143 tube olfactometer (3 cm diameter, main arm 15 cm long, side arms 13 cm long, 70°  
144 angle between the side arms) (Analytical Research Systems, Gainesville, FL, USA).  
145 Each olfactometer side arm was connected via plastic tubes to two identical 5L glass  
146 chambers: one contained a tested odor source and the other contained control odour  
147 source. Compressed air was provided by an air pump that pulled charcoal-filtered  
148 and humidified air into the glass chambers and olfactometer. Airflow was adjusted to

1491.2 l/min\*arm with a flowmeter (Flo-Rite™, Treviso, PA, USA) (Pérez-Hedo &  
150Urbaneja, 2015). Four 60-cm fluorescent tubes (OSRAM, L18W/765, OSRAM  
151GmbH, Germany) were positioned 40 cm above the horizontal Y-shaped glass tube.  
152The light intensity registered 2,516 lux over the Y-tube and was measured using a  
153ceptometer (LP-80 AccuPAR, Decagon Devices, Inc. Pullman, WA, USA). All Y-tube  
154experiments were conducted between 9:00 and 17:00 under the following  
155environmental conditions,  $24 \pm 1^\circ\text{C}$ ,  $60 \pm 10\%$  r.h.

156 A single mirid female was placed inside the main arm of the olfactometer and  
157observed for up to 15 min. Females were considered to have made a choice when  
158they walked up at least 10 cm from the branching point of the Y-tube. Females not  
159choosing a side arm within 15 min were considered to be unresponsive and were  
160excluded from data analysis. Each female was tested only once. Thirty replicates  
161(responses) in total were performed for each treatment and each mirid species. After  
162every pair (two) of replicates, the olfactometer side arms were switched to minimize  
163positional bias. After testing ten females, the Y-tube and glass chambers were  
164washed with neutral soap and acetone and left to dry for 5 min and the sources of  
165volatile emission were changed.

166

#### 167Synthetic compounds tested

168 For the three mirid predators, assessment of olfactory responses was carried  
169out with ten synthetic volatile compounds previously found on plants induced by *N.*  
170*tenuis* and *M. pygmaeus* (Bouagga et al., 2017; Pérez-Hedo et al., 2018). The  
171chemical compounds belong to three chemical groups (i) GLVs: (Z)-3-hexenyl  
172acetate, (Z)-3-hexen-1-ol, (Z)-3-hexenyl butanoate, hexyl butyrate, and (Z)-3-hexenyl  
173propanoate, (ii) Esters: methyl jasmonate (MeJA) and MeSA and (iii) Terpenes:

174ocimene, caryophyllene, and limonene. The volatiles were released into the glass  
175chamber through a piece of filter paper (4 x 3 cm) containing 20 µl of each synthetic  
176compound or water as control (for all, 1:5000 dilution in methanol). The filter paper  
177was positioned inside of a Petri dish (5 cm diameter) and placed at the bottom of the  
178glass chamber. Pérez-Hedo et al. (2018) reported the dilutions of 1:10000 of pure  
179compounds to be the same order of magnitude as those emitted by mirid-induced  
180tomato plants. Nevertheless, in preliminary experiments, none of the three mirid  
181species responded to this concentration; thus, we used the greater concentration of  
1821:5000. All synthetic standards of the tomato volatile compounds were purchased  
183from Sigma-Aldrich (St. Louis, MO, USA).

184

#### 185Data analysis

186 To investigate whether mirid females are attracted to the synthetic  
187compounds odours tested, the data were analyzed. The response variable was the  
188proportion of insects responding to one of the volatile sources with the null  
189hypothesis of odor sources being chosen with equal frequency.

190

#### 191Results

192

193 The 1:5000 dilutions of pure compounds used in this study elicited the  
194attraction of all three mirid predators: 78% of *N. tenuis* females, 79% of *D. bolivari*  
195females, and 79% of *M. pygmaeus* responded.

196 The two green leaf volatiles, (Z)-3-hexenyl propanoate, and (Z)-3-hexenyl  
197acetate and the ester MeSA were attractive for all three mirid species: *N. tenuis* *M.*  
198*pygmaeus* and *D. bolivari* (for each of the three compounds and mirid species,  $p <$

1990.001). Nevertheless, *N. tenuis* females responded to four out of five GLVs: (Z)-3-  
200hexenyl propanoate ( $\chi^2 = 13.6, p < 0.001$ ), hexyl butyrate ( $\chi^2 = 14.9, p < 0.001$ ), (Z)-  
2013-hexen-1-ol ( $\chi^2 = 32.7, p < 0.001$ ) and (Z)-3-hexenyl acetate ( $\chi^2 = 37.3, p < 0.001$ )  
202(Figure 1). While *M. pygmaeus* females responded to (Z)-3-hexenyl propanoate ( $\chi^2 =$   
20328.9,  $p < 0.001$ ), (Z)-3-hexen-1-ol ( $\chi^2 = 35.4, p < 0.001$ ), and (Z)-3-hexenyl acetate  
204( $\chi^2 = 12.2, p < 0.001$ ) (Figure 2). *Dicyphus bolivari* females were attracted to MeSA  
205( $\chi^2 = 11.2, p < 0.001$ ), (Z)-3-hexenyl propanoate ( $\chi^2 = 26.6, p < 0.001$ ), (Z)-3-hexen-1-  
206ol ( $\chi^2 = 25.5, p < 0.001$ ) (Figure 3) and MeJA ( $\chi^2 = 26.9, p < 0.001$ ). MeJA did not  
207elicit behavioral response in *N. tenuis* nor *M. pygmaeus* ( $p > 0.05$ ) (Figures 1, 2, 3).  
208A lack of preference was observed for the green leaf volatile (Z)-3-hexenyl butanoate  
209and for the terpenes: ocimene, caryophyllene, and limonene by all three mirid  
210species ( $p > 0.05$ ) (Figures 1, 2, 3).

211

## 212 Discussion

213

214 Plant volatiles induced by herbivore pests play an important role in attracting  
215 natural enemies. However, beneficial arthropods like zoophytophagous predators  
216 due to their phytophagy behavior can also induce plant defense mechanisms  
217 resulting in the emission of (Z)HIPVs (Pérez-Hedo et al., 2018). Mirid predators and  
218 parasitoids have been demonstrated to be attracted to plant volatiles when colonized  
219 by conspecifics or heterospecific mirid predators (Lins Jr. et al., 2014; Pérez-Hedo et  
220 al., 2015). In an additional step, the synthetic version of some of those HIPVs  
221 resulted to be repellent to *B. tabaci* and highly attractive for the parasitic wasp, *E.*  
222 *formosa*. In our study the same compounds were also tested on the mirid species.  
223 The concentration of those synthetic HIPV compounds were very low, approximately

224the same quantity as released by tomato plants (Pérez-Hedo et al., 2018). Attraction  
225to volatile chemicals presented at small concentrations eludes to the importance of  
226these molecules for long-distance orientation (De Backer et al., 2017). Our results  
227are consistent with the hypothesis that the HIPVs, mainly green leaf volatiles such as  
228(Z)-3-hexenyl propanoate, (Z)-3-hexenyl acetate, (Z)-3-hexen-1-ol, hexyl butyrate,  
229and the esters: MeSA and MeJA are effective for manipulating mirid predators'  
230behavior. On the contrary, the three terpenoids tested were not attractive for *N.*  
231*tenuis*, *M. pygmaeus* nor *D. bolivari*.

232       The above-mentioned classes of compounds have an important ecological  
233role on several crops, by among others, attracting parasitoids and predators  
234(Turlings & Erb, 2018). The fatty acid derivate group, commonly called green leaf  
235volatiles (GLVs), is a well-studied group of compounds released by plants  
236immediately after mechanical damage, herbivore or zoophytophagous feeding.  
237Therefore, GLVs are important components of a blend of volatiles, which rapidly  
238provide information about the exact location of a feeding herbivore (Yu et al., 2008).  
239Synthetic versions of some of those compounds have been tested as important cues  
240to several insect predators from different families such as Chrysopidae,  
241Anthocoridae, Geocoridae, and Coccinellidae (James, 2003b; James & Grasswitz,  
2422005; Kaplan, 2012; Maeda et al., 2015; Gebreziher, 2018). This class of  
243compounds was highly attractive for *N. tenuis*, since four out of the five GLV's tested  
244resulted attractive. In addition, (Z)-3-hexenyl propanoate and (Z)-3-hexenyl acetate  
245compounds derived by the breakdown of membrane lipids were attractive for all  
246three mirid species, suggesting their potential in integrated pest management  
247strategies despite, to date, the little attention these volatile chemicals have received  
248(James et al., 2003; Kaplan, 2012; Turlings & Erb, 2018).

249 MeSA, one of the two aromatic benzenoids tested, has been the center of  
250attention for manipulation of natural enemies' behavior (Rodríguez-Saona et al.,  
2512011). MeSA emission is often induced after phytophagy by zoophytophagous  
252predators, herbivore, or pathogen attack through the activation of the shikimate  
253pathway. It is effective in direct and indirect defense against pathogens and  
254herbivores (Silva et al., 2017; Salamanca et al., 2017; Bouagga et al., 2018a, 2018b;  
255Pérez-Hedo et al., 2018). Both laboratory and field studies demonstrated the  
256attractiveness of the synthetic version of MeSA for a number of natural enemies.  
257Insects attracted to MeSA included, among others, the minute pirate bug *Orius*  
258*insidiosus* White (Hemiptera: Anthocoridae), the green lacewing, *Chrysopa*  
259*nigricornis* Burmeister, and *C. carnea*, the big eyed bug, *Geocoris pallens* Stal  
260(Hemiptera: Geocoridae), the ladybird, *A. bipunctata*, *Lygus hesperus* Knight  
261(Hemiptera: Miridae) and syrphid species (Diptera: Syrphidae) (James, 2003;  
262Kaplan, 2012; Salamanca et al., 2017; Laznik & Trdan, 2018).

263 Besides the previously mentioned GLVs, (Z)-3-hexenyl acetate and (Z)-3-  
264hexenyl propanoate, this is the first evidence that *N. tenuis*, *M. pygmaeus* and *D.*  
265*bolivari* are attracted to synthetic MeSA. Interestingly, previous studies showed mirid  
266species to be attracted to whitefly and *T. absoluta* infested plants (Lins Jr. et al.,  
2672014; Silva et al., 2018) and all three attractive compounds are often emitted by  
268tomato plants attacked by these pests (López et al., 2012; Silva et al., 2017). It is,  
269thus, possible that the application of those compounds in the field could serve as an  
270important cue in guiding these predators to tomato crop areas.

271 There were uniformly low attraction rates across all treatments with terpenes  
272for all three mirid species. We speculate that the low attraction to terpenes by the  
273mirids could be due to their generalist behavior. Mirids have not been found to have

274a high level of compound specificity for foraging; most of them are guided by the  
275green leaf volatiles which are released by the plant as soon as it has been damaged;  
276thus, quickly providing information about the prey location (War et al., 2011).  
277Terpenes are released later, as observed by Erb et al. (2015). It is also possible that  
278attraction to terpene compounds depends on others factors like: Predator identity  
279and changes in compounds ratios. For instance, the ladybird *Harmonia axyridis*  
280Pallas (Coleoptera: Coccinellidae), responds to caryophyllene (Leroy et al., 2012),  
281while another ladybird, *Coleomegilla maculata* Fitch (Coleoptera: Coccinellidae),  
282does not (Zhu et al., 1999); changes in the proportions of the major compounds (*E*-  
283 $\beta$ -farnesene and (-)- $\beta$ -caryophyllene decreased the attractiveness of *Coccinella*  
284*septempunctata* L. (Coleoptera: Coccinellidae) (Abassi et al., 2000). In addition,  
285some natural enemies require a brief learning period before they illustrate a  
286preference to an odor (Drukker et al., 2000; De Boer et al., 2005; Lins Jr. et al.,  
2872014). In our study we used inexperienced (previously unexposed) adult females,  
288whereas experienced individuals might be more responsive due to learning. In fact,  
289Lins Jr. et al. (2014) observed that the attraction of *N. tenuis* to HIPVs was only  
290positive after experience which indicates associative learning.

291 In summary, our results demonstrated that *N. tenuis*, *M. pygmaeus*, and *D.*  
292*bolivari* can detect and are attracted to HIPVs under controlled laboratory conditions.  
293To the best of our knowledge, this is the first study to show the role synthetic HIPVs  
294on mirid location behavior and that the two GLVs, (*Z*)-3-hexenyl propanoate and (*Z*-  
2953-hexenyl acetate and the ester MeSA are potential compounds to change mirid  
296behavior leading to their recruitment in agroecosystems. Our results have direct  
297ecological implications in the context of pest control. We have demonstrated that  
298some of the tested compounds resulted attractive to predatory mirids. However,

299these same compounds were previously shown to be repellent against certain  
300herbivore pests such as *B. tabaci*, *F. occidentalis* or *T. absoluta* (Pérez-Hedo 2018).  
301Therefore, in the case of field application of these HIPV's in tomatoes to increase the  
302appearance of natural-occurring populations of mirids, a possible counterproductive  
303effect should be carefully addressed. On the one hand, the attraction of predatory  
304mirids and the repellence of pests could result that predatory mirids did not have  
305enough food to establish and develop in the crop. On the other hand, the risk of plant  
306damage caused by predatory mirids would increase. Currently, there are strategies  
307that could be used to limit this problem. It has recently been shown that the  
308application of sugars, either using hydrocapsules filled with sugars or by directly  
309spraying sugars in field conditions, can reduce plant damage caused by *N. tenuis*  
310(Urbaneja-Bernat et al. 2019) and facilitate its establishment (Urbaneja et al., 2013;  
3112015). Similar results have been obtained with the use of sesame *Sesamum indicum*  
312(l.) (Pedaliaceae) as companion plant (Biondi et al 2016). Therefore, the use of  
313HIPV's could be strengthened by integrating it with any of these two strategies for  
314managing mirids mentioned above. In any case, further field research is needed to  
315elucidate under what growing conditions this would be necessary.

316        This research is just another step in understanding how to better use HIPVs in  
317the biological control of solanaceous crop pests. However, research is still required  
318to obtain knowledge on the practical use of HIPVs in attracting mirid predators in the  
319field. As a follow up study, two strategies were planned to elucidate the role of HIPVs  
320in indirect plant defense. The first one is mirid attraction through synthetic application  
321of those HIPVs in the field. This strategy is currently been developed but no  
322consistent results are yet available (Silva et al., unpublished data). In addition, the  
323second study is based on the stimulation of plants to produce their own HIPV blends

324through the use of an airborne chemical signals (synthetic compounds). Exposing  
325plants to chemical compounds would likely be interpreted by the plants as herbivory  
326of nearby plants warning them to defend themselves and consequently benefiting  
327with the attraction of natural enemies (Pérez-Hedo et al., 2015). These strategies will  
328provide important information on the role of indirect plant defense in enhancing the  
329effectiveness of bio-control agents for integrate pest management.

### 330**Acknowledgements:**

331The authors thank Dr. Felipe Madeira and Prof. Ramon Albajes (UdL, Spain) for the  
332individuals to establish the colony of *D. bolivari*, Dr Javier Calvo (Koppert Biological  
333Systems, S.L., Spain) for supplying *N. tenuis* and *E. kuehniella*, and Miquel Alonso  
334(IVIA) for technical assistance. The research leading to these results was partially  
335funded by the Spanish Ministry of Economy and Competitiveness MINECO  
336(RTA2017-00073-00-00), the Conselleria d'Agricultura, Pesca i Alimentació de la  
337Generalitat Valenciana, and by FAPESP (Process 2018/25151-5).

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#### 570**Figure legends**

571Figure 1 Response (%  $\pm$  SE) of *Nesidiocoris tenuis* females (n = 30) in a Y-tube  
572olfactometer when exposed to ten synthetic HIPVs or water (for all, 1:5,000 dilution  
573in methanol). The horizontal axis represents the percentage of predators that moved  
574towards the volatile sources in the corresponding choice trials. NR indicates the  
575number of tested individuals that did not make a choice. Chi-square test ( $\chi^2$ ): \* $P \leq$   
5760.05.

577Figure 2 Response (%  $\pm$  SE) of *Macrolophus pygmaeus* females (n = 30) in a Y-tube  
578olfactometer when exposed to ten synthetic HIPVs or water (for all, 1:5,000 dilution  
579in methanol). The horizontal axis represents the percentage of predators that moved  
580towards the volatile sources in the corresponding choice trials. NR indicates the  
581number of tested individuals that did not make a choice. Chi-square test ( $\chi^2$ ): \* $P \leq$   
5820.05.

583 Figure 3 Response ( $\% \pm \text{SE}$ ) of *Dicyphus bolivari* females ( $n = 30$ ) in a Y-tube  
584 olfactometer when exposed to ten synthetic HIPVs or water (for all, 1:5,000 dilution  
585 in methanol). The horizontal axis represents the percentage of predators that moved  
586 towards the volatile sources in the corresponding choice trials. NR indicates the  
587 number of tested individuals that did not make a choice. Chi-square test ( $\chi^2$ ):  $*P \leq$   
588 0.05.

589