

2 **Defensive plant responses induced by *Nesidiocoris tenuis***  
3 **(Hemiptera: Miridae) on tomato plants**

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8 **Abstract** In the last decade, biological control programs  
9 for greenhouse tomatoes and other crops have been suc-  
10 cessfully implemented using zoophytophagous plant bugs  
11 (Miridae), which can feed on both plant tissues and insect  
12 prey. It is well known that plants respond to herbivore  
13 attacks by releasing volatile compounds through diverse  
14 pathways triggered by phytohormones. These herbivore-  
15 induced plant volatiles can alert neighboring plants, repel  
16 or attract herbivores, and attract natural enemies of these  
17 herbivores. Nevertheless, the possible benefits of induced  
18 plant responses by zoophytophagous predators that could  
19 add to their usefulness as biocontrol agents have not been  
20 studied until now. Here we show that the zoophytophagous  
21 predator *Nesidiocoris tenuis* activated abscisic acid and  
22 jasmonic acid (JA) signaling pathways in tomato plants,  
23 which made them less attractive to the whitefly *Bemisia*  
24 *tabaci*, a major tomato pest worldwide, and more attractive  
25 to the whitefly parasitoid, *Encarsia formosa*. We also found  
26 that intact tomato plants exposed to volatiles from *N. ten-*  
27 *uis*-punctured plants activated the JA pathway, and as a

consequence, *E. formosa* was also attracted to these intact 28  
plants with activated defense systems. Thus, our results 29  
demonstrate that *N. tenuis* not only benefits tomato plants 30  
directly by entomophagy but also indirectly by phyto- 31  
phagy, which induces a physiological response in the 32  
tomato plant. **AQ1** 34

**Keywords** *Bemisia tabaci* · *Encarsia formosa* · 35  
Induced plant responses · Biological control 36

**Key message** 37

We have proved that the zoophytophagous predator *Nesi-* 38  
*diocoris tenuis* induces plant benefits directly by its ento- 39  
mophagy and also indirectly by its phytophagy, which 40  
induces the attraction of a whitefly parasitoid (*Encarsia* 41  
*formosa*) and antixenosis to the whitefly *Bemisia tabaci*. 42  
Furthermore, *N. tenuis*-punctured plants induce plant 43  
defenses in intact plants that result in attraction of *E. for-* 44  
*mosa*. Our results might be one reasonable explanation for 45  
the great success achieved by *N. tenuis* as a key biocontrol 46  
agent in tomatoes. 47

**Introduction** 48

In plants, arthropod herbivory activates different responses 49  
that are generally triggered by receptor complexes that 50  
recognize herbivore-associated elicitors (HAEs) and fatty 51  
acid-amino acid conjugates (FACs) (Bonaventure et al. 52  
2011). Once the plant has identified an attack, it can 53  
respond through the activation of diverse signaling path- 54  
ways. One set produces antibiotic and antixenotic com- 55  
pounds that exert a negative effect on the herbivore 56

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57 (Bennett and Wallsgrove 1994; Chen 2008) and systemic  
58 signals that warn other parts of the plant (Davis et al. 1991;  
59 Zhang and Baldwin 1997; Stratmann 2003). Another set  
60 causes the release of volatiles (so-called herbivore-induced  
61 plant volatiles or HIPVs) that play a double role in defense  
62 by priming both distal parts of the same plant and its  
63 neighbors (Frost et al. 2008) and attracting secondary  
64 consumers such as parasitoids and predators (Heil and Ton  
65 2008) or repelling herbivores. Indeed, these HIPVs may  
66 increase plant productivity through a trophic cascade  
67 effect, which constitutes the basis of modern biological  
68 control science (Hairston et al. 1960; Oksanen et al. 1981).

69 Zoophytophagous predators are a special case of natural  
70 enemies (Coll and Guershon 2002). These omnivorous  
71 predators feed on plants and prey during the same devel-  
72 opmental stage (Castañé et al. 2011). Interestingly, under  
73 certain conditions, omnivory has been demonstrated to be a  
74 stabilizing feature of complex natural systems (Kratina  
75 et al. 2012). Indeed, this plasticity facilitates the estab-  
76 lishment of zoophytophagous predators in the crop prior to  
77 pest infestation and their conservation in periods of prey  
78 scarcity. As a result, crops in which zoophytophagous  
79 predators have been established become highly resilient to  
80 pest invasions (Ramakers and Rabasse 1995; Messelink  
81 et al. 2008; Lu et al. 2012). Zoophytophagous predators  
82 such as Miridae and Anthocoridae (Heteroptera) are  
83 becoming increasingly important for the biological control  
84 of important agricultural pests (Bueno et al. 2013; Pérez-  
85 Hedo and Urbaneja 2014) even though they exploit plants  
86 for both feeding and oviposition (Coll 1996; Coll and  
87 Guershon 2002). They use their flexible stylets to extract  
88 liquid food from their prey and the plants on which they  
89 live. Females use their ovipositor to insert their eggs in the  
90 same plants. By wounding, these natural enemies can  
91 activate the same defense mechanisms as strict herbivores  
92 (Kessler and Baldwin 2004; Halitschke et al. 2011).  
93 Indeed, De Puyseleir et al. (2011) demonstrated that  
94 *Orius laevigatus* (Fieber) (Heteroptera: Miridae), a widely  
95 used biological control agent for Thripidae, which are of  
96 economic importance, increased tomato (*Solanum lyc-*  
97 *opersicum* L.) resistance to pestiferous *Frankliniella occi-*  
98 *dentalis* (Pergande) (Thysanoptera: Thripidae) feeding by  
99 inducing jasmonic acid (JA)-mediated wound response  
100 during oviposition. However, the same authors noted that  
101 *O. laevigatus* is not naturally occurring or commercially  
102 used in tomato crops.

103 Among the different mirid bugs that can be found natu-  
104 rally feeding on tomato plants (Zappala et al. 2013), the  
105 cosmopolitan *Nesidiocoris tenuis* (Reuter) (Hemiptera: Mir-  
106 idae) has been extremely effective in controlling the inva-  
107 sive South American tomato pinworm *Tuta absoluta*  
108 (Meyrick) (Lepidoptera: Gelechiidae), an important tomato  
109 pest first detected in the Old World in 2007 (Desneux et al.

2010). Furthermore, the most threatening whitefly world-  
wide, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae),  
is also effectively controlled by this mirid bug (Calvo et al.  
2012a; Urbaneja et al. 2012). Our research group has regu-  
larly observed over the last few years that the incidence of  
whiteflies, in particular *B. tabaci*, was very low in both  
protected and open-field tomato crops where *N. tenuis* was  
successfully established. At first, we attributed this result to  
active predation by *N. tenuis*, which typically lives in and  
feeds on the upper growing parts of tomato plants, on  
immature *B. tabaci* and, to a lesser extent, on *B. tabaci*  
adults (Calvo et al. 2009). However, we thought that pre-  
dation alone could not explain the extremely low densities of  
*B. tabaci* adults landing on the apical parts of plants com-  
pared to conventional crops where pesticides were used.  
This observation led us to hypothesize that the presence of  
*N. tenuis* on plants could be the result of not only direct  
predation of this mirid on *B. tabaci* populations but also of  
indirect defense mechanisms, such as the attraction of other  
natural enemies, and the induction of plant defenses (anti-  
xenosis and antibiosis). However, to our knowledge, whe-  
ther *N. tenuis*, which is not a strict herbivore, can activate  
plant responses and whether these responses can be an added  
benefit to its effectiveness as an arthropod predator remain  
unknown.

In this work, we hypothesized that tomato plants with *N.*  
*tenuis* were less attractive to the whitefly *B. tabaci* than  
plants without *N. tenuis*. Therefore, we studied whether the  
plant-feeding activity of *N. tenuis* could induce plant  
responses in tomato plants using hormonal profiling and  
gene-expression analysis of the main defensive signaling  
pathways. We also studied the role of selected phytohor-  
mones on host plant selection by the whitefly *B. tabaci* and  
the parasitoid *Encarsia formosa* (Gahan) (Hymenoptera:  
Aphelinidae), which is used commercially worldwide to  
control whiteflies in tomato crops (van Lenteren 2012)  
using hormone-deficient mutant tomato plants. Finally,  
because HIPVs can activate rapid defense responses in both  
distal plant parts and neighboring conspecific plants (Choh  
and Takabayashi 2006; Frost et al. 2008), we investigated  
whether HIPVs from *N. tenuis*-infested plants induce  
defensive responses in neighboring, uninfested tomato  
plants.

## Materials and methods

### Plant material and insects

*S. lycopersicum* (cv. Optima), abscisic acid (ABA)-defi-  
cient (*Sitiens*) and jasmonic acid (JA)-deficient tomato  
mutants (*def-1*) and their respective near-isogenic wild-  
type (cvs. Rheinlands Rhum and Castlemart) parental lines

159 were used to determine the responses of *B. tabaci* and the  
160 whitefly parasitoid *E. formosa* to the different experimental  
161 treatments described below. Plants were used for experi-  
162 ments at 6 weeks of age, when they had seven to eight fully  
163 expanded leaves. All plant genotypes were germinated in  
164 soil, and 2 weeks after germination, the seedlings were  
165 individually transferred to pots and maintained at  
166  $25 \pm 2$  °C and high relative humidity (>60 %) under a  
167 16:8 h L:D photoperiod.

168 *B. tabaci*, *E. formosa* and *N. tenuis* individuals were  
169 directly provided from the mass rearings of Koppert Bio-  
170 logical Systems, S.L. (Águilas, Murcia, Spain). *E. formosa*  
171 pupae were isolated in a petri dish (9 cm diameter) where a  
172 small drop of honey was provided on the sides of the dish  
173 as a food source. Adult females less than 2 days old were  
174 used in all trials. In the case of *B. tabaci*, newly emerged  
175 adults were released on four tomato plants placed in a  
176  $60 \times 60 \times 60$ -cm plastic cage (BugDorm-2; MegaView  
177 Science Co., Ltd.; Taichung, Taiwan) for 48 h. Female  
178 adults less than 5 days old were collected from those plants  
179 and used in all trials.

#### 180 Y-tube bioassays

181 The behavioral responses of *B. tabaci* and *E. formosa*  
182 females to plant volatiles were investigated in a Y-tube  
183 olfactometer (Analytical Research Systems, Gainesville,  
184 FL) consisting of a 2.4-cm-diameter Y-shaped glass tube  
185 with a 13.5-cm-long base and two 5.75-cm-long arms. The  
186 base of the Y-tube was connected to an air pump that  
187 produced a unidirectional airflow at 150 ml/min from the  
188 arms to the base of the tube. The arms were connected via  
189 plastic tubes to two identical glass jars (5-l volume), each  
190 of which contained a test odor source. Each odor source  
191 vial was connected to a flow meter and a water filter. Four  
192 60-cm-long fluorescent tubes (OSRAM, L18 W/765, OS-  
193 RAM GmbH, Germany) were positioned 40 cm above the  
194 arms. The light intensity over the Y-tube was measured  
195 with a ceptometer (LP-80 AccuPAR, Decagon Devices,  
196 Inc., Pullman, WA) at 2,516 lux. The environmental con-  
197 ditions in the Y-tube experiments were  $23 \pm 2$  °C and  
198  $60 \pm 10$  % RH.

199 Each female was observed until she had walked at least  
200 3 cm up one of the side arms or until 15 min had elapsed.  
201 Females that did not choose a side arm within 15 min  
202 were considered to be 'non-responders' and were not  
203 included in the subsequent data analysis. Each individual  
204 was used only once. After five individual females had  
205 been tested, the olfactometer arms were flipped around  
206 ( $180^\circ$ ) to minimize the spatial effect on arm choice. After  
207 ten females had been bioassayed, the olfactometer setup  
208 was rinsed with soap, water and acetone and then air  
209 dried.

#### *B. tabaci* plant selection mediated by *N. tenuis*

210  
211 To confirm our initial hypothesis that tomato plants with *N.*  
212 *tenuis* were less attractive to the whitefly *B. tabaci* than  
213 plants without *N. tenuis*, two different two-choice experi-  
214 ments were conducted. The first took place in the Y-tube  
215 olfactometer described above. A combination of the fol-  
216 lowing experimental treatments was assayed: (1) intact  
217 plants, (2) *N. tenuis*-bagged plants, which were tomato  
218 plants holding two double-layer gauze bags (to prevent  
219 plant feeding) containing two *N. tenuis* pairs each, and (3)  
220 *N. tenuis*-punctured plants, which were obtained by  
221 enclosing four intact tomato plants in a  $60 \times 60 \times 60$ -cm  
222 plastic cage (BugDorm-2; MegaView Science Co., Ltd.;  
223 Taichung, Taiwan) in which 100 *N. tenuis* had been pre-  
224 viously introduced for 24 h. All *N. tenuis* specimens were  
225 removed from *N. tenuis*-punctured plants before being  
226 subjected to this Y-tube choice assay.

227 The second choice experiment consisted of releasing  
228 100 *B. tabaci* in the middle of a  $60 \times 60 \times 60$ -cm plastic  
229 cage (BugDorm-2, MegaView Science Co., Ltd.; Tai-  
230 chung, Taiwan) containing three intact plants and three  
231 plants that had each been previously in contact with two  
232 pairs of *N. tenuis* for 7 days. *N. tenuis*-punctured plants  
233 were obtained simulating the standard commercial method  
234 of *N. tenuis* release in which 0.25–0.5 *N. tenuis* pairs per  
235 plant are inoculated in the nursery for 7 days before  
236 transplanting to the greenhouse (Calvo et al. 2012a;  
237 Urbaneja et al. 2012). Twenty-four hours after the release  
238 of *B. tabaci*, the number of whitefly individuals per plant  
239 was counted. The experiment was replicated five times.  
240 This experiment was conducted in a glasshouse located at  
241 the Instituto Valenciano de Investigaciones Agrarias IVIA  
242 (Moncada, Valencia, Spain). The climatic conditions were  
243  $25 \pm 2$  °C and  $65 \pm 10$  % RH and a natural photoperiod  
244 (approximately 14L:10D).

#### Phytohormone analysis

245  
246 Because HIPV release is the result of a signaling cascade in  
247 response to an herbivore attack that triggers the activation  
248 of diverse defensive signaling pathways controlled by  
249 phytohormones, we determined the levels of different  
250 phytohormones in the apical part (apical bud with tender  
251 developing stem and leaves) of *N. tenuis*-punctured tomato  
252 plants (plants exposed to 25 *N. tenuis* adults for 24 h prior  
253 to the assay) compared to intact plants. The hormones  
254 ABA, indole-3-acetic acid (IAA), salicylic acid (SA), JA,  
255 12-oxo-phytodienoic acid (OPDA) and JA-isoleucine (JA-  
256 Ile) were analyzed by ultra-performance liquid chroma-  
257 tography coupled to mass spectrometry (UPLC-MS) (Flors  
258 et al. 2008; Forcat et al. 2008). Fresh material from intact  
259 and *N. tenuis*-punctured plants was frozen in liquid

260 nitrogen and lyophilized. Before extraction, a mixture of  
261 internal standards containing 100 ng d6ABA, 100 ng  
262 d6IAA and 100 ng dhJA was added. Dry tissue (0.05 g)  
263 was immediately homogenized in 2.5 ml of ultrapure  
264 water.

265 After centrifugation (5,000×g, 40 min), the supernatant  
266 was recovered and adjusted to pH 2.8 with 6 % acetic acid  
267 and subsequently partitioned twice against an equal volume  
268 of diethyl ether. The aqueous phase was discarded, and the  
269 organic fraction was evaporated in a Speed Vacuum Con-  
270 centratator (Eppendorf; <http://www.eppendorf.com>) at room  
271 temperature. The solid residue was re-suspended in 1 ml of a  
272 methanol/water (10:90) solution and filtered through a 0.22-  
273 µm cellulose acetate filter (13 mm pk/100 TR-200430.  
274 Olimpeak. Teknokroma, Barcelona, Spain). A 20-µl aliquot  
275 of this solution was then directly injected into the HPLC  
276 system. Analyses were carried out using a Waters Alliance  
277 2690 HPLC system (Waters, <http://www.waters.com/>) with a  
278 Kromasil reversed phase column (100 2 mm i.d.; 5 µm;  
279 Scharlabl, <http://www.scharlab.es>). The chromatographic  
280 system was interfaced with a Quatro LC (quadrupole-hexa-  
281 pole-quadrupole) mass spectrometer (Micromass; [http://](http://www.micromass.co.uk)  
282 [www.micromass.co.uk](http://www.micromass.co.uk)). MASSLYNX NT software version  
283 4.1 (Micromass) was used to process the quantitative data  
284 from calibration standards and the plant samples. The cali-  
285 bration curves were obtained by using solutions containing  
286 increasing amounts of ABA, JA, SA, IAA and OPDA  
287 commercial standards (Sigma-Aldrich, [http://www.sigma-](http://www.sigma-aldrich.com/)  
288 [aldrich.com/](http://www.sigma-aldrich.com/)) and JA-Ile (kindly provided by Edward  
289 Farmer, University of Lausanne, Switzerland) and a fixed  
290 amount of the corresponding internal standard.

## 291 ABA- and JA-induced responses

292 Because the ABA pathway is mainly activated in response  
293 to abiotic stresses such as water stress or desiccation (Kahn  
294 et al. 1993; Maskin et al. 2001; Ramirez et al. 2009), and  
295 this is a symptom that *N. tenuis* produces in tomato plants  
296 (Calvo et al. 2009), we decided to explore the effect of  
297 ABA-induced responses on the preference of the herbivore  
298 *B. tabaci*. For this purpose, the ABA-deficient tomato  
299 mutant *Sitiens* and its near-isogenic wild-type (*wt*) parental  
300 line were assessed (Asselbergh et al. 2007; Rodriguez et al.  
301 2010) in the laboratory using an olfactometer. We also  
302 compared the response of whiteflies to the volatiles emitted  
303 from intact *wt* tomato plants and intact *wt* tomato plants  
304 treated with exogenous ABA. Ten milliliters of 100 µM  
305 ABA solution (Sigma, St Louis, MO, USA) per plant was  
306 applied as a soil drench to 6-week-old plants to mimic the  
307 response induced by *N. tenuis*-punctured plants. Twenty-  
308 four hours later, plants were used for the Y-tube experi-  
309 ments. Additionally, the *ASRI* (abscisic acid stress ripening  
310 protein) transcriptional response of the apical part of intact

*wt* and *N. tenuis*-punctured tomato plants (var. Rheinlands) 311  
was obtained. Total RNA was extracted from the leaves of 312  
three plants, converted to cDNA and subjected to quanti- 313  
tative RT-PCR analysis (see below for more details). 314

315 Because many previous studies have demonstrated that 315  
the JA signaling pathway is involved in the attraction of 316  
natural enemies (Erb et al. 2012), we decided to investigate 317  
whether the JA signaling pathway induced by the plant- 318  
feeding behavior of *N. tenuis* might be attractive to the 319  
whitefly parasitoid *E. formosa*. For this purpose, we used 320  
the JA-deficient tomato mutant *def-1* and its near-isogenic 321  
wild-type (*wt*) parental line (Vicedo et al. 2009; O'Donnell 322  
et al. 2003) with or without *N. tenuis* feeding punctures. 323  
Additionally, the *PIN2* (a JA-regulated defense protein) 324  
transcriptional response of the apical part of intact *wt* and 325  
*N. tenuis*-punctured tomato plants (var. Castlemart) was 326  
determined. Total RNA was extracted from the leaves of 327  
three plants, converted to cDNA and subjected to quanti- 328  
tative RT-PCR analysis (see below for more details). 329

## 330 Induction of defensive responses in neighboring plants

331 The preference of *B. tabaci* and *E. formosa* for plants that 331  
had not been in contact with the mirid but had been placed 332  
in close contact with *N. tenuis*-punctured plants or intact 333  
plants was investigated in the laboratory using an olfacto- 334  
meter. We placed tomato plants that had been exposed to 335  
*N. tenuis* the day prior together with tomato plants that had 336  
not been exposed to *N. tenuis* (hereafter HIPV-exposed 337  
plants) for 24 h following the methodology described 338  
above. Five independent replicates were performed. The 339  
*ASRI* (abscisic acid stress ripening protein) and *PIN2* (a 340  
JA-regulated defense protein) transcriptional response of 341  
the apical part of intact, HIPV-exposed and *N. tenuis*- 342  
punctured tomato plants was determined. Total RNA was 343  
extracted from the apical part of the plants, converted to 344  
cDNA and subjected to quantitative RT-PCR analysis (see 345  
the following section for more details). 346

## 347 Quantification of plant gene expression

348 Transcription of the genes *ASRI* and *PIN2*, a proteinase 348  
inhibitor, was analyzed (Lopez-Raez et al. 2010). The 349  
apical part of the tomato plants (as explained above) was 350  
ground in liquid nitrogen, and a portion was used for RNA 351  
extraction. Total RNA (1.5 µg) extracted by the Plant RNA 352  
Kit (Omega Bio-Tek Inc., Doraville, GA, USA) was treated 353  
with RNase-free DNase (Promega Corp., Madison, WI, 354  
USA) to eliminate genomic DNA contamination. The RT 355  
reaction was performed by adding 2 µl of RT buffer, 2 µl 356  
of 5 mM dNTP, 2 µl of 10 µM Oligo(dT) 15 primer 357  
[Promega, Oligo(dT)15 Primer], 1 µl of 10 U/µl RNase 358  
inhibitor (Promega RNasin RNase inhibitor) and 1 µl of 359

360 Omniscript reverse transcriptase (Qiagen, Barcelona,  
361 Spain). The reaction mixture was incubated at 37 °C for  
362 60 min. Complementary DNA from the RT reaction,  
363 diluted ten-fold, was used for qPCR. Forward and reverse  
364 primers (0.3 μM) were added to 12.5 μl of PCR SYBR  
365 reaction buffer and 2 μl of cDNA, then brought to 25 μl  
366 total volume by Milli-Q sterile water (Takara Bio, Kyoto,  
367 Japan). Quantitative PCR was carried out using the Smart  
368 Cycler II (Cepheid, Sunnyvale, CA USA) sequence  
369 detector with standard PCR conditions. There were dif-  
370 ferences in the cycle numbers during the linear amplifica-  
371 tion phase for different samples. The data were transformed  
372 with the formula  $2\Delta Ct$ . RT-qPCR analysis was performed  
373 at least three times using sets of cDNA samples of inde-  
374 pendent experiments. Expression of *EFL* (elongation fac-  
375 tor-1) was used as a standard control gene for  
376 normalization. The nucleotide sequences of the gene-spe-  
377 cific primers are described in Table S1.

### 378 Data analyses

379  $\chi^2$  Tests were used to test the hypothesis that the distri-  
380 bution of side-arm choices between pairs of odors deviated  
381 from a null model where odor sources were chosen with  
382 equal frequency. Females that did not make a choice were  
383 excluded from the statistical analysis. The results were  
384 expressed as the mean  $\pm$  SE. Significant differences  
385 ( $P < 0.05$ ) were determined with a one-tailed Student's  
386  $t$  test performed in a pairwise manner for the concentration  
387 of each phytohormone. One-way ANOVA followed by a  
388 comparison of means (Tukey's test) was applied to identify  
389 differences in the transcriptional responses of the *ASR1* and  
390 *PIN2* genes in the apical parts of intact, induced and *N.*  
391 *tenuis*-feeding punctured tomato plants.

## 392 Results

### 393 *N. tenuis* feeding influences *B. tabaci* plant selection

394 Whitefly females were attracted to the odor of tomato plants  
395 over clean air ( $\chi^2 = 18.29$ ,  $P < 0.0001$ ; Fig. 1a) in a Y-tube  
396 olfactometer. Plants experiencing *N. tenuis* feeding activity  
397 proved to be less attractive to *B. tabaci* than intact plants  
398 ( $\chi^2 = 6.25$ ,  $P = 0.0124$ ; Fig. 1a). The repellence effect of  
399 *N. tenuis* per se was discarded based on the results of the  
400 first test where whitefly females were offered intact tomato  
401 plants that were either empty or contained two couples of  
402 *N. tenuis* each in two double-layer gauze bags (to prevent  
403 plant feeding) ( $\chi^2 = 1.724$ ,  $P = 0.1892$ ; Fig. 1a), indicating  
404 that whiteflies were not able to detect the mere presence of

*N. tenuis* on plants. Furthermore, intact plants on which *N.*  
*tenuis* was bagged were preferred relative to *N. tenuis*-  
punctured plants ( $\chi^2 = 16.20$ ,  $P < 0.0001$ ; Fig. 1a).

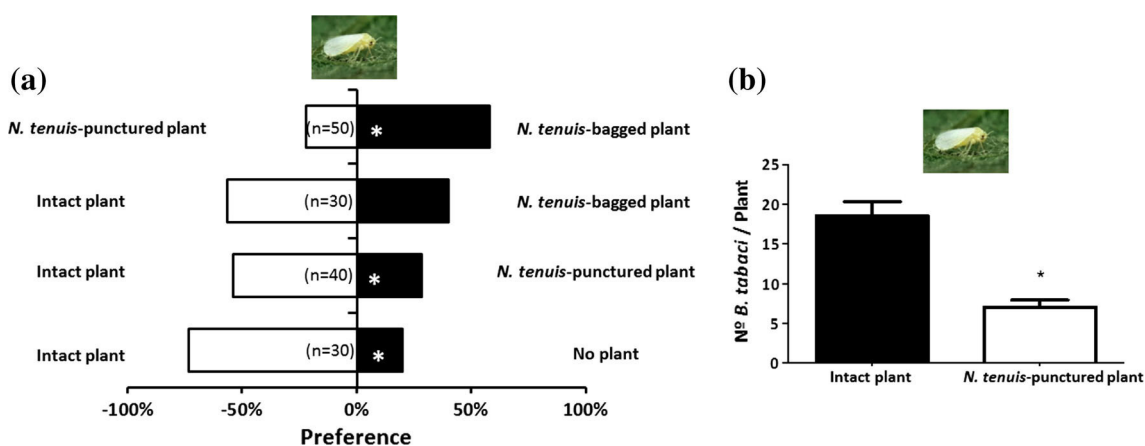
408 An additional semi-field choice test simulating com-  
409 mercial *N. tenuis* releases in tomato crops confirmed that  
410 whiteflies avoided *N. tenuis*-punctured tomato plants  
411 ( $t = 5.724$ ,  $P < 0.0001$ ; Fig. 1b).

### 412 *N. tenuis* plant feeding modifies the plant 413 phytohormone profile

414 The endogenous levels of ABA ( $t = 3.459$ ,  $P = 0.0086$ ;  
415 Fig. 2a) and the components of the JA pathway 12-oxo-  
416 phytodienoic acid (OPDA, a precursor of JA; Fig. 2b) and  
417 isoleucine conjugate of JA (JA-Ile, the bioactive form of  
418 JA; Fig. 2c) were higher in the apical part of *N. tenuis*-  
419 punctured plants ( $t = 2.472$ ;  $P = 0.0386$  and  $t = 3.936$ ;  
420  $P = 0.0043$  for OPDA and JA-Ile, respectively). Despite  
421 the trend of increased JA concentration in *N. tenuis*-  
422 punctured plants, the difference was not significant  
423 ( $t = 1.410$ ,  $P = 0.1962$ ; Fig. 2d), probably as a conse-  
424 quence of its conversion to other metabolic sinks such as  
425 JA-Ile (Fig. 2c). The levels of salicylic acid (SA) were  
426 similar in both treatments ( $t = 0.9849$ ,  $P = 0.1760$ ;  
427 Fig. 2f). In contrast, the indole-3-acetic acid (IAA) content  
428 was lower in *N. tenuis*-punctured plants ( $t = 2.662$ ,  
429  $P = 0.0287$ ; Fig. 2e). Therefore, alteration of the phyto-  
430 hormone profiling of tomato plants by *N. tenuis* activity  
431 was demonstrated.

### 432 ABA-induced repellence on whiteflies

433 Given a choice between intact *wt* plants and *N. tenuis*-  
434 punctured *wt* plants, *B. tabaci* chose the plant not in contact  
435 with the mirid ( $\chi^2 = 22.22$ ,  $P < 0.001$ ; Fig. 3a), as  
436 expected from the results above. The ABA mutant tomato  
437 plants were preferred over the intact *wt* plants by whiteflies  
438 ( $\chi^2 = 10.29$ ,  $P = 0.0013$ ; Fig. 3a). Accordingly, whiteflies  
439 did not show a significant preference ( $\chi^2 = 0.2857$ ,  
440  $P = 0.5930$ ; Fig. 3a) for ABA-mutant plants that were or  
441 were not exposed to mirids. The ABA-mutant tomato  
442 plants with *N. tenuis* feeding punctures were preferred over  
443 *N. tenuis*-punctured *wt* plants ( $\chi^2 = 18.00$ ,  $P < 0.001$ ;  
444 Fig. 3a). A strongly significant *B. tabaci* preference was  
445 observed for plants that were not watered with exogenous  
446 ABA ( $\chi^2 = 30.41$ ,  $P < 0.001$ ; Fig. 3a). Transcriptional  
447 analysis showing that *N. tenuis*-punctured plants expressed  
448 higher levels of the ABA-responsive *ASR1* gene than intact  
449 plants confirmed that the insect-infested plants contained  
450 higher levels of the phytohormone ABA ( $t = 2.228$ ,  
451  $P = 0.0449$ ; Fig. 3b).



**Fig. 1** *Bemisia tabaci* plant selection mediated by *Nesidiocoris tenuis*. **a** Response of the herbivore *B. tabaci* females in a Y-tube olfactometer when exposed to intact tomato plants, intact tomato plants containing two pairs of the zoophytophagous *N. tenuis* in two double-layer gauze bags (to prevent plant feeding and oviposition) (*N. tenuis*-bagged plant) or tomato plants that had been exposed to 25 *N. tenuis* adults for 24 h prior to the assay (*N. tenuis*-punctured plants).

Significant differences based on a  $\chi^2$  test are marked with (\*) ( $P < 0.001$ ). **b** Number of *B. tabaci* adults per plant ( $X \pm SE$ ) captured 24 h after releasing 100 *B. tabaci* in the center of a circle in which three intact plants and three *N. tenuis*-punctured plants were evenly distributed inside a cage. Significant differences based on a *t* test are marked with (\*) ( $P < 0.001$ )

452 JA-induced attraction of the parasitoid *Encarsia*  
453 *formosa*

454 The wasp *E. formosa* significantly chose *N. tenuis*-punctured  
455 *wt* plants or intact *wt* plants (Fig. 4a;  $\chi^2 = 30.41$ ,  
456  $P < 0.001$ ) over JA-deficient mutant plants whether in  
457 contact with the mirids ( $\chi^2 = 30.41$ ,  $P < 0.001$ ; Fig. 4a) or  
458 not ( $\chi^2 = 30.41$ ,  $P < 0.001$ ; Fig. 4a). To confirm that *N.*  
459 *tenuis*-punctured plants had higher JA expression, the *PIN2*  
460 transcriptional response of the apical part of both types of  
461 tomato plants was analyzed ( $t = 5.112$ ,  $P = 0.035$ ;  
462 Fig. 4b). This clear effect showed that *N. tenuis* activity  
463 resulted in attraction of the parasitoid *E. formosa*.

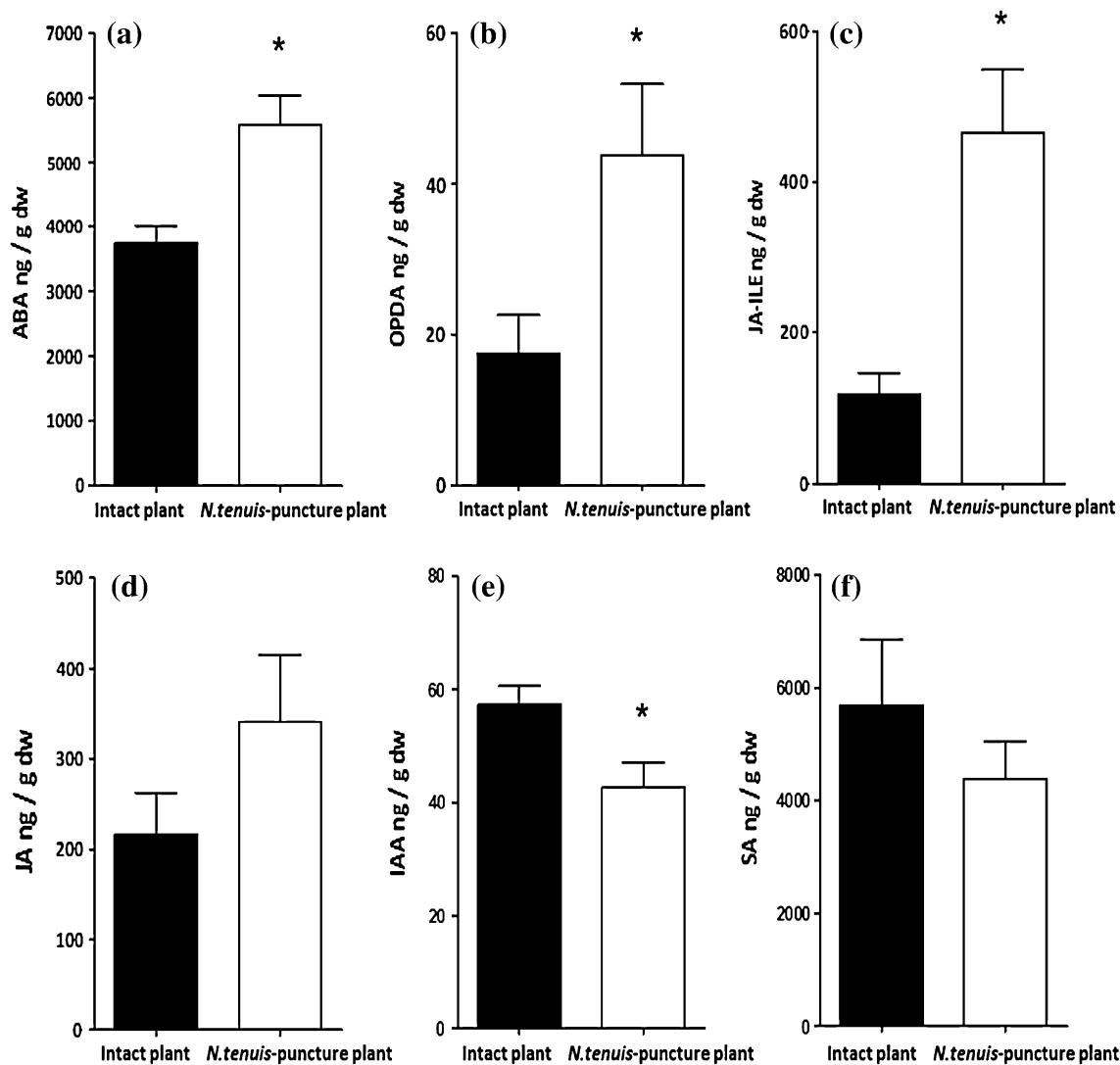
464 *N. tenuis*-punctured plants induce plant defenses  
465 in intact plants

466 The whitefly *B. tabaci* did not show any preference between  
467 HIPV-exposed plants or intact plants ( $\chi^2 = 0.00$ ,  $P = 1$ ;  
468 Fig. 5a). However, the parasitoid *E. formosa* was significantly  
469 attracted to HIPV-exposed tomato plants relative to intact ones  
470 ( $\chi^2 = 14.00$ ,  $P = 0.0002$ ; Fig. 5a). To confirm the hypothesis  
471 that exposure to HIPVs from *N. tenuis*-damaged plants indu-  
472 ces defenses of intact plants, we measured the transcriptional  
473 response of the genes *ASR1* and *PIN2* as a measure of ABA  
474 and JA expression, respectively, for intact, HIPV-exposed and  
475 *N. tenuis*-punctured plants as in the above experiments. The  
476 two studied genes, *ASR1* ( $F = 19.33$ ,  $P = 0.0009$ ; Fig. 5b)  
477 and *PIN2* ( $F = 20.79$ ,  $P = 0.0004$ ; Fig. 5c), were upregu-  
478 lated when the tomato plant was exposed to HIPVs from *N.*  
479 *tenuis*-damaged plants, as demonstrated above. More inter-  
480 estingly, and in accordance with the results obtained in the

481 olfactometer, the amounts of these two transcripts of defense-  
482 related genes were different in HIPV-exposed plants com-  
483 pared to *N. tenuis*-punctured plants. The induction of defenses  
484 had no effect on *ASR1* expression compared with intact plants,  
485 while *PIN2* reached the same levels in HIPV-exposed and *N.*  
486 *tenuis*-punctured plants, confirming the potential of HIPVs  
487 from *N. tenuis*-damaged plants to activate plant defenses in  
488 neighboring, undamaged plants via JA, resulting in attraction  
489 of parasitoids.

## 490 Discussion

491 During the last decade, biological control programs using  
492 mirids (Calvo et al. 2012a), which can feed on both plant  
493 tissues and insect prey (Castañé et al. 2011), have been  
494 effectively implemented in greenhouse tomatoes and other  
495 crops. To date, the success of these predators has been  
496 mainly attributed to their efficient predation of a wide  
497 range of important pests (Urbaneja et al. 2009; Calvo et al.  
498 2012b; Pérez-Hedo and Urbaneja 2014) and to their phy-  
499 topathy (Calvo et al. 2009), which allows them to become  
500 established prior to pest appearance and to maintain their  
501 populations in periods of prey scarcity. Remarkably, *N.*  
502 *tenuis* was formerly considered a tomato pest because of  
503 feeding-based damage such as necrotic rings in apical  
504 stems (Raman and Sanjayan 1984; Calvo et al. 2009) when  
505 prey is scarce. However, thanks to proper management  
506 (exhaustive monitoring and adoption of corrective mea-  
507 sures when needed), this predator has shifted from being  
508 considered a pest to becoming a key biological control  
509 agent for successful pest management (Calvo et al. 2012a).



**Fig. 2** Effect of *Nesidiocoris tenuis* injury on different phytohormone levels of **a** ABA, **b** OPDA, **c** JA-Ile **d** JA, **e** IAA and **f** SA in the apical part of tomato plants. The results shown are mean hormone

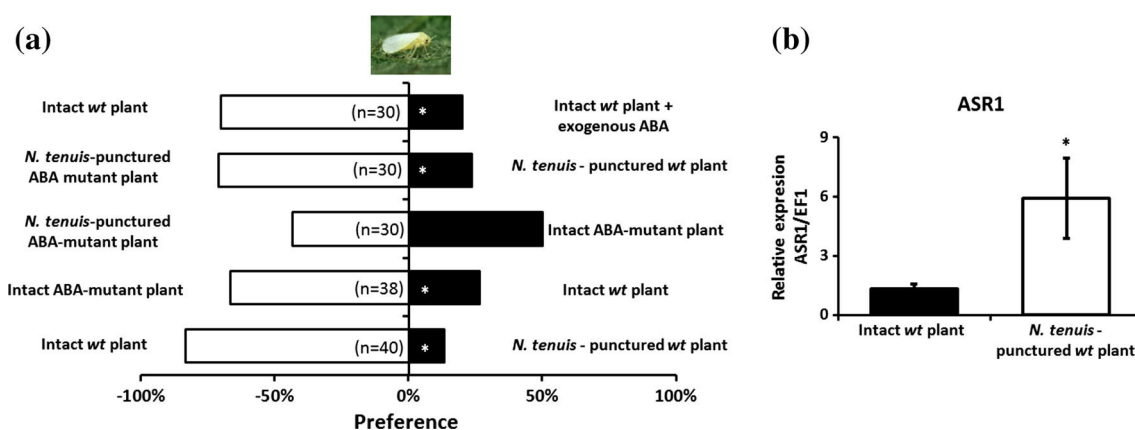
levels of five independent analyses  $\pm$  SE ( $n = 5$ ). Significant differences based on a *t* test are marked with (\*) ( $P < 0.05$ )

510 Our results (see Fig. 6 for a graphical summary) confirm  
 511 that the activity of a zoophytophagous insect induces a  
 512 physiological response in plants (Kessler and Baldwin  
 513 2004; Halitschke et al. 2011) similar to that induced by  
 514 strictly phytophagous mirid species (Rodriguez-Saona  
 515 et al. 2002). Specifically, the insect triggers synthesis of  
 516 HIPVs, which make plants less attractive to herbivores,  
 517 attract natural enemies and induce defenses in neighboring  
 518 plants, which undoubtedly strongly contribute to the suc-  
 519 cess of these predators as invertebrate biological control  
 520 agents.

521 Our results confirmed that the plant-feeding behavior of  
 522 *N. tenuis* significantly changed the phytohormone levels of  
 523 tomato plants. The zoophytophagous predator activates the  
 524 ABA, IAA and JA signaling pathways. However, levels of

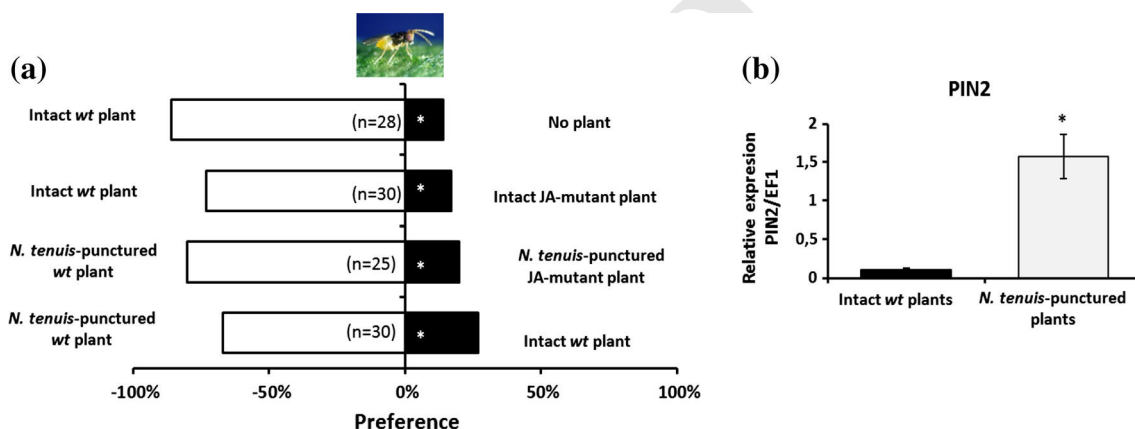
the phytohormone SA, which has been considered an her-  
 525 bivore repellent in many previous studies (Erb et al. 2012),  
 526 were not significantly different between *N. tenuis*-punc-  
 527 tured plants and intact plants. Wei et al. (2014) demon-  
 528 strated that there are antagonistic effects of SA-mediated  
 529 responses on JA-mediated responses and vice versa. In  
 530 addition, the dose and timing of phytohormone levels may  
 531 affect the behavioral responses of an herbivore. Therefore,  
 532 the crosstalk between SA- and JA-dependent defense  
 533 responses to plant feeding by *N. tenuis* deserves further  
 534 research.  
 535

536 Although ABA involvement in multiple physiological  
 537 processes in response to abiotic stresses and pathogen  
 538 attacks has been shown (Leung and Giraudat 1998; Erb  
 539 et al. 2012), its relationship to herbivory is still poorly



**Fig. 3** ABA-induced non-attraction of whiteflies. **a** Response of the herbivore *Bemisia tabaci* females in a Y-tube olfactometer when exposed to ABA-deficient mutant tomato plants or their near isogenic wild type (*wt* plant), which were with the zoophytophagous *Nesidiocoris tenuis* (*N. tenuis*-punctured plants) or without (intact plants) contact with *N. tenuis* or *wt* plant irrigated with 10 ml of 100  $\mu$ M ABA 24 h before the assay. Significant differences using a  $\chi^2$  test are marked with (\*) ( $P < 0.001$ ). **b** Transcriptional response of the apical

part of intact *wt* and *N. tenuis*-punctured tomato plants (var. Rheinlands) for the *ASR1* gene, which is ABA responsive. Transcript levels were normalized to the expression of *EF1 $\alpha$*  measured in the same sample. Data are presented as a mean of three independent analyses of transcript expression relative to the housekeeping gene plants  $\pm$  SE ( $n = 3$ ). Significant differences using a *t* test are marked with (\*) ( $P < 0.05$ )



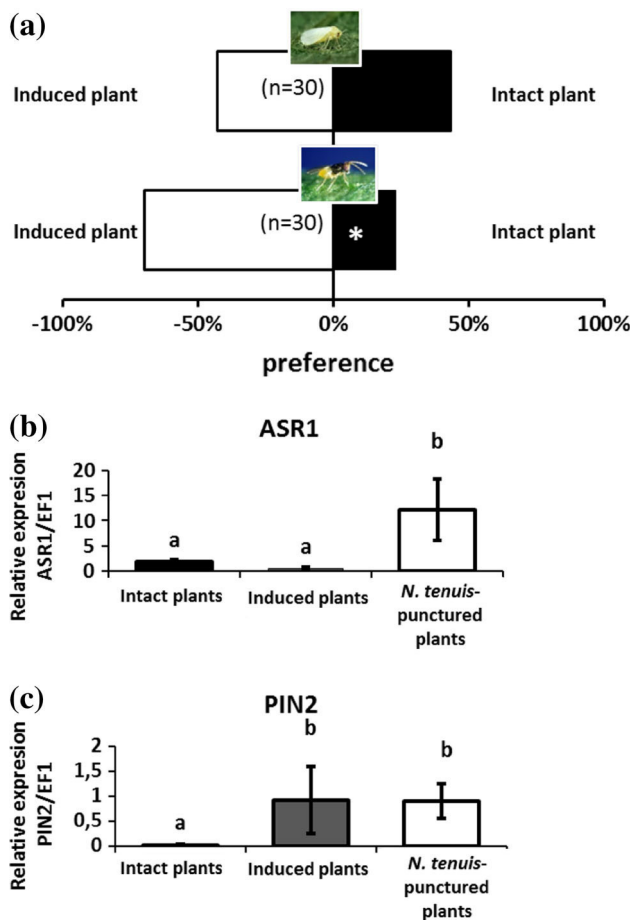
**Fig. 4** JA-induced attraction to the parasitoid *Encarsia formosa*. **a** Response of *E. formosa* females in a Y-tube olfactometer when exposed to JA-mutant tomato plants or their near isogenic wild type (*wt* plants) in contact with the zoophytophagous *Nesidiocoris tenuis* (*N. tenuis*-punctured plants) or not in contact (intact plants) with *N. tenuis*. Significant differences using a  $\chi^2$  test are marked with (\*)

( $P < 0.001$ ). **b** *PIN2* transcriptional response, which is JA responsive, in the apical part of intact *wt* and *N. tenuis*-punctured tomato plants (var. Castlemart). The data are presented as the mean of three independent analyses of transcript expression relative to housekeeping gene plants  $\pm$  SE ( $n = 3$ ). Significant differences based on a *t* test are marked with (\*) ( $P < 0.05$ )

540 documented (Bodenhausen and Reymond 2007). Our  
541 results show that *B. tabaci* did not reject induced tomato  
542 plants where the ABA pathway, as opposed to the JA  
543 pathway, had not been altered. We have demonstrated that  
544 an intact ABA pathway, which is the pathway activated by  
545 *N. tenuis* activity, is needed to make the plant less attrac-  
546 tive to whiteflies, while JA is not directly related to this  
547 antixenotic response. The ABA pathway is mainly acti-  
548 vated in response to abiotic stresses such as water stress or  
549 desiccation (Kahn et al. 1993; Maskin et al. 2001; Ramirez

et al. 2009). Therefore, the ABA pathway signaling acti- 550  
vated by *N. tenuis* could simply be the response of the 551  
tomato plant to water-content reduction (and logically 552  
other supplementary nutrients) caused by feeding of *N.* 553  
*tenuis*, which is mostly detectable in the form of necrotic 554  
rings in the apical stems of the plant (Castañé et al. 2011). 555  
Therefore, it might be reasonable that whiteflies recognize 556  
plants emitting HIPVs triggered through the ABA pathway 557  
as stressed plants and consequently as less suitable for the 558  
progeny. Another possible explanation for *B. tabaci* 559





**Fig. 5** *Nesidiocoris tenuis*-punctured plant induces plant defenses in intact plants. **a** Response of the herbivore *Bemisia tabaci* and the parasitoid *Encarsia formosa* females in a Y-tube olfactometer when exposed to intact and induced (plants that had not been in contact with the mirid but had been placed in close contact with *N. tenuis*-punctured plants for 24 h) tomato plants. Significant differences based on a  $\chi^2$  test are marked with (\*) ( $P < 0.001$ ). **b** and **c** *ASR1* (**b**) and *PIN2* (**c**) transcriptional responses, which are ABA and JA responsive, respectively, in the apical part of intact, induced and *N. tenuis*-punctured tomato plants. Data are presented as the mean of four independent analyses of transcript expression relative to a housekeeping gene  $\pm$  SD ( $n = 4$ ). Different letters over the bars indicate significant differences ( $P < 0.05$ ) based on Tukey comparisons

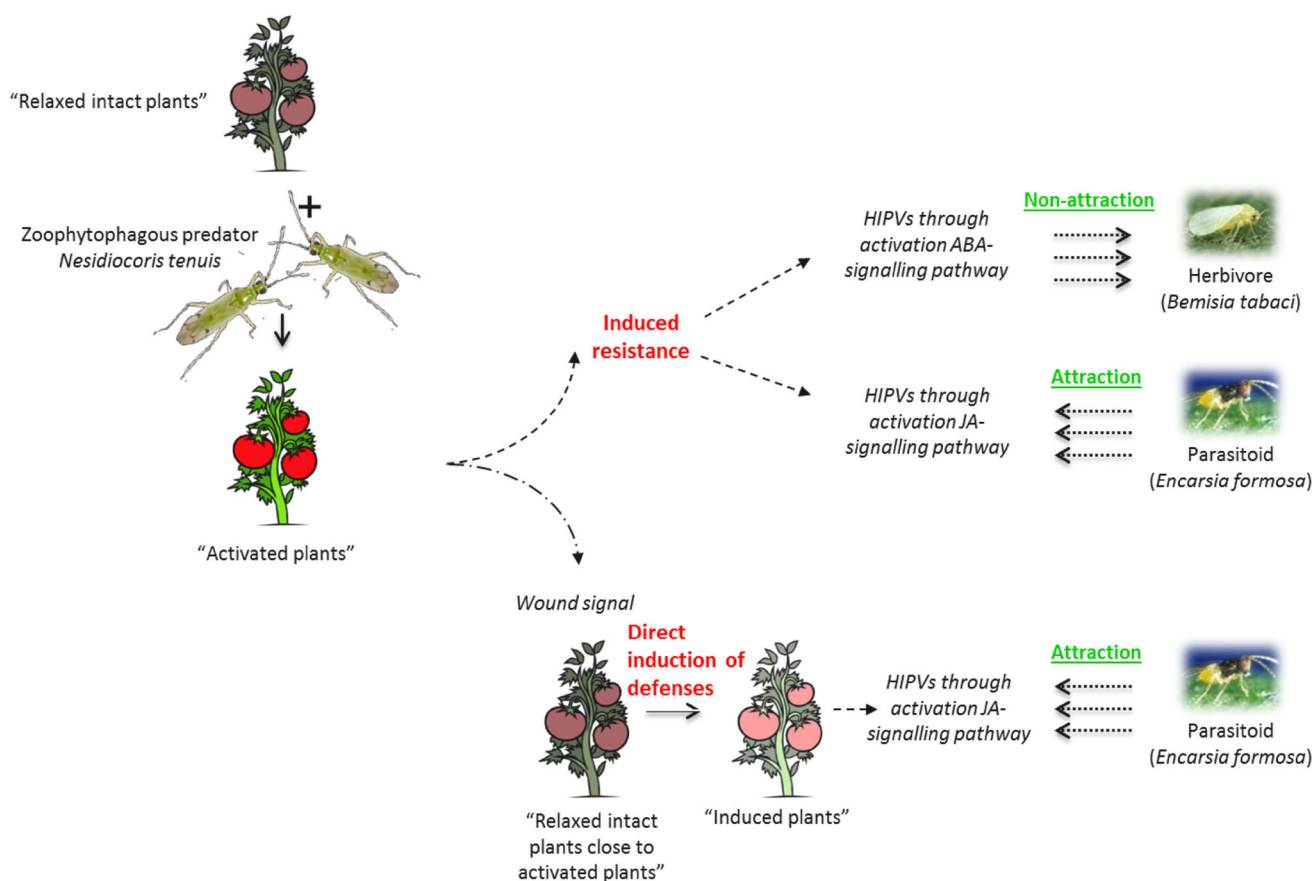
560 rejection is that heavily *B. tabaci*-infested tomato plants  
 561 could induce a plant response similar to that caused by *N.*  
 562 *tenuis*, i.e., activation of the ABA pathway, given that both  
 563 hemipterans have piercing-sucking mouthparts and feed on  
 564 vascular bundles, particularly phloem tissue and the  
 565 neighboring parenchyma cells (Raman and Sanjayan 1984;  
 566 Walker 2010). Thus, whiteflies could also identify plants  
 567 emitting HIPVs triggered by the ABA pathway signaling as  
 568 plants already highly populated by conspecific whiteflies,  
 569 which would impair the successful development of their  
 570 progeny through increased competition. However, further  
 571 research is required to distinguish between these two  
 572 hypotheses.

The endogenous JA levels of the tomato plant strongly  
 affected the response of the parasitoid *E. formosa*. This  
 parasitoid significantly exhibited a preference for *N. tenuis*-  
 punctured plants, which have higher JA expression  
 relative to intact plants. Previous studies have demon-  
 strated the role of JA in indirect defense mechanisms,  
 which results in attraction of natural enemies to plants  
 (Heil 2008; Dicke 2009). The reason why this whitefly  
 parasitoid is capable of detecting *N. tenuis*-punctured  
 plants is unlikely to be related to the presence of the  
 zoophytophagous predator, given that on those plants the  
 parasitoid would encounter a lower whitefly population.  
 Therefore, we believe that the parasitoid is able to relate  
 the presence of HIPVs triggered by the activation of JA  
 pathway with a high presence of suitable hosts on these  
 plants, which induces physiological defense responses as  
 we hypothesized above.

We have observed that tomato plants activate defense  
 systems because of the wounding by *N. tenuis*. It is known  
 that some plants appear to respond to environmental cues  
 that reliably indicate an increased probability of attack  
 before they actually experience an herbivore or pathogen  
 (Frost et al. 2008; Muroi et al. 2011; Shiojiri et al. 2012).  
 We initially wondered whether HIPVs from *N. tenuis*-  
 infested plants could induce plant defenses in neighboring,  
 uninfested tomato plants and therefore could activate the  
 mechanisms of avoidance of *B. tabaci* and attraction of *E.*  
*formosa*. As noted earlier, our results show that *B. tabaci*  
 did not reject HIPV-exposed plants, while the parasitoid  
 was strongly attracted by HIPV-exposed plants. Further  
 research is needed to better understand the variables  
 associated with this interesting phenomenon both from a  
 basic point of view (why only the JA pathway is activated)  
 and for application in crop protection practices (how long  
 the plant's response to HIPVs is effective).

The apical IAA content was also increased in *N. tenuis*-  
 punctured plants. This phytohormone coordinates devel-  
 opment in plants (Sachs and Thimann 1967). Therefore, we  
 hypothesize that *N. tenuis* feeding on the apex, which may  
 affect plant growth, partially blocks auxin-mediated apical  
 dominance. However, whether IAA is mediating an effect  
 (repellence or attraction) on herbivores or natural enemies  
 needs further research.

In summary, we have proven that the zoophytophagous  
 predator *N. tenuis* induces plant benefits not only directly  
 by its entomophagy but also indirectly by its phytophagy  
 through an increase in the attraction of the whitefly para-  
 sitoid *E. formosa* (an indirect mechanism of defense) and  
 antixenosis to *B. tabaci* (a direct mechanism of resistance).  
 Furthermore, chemical attraction of a natural enemy could  
 be induced in neighboring plants. Our results might be one  
 reasonable explanation for the great success achieved by *N.*  
*tenuis* as a key biocontrol agent in tomatoes.



**Fig. 6** A conceptual model of plant benefits indirectly caused by the zoophytophagous predator *Nesidiocoris tenuis*. At the top left of the flow chart, a relaxed tomato plant is induced by *N. tenuis* feeding. *N. tenuis* feeding activated abscisic acid (ABA) and jasmonic acid (JA)-signaling pathways in tomato plants, which resulted in a non-

preference effect on the whitefly *B. tabaci* and in attraction of the whitefly parasitoid *Encarsia formosa*. Some of the chemical changes in the punctured plant may act as wound signals to undamaged adjacent tomato plants. The JA pathway is activated in induced tomato plants, which results in attraction to the parasitoid *E. formosa*

## 626 Author contribution statement

627 MP-H and AU designed the research. All authors per-  
628 formed the research, and MP-H and AU wrote the paper.  
629 MP-H, VF and AU analyzed the data. All authors com-  
630 mented on the manuscript.

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641 **Conflict of interest** The authors declare that they have no conflict  
642 of interest.  
643

## References

- 644  
645 Asselbergh B, Curvers K, Franca SC, Audenaert K, Vuylsteke M, Van  
646 Breusegem F, Hofte M (2007) Resistance to *Botrytis cinerea* in  
647 sitiens, an abscisic acid-deficient tomato mutant, involves timely  
648 production of hydrogen peroxide and cell wall modifications in  
649 the epidermis. *Plant Physiol* 144:1863–1877  
650 Bennett RN, Wallsgrave RM (1994) Secondary metabolites in plant  
651 defense-mechanisms. *New Phytol* 127:617–633  
652 Bodenhausen N, Reymond P (2007) Signaling pathways controlling  
653 induced resistance to insect herbivores in *Arabidopsis*. *Mol Plant  
654 Microbe Interact* 20:1406–1420  
655 Bonaventure G, VanDoom A, Baldwin IT (2011) Herbivore-associated  
656 elicitors: FAC signaling and metabolism. *Trends Plant Sci* 16:294–299  
657 Bueno VHP, van Lenteren JC, Lins JC, Calixto AM, Montes FC,  
658 Silva DB, Santiago LD, Pérez LM (2013) New records of *Tuta  
659 absoluta* (Meyrick) (Lepidoptera:Gelechiidae) predation by  
660 Brazilian Hemipteran predatory bugs. *J App Entomol* 137:29–34  
661 Calvo J, Blockmans K, Stansly PA, Urbaneja A (2009) Predation by  
662 *Nesidiocoris tenuis* on *Bemisia tabaci* and injury to tomato.  
663 *Biocontrol* 54:237–246

- 664 Calvo FJ, Lorente MJ, Stansly PA, Belda JE (2012a) Preplant release  
665 of *Nesidiocoris tenuis* and supplementary tactics for control of  
666 *Tuta absoluta* and *Bemisa tabaci* in greenhouse tomato. Entomol  
667 Exp Appl 143:111–119
- 668 Calvo FJ, Soriano J, Bolckmans K, Belda JE (2012b) A successful  
669 method for whitefly and *Tuta absoluta* control in tomato.  
670 Evaluation after two years of application in practice. IOBC/  
671 WPRS Bull 80:237–244
- 672 Castañé C, Arnó J, Gabarra R, Alomar O (2011) Plant damage to  
673 vegetable crops by zoophytophagous mirid predators. Biol  
674 Control 59:22–29
- 675 Chen MS (2008) Inducible direct plant defense against insect  
676 herbivores: a review. Insect Sci 15:101–114
- 677 Choh Y, Takabayashi J (2006) Herbivore-induced extrafloral nectar  
678 production in lima bean plants enhanced by previous exposure to  
679 volatiles from infested conspecifics. J Chem Ecol 32:2073–2077
- 680 Coll M (1996) Feeding and ovipositing on plants by an omnivorous  
681 insect predator. Oecologia 105:214–220
- 682 Coll M, Guershon M (2002) Omnivory in terrestrial arthropods:  
683 mixing plant and prey diets. Annu Rev Entomol 47:267–297
- 684 Davis JM, Gordon MP, Smit BA (1991) Assimilate movement  
685 dictates remote sites of wound-induced gene-expression in  
686 poplar leaves. Proc Natl Acad Sci USA 88:2393–2396
- 687 De Puyseleir V, Hofte M, De Clercq P (2011) Ovipositing *Orius*  
688 *laevigatus* increase tomato resistance against *Frankliniella*  
689 *occidentalis* feeding by inducing the wound response. Arth-  
690 Plant Int 5:71–80
- 691 Desneux N, Wajnberg E, Wyckhuys K, Burgio G, Arpaia S, Narváez-  
692 Vasquez C, González-Cabrera J, Catalán-Ruescas D, Tabone E,  
693 Frandon J, Pizzol J, Poncet C, Cabello T, Urbaneja A (2010)  
694 Biological invasion of European tomato crops by *Tuta absoluta*:  
695 ecology, geographic expansion and prospects for biological  
696 control. J Pest Sci 83:197–215
- 697 Dicke M (2009) Behavioural and community ecology of plants that  
698 cry for help. Plant Cell Environ 32:654–665
- 699 Erb M, Meldau S, Howe GA (2012) Role of phytohormones in insect-  
700 specific plant reactions. Trends Plant Sci 17(5):250–259
- 701 Flors V, Ton J, van Doorn R, Jakab G, Garcia-Agustin P, Mauch-  
702 Mani B (2008) Interplay between JA, SA and ABA signalling  
703 during basal and induced resistance against *Pseudomonas*  
704 *syringae* and *Alternaria brassicicola*. Plant J 54:81–92
- 705 Forcat S, Bennett MH, Mansfield JW, Grant MR (2008) A rapid and  
706 robust method for simultaneously measuring changes in the  
707 phytohormones ABA, JA and SA in plants following biotic and  
708 abiotic stress. Plant Methods 4:16
- 709 Frost CJ, Mescher MC, Carlson JE, De Moraes CM (2008) Plant  
710 defense priming against herbivores: getting ready for a different  
711 battle. Plant Physiol 146:818–824
- 712 Hairston NG, Smith FE, Slobodkin LB (1960) Community structure,  
713 population control, and competition. Am Nat 94:421–425
- 714 Halitschke R, Hamilton JG, Kessler A (2011) Herbivore-specific  
715 elicitation of photosynthesis by mirid bug salivary excretions in  
716 the wild tobacco *Nicotiana attenuata*. New Phytol 191:528–535
- 717 Heil M (2008) Indirect defence via tritrophic interactions. New Phytol  
718 178:41–61
- 719 Heil M, Ton J (2008) Long-distance signalling in plant defence.  
720 Trends Plant Sci 13:264–272
- 721 Kahn TL, Fender SE, Bray EA, Oconnell MA (1993) Characterization  
722 of expression of drought and abscisic acid-regulated tomato  
723 genes in the drought-resistant species *Lycopersicon pennellii*.  
724 Plant Physiol 103:597–605
- 725 Kessler A, Baldwin I (2004) Herbivore-induced plant vaccination.  
726 Part I. The orchestration of plant defenses in nature and their  
727 fitness consequences in the wild tobacco *Nicotiana attenuata*.  
728 The Plant J 38:639–649
- Kratina P, LeCraw RM, Ingram T, Anholt BR (2012) Stability and  
729 persistence of food webs with omnivory: is there a general  
730 pattern? Ecosphere 3:50
- 731 Leung J, Giraudat J (1998) Abscisic acid signal transduction. Annu  
732 Rev Plant Phys 49:199–222
- 733 Lopez-Raez JA, Verhage A, Fernandez I, Garcia JM, Azcon-Aguilar  
734 C, Flors V, Pozo MJ (2010) Hormonal and transcriptional  
735 profiles highlight common and differential host responses to  
736 arbuscular mycorrhizal fungi and the regulation of the oxylipin  
737 pathway. J Exp Bot 61:2589–2601
- 738 Lu YH, Wu KM, Jiang YY, Guo YY, Desneux N (2012) Widespread  
739 adoption of Bt cotton and insecticide decrease promotes  
740 biocontrol services. Nature 487:362–365
- 741 Maskin L, Gudesblat GE, Moreno JE, Carrari FO, Frankel N,  
742 Sambade A, Rossi M, Iusem ND (2001) Differential expression  
743 of the members of the Asr gene family in tomato (*Lycopersicon*  
744 *esculentum*). Plant Sci 161:739–746
- 745 Messelink GJ, van Maanen R, van Steenpaal SEF, Janssen A (2008)  
746 Biological control of thrips and whiteflies by a shared predator:  
747 two pests are better than one. Biol Control 44:372–379
- 748 Muroi A, Ramadan A, Nishihara M, Yamamoto M, Ozawa R,  
749 Takabayashi J, Arimura G (2011) The composite effect of  
750 transgenic plant volatiles for acquired immunity to herbivory  
751 caused by inter-plant communications. PLoS One  
752 6:e24594
- 753 O'Donnell PJ, Schmelz E, Block A, Miersch O, Wasternack C, Jones  
754 JB, Klee HJ (2003) Multiple hormones act sequentially to  
755 mediate a susceptible tomato pathogen defense response. Plant  
756 Physiol 133:1181–1189
- 757 Oksanen L, Fretwell SD, Arruda J, Niemela P (1981) Exploitation  
758 ecosystems in gradients of primary productivity. Am Nat  
759 118:240–261
- 760 Pérez-Hedo M, Urbaneja A (2014) Prospects for predatory mirid bugs  
761 as biocontrol agents of aphids in sweet peppers. J Pest Sci.  
762 doi:10.1007/s10340-014-0587-1
- 763 Ramakers PMJ, Rabasse JM (1995) Integrated pest management in  
764 protected cultivation. Novel approaches to integrated pest  
765 management. CRC Press, Florida
- 766 Raman K, Sanjayan KP (1984) Histology and Histopathology of the  
767 Feeding Lesions by *Cyrtopeltis tenuis* Reut (Hemiptera, Miri-  
768 dae) on *Lycopersicon esculentum* Mill (Solanaceae). Proc Indian  
769 Acad Sci Anim Sci 93:543–547
- 770 Ramirez V, Coego A, Lopez A, Agorio A, Flors V, Vera P (2009)  
771 Drought tolerance in Arabidopsis is controlled by the OCP3  
772 disease resistance regulator. Plant J 58:578–591
- 773 Rodriguez JAM, Morcillo RL, Vierheilig H, Ocampo JA, Ludwig-  
774 Muller J, Garrido JMG (2010) Mycorrhization of the notabilis  
775 and sitiens tomato mutants in relation to abscisic acid and  
776 ethylene contents. J Plant Physiol 167:606–613
- 777 Rodriguez-Saona C, Crafts-Brandner SJ, Williams L III, Paré PW  
778 (2002) *Lygus hesperus* feeding and salivary gland extracts  
779 induce volatile emissions in plants. J Chem Ecol 28:1733–1747
- 780 Sachs T, Thimann V (1967) Role of auxins and cytokinins in release  
781 of buds from dominance. Am J Bot 54(1):136–144
- 782 Shiojiri K, Ozawa R, Matsui K, Sabelis MW, Takabayashi J (2012)  
783 Intermittent exposure to traces of green leaf volatiles triggers a  
784 plant response. Sci Rep 2:378
- 785 Stratmann JW (2003) Long distance run in the wound response—  
786 jasmonic acid is pulling ahead. Trends Plant Sci 8:247–250
- 787 Urbaneja A, Monton H, Molla O (2009) Suitability of the tomato  
788 borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and  
789 *Nesidiocoris tenuis*. J Appl Entomol 133:292–296
- 790 Urbaneja A, Gonzalez-Cabrera J, Arno J, Gabarra R (2012) Prospects  
791 for the biological control of *Tuta absoluta* in tomatoes of the  
792 Mediterranean basin. Pest Manag Sci 68:1215–1222
- 793

- 794 van Lenteren JC (2012) The state of commercial augmentative  
795 biological control: plenty of natural enemies, but a frustrating  
796 lack of uptake. *Biocontrol* 57:1–20
- 797 Vicedo B, Flors V, Leyva MD, Finiti I, Kravchuk Z, Real MD,  
798 Garcia-Agustin P, Gonzalez-Bosch C (2009) Hexanoic acid-  
799 induced resistance against *Botrytis cinerea* in tomato plants. *Mol*  
800 *Plant Microbe Interact* 22:1455–1465
- 801 Walker GPP, TM, Freeman, TP (2010) Life history, functional  
802 anatomy, feeding and mating behavior. In: Stanly PAN, S.E.  
803 (ed) *Bemisia*: bionomics and management of global pest.  
804 Springer Dordrecht
- 805 Wei JN, van Loon JJA, Gols R, Menzel TR, Li N, Kang L, Dicke M  
806 (2014) Reciprocal crosstalk between jasmonate and salicylate  
807 defence-signalling pathways modulates plant volatile emission  
808 and herbivore host-selection behaviour. *J Exp Bot* 65:3289–3298
- Zappala L, Biondi A, Alma A, Al-Jboory IJ, Arno J, Bayram A, 809  
Chailleux A, El-Arnaouty A, Gerling D, Guenaoui Y, Shaltiel- 810  
Harpaz L, Siscaro G, Stavrinides M, Tavella L, Aznar RV, 811  
Urbaneja A, Desneux N (2013) Natural enemies of the South 812  
American moth, *Tuta absoluta*, in Europe, North Africa and 813  
Middle East, and their potential use in pest control strategies. 814  
*J Pest Sci* 86:635–647 815
- Zhang ZP, Baldwin IT (1997) Transport of [2-C-14] jasmonic acid 816  
from leaves to roots mimics wound-induced changes in endog- 817  
enous jasmonic acid pools in *Nicotiana sylvestris*. *Planta* 818  
203:436–441 819

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