

# Terpenes from herbivore-induced tomato plant volatiles attract *Nesidiocoris tenuis* (Hemiptera: Miridae), a predator of major tomato pests

Pascal M Ayelo,<sup>a,b</sup> Abdullahi A Yusuf,<sup>b</sup> Christian WW Pirk,<sup>b</sup> Anaïs Chailleux,<sup>c,d</sup> Samira A Mohamed<sup>a</sup> and Emilie Deletre<sup>a,c\*</sup> 

## Abstract

**BACKGROUND:** Biological control plays a key role in reducing crop damage by *Tuta absoluta* (Meyrick) and *Trialeurodes vaporariorum* (Westwood), which cause huge yield losses in tomato (*Solanum lycopersicum* L.). The mirid predator *Nesidiocoris tenuis* (Reuter) preys heavily on these pests, with satisfying control levels in tomato greenhouses. Although *N. tenuis* is known to be attracted to volatiles of tomato plants infested by *T. absoluta* and whitefly, little is known about the specific attractive compounds and the effect of prey density on the predator response.

**RESULTS:** Y-tube olfactometer bioassays revealed that the attraction of *N. tenuis* to tomato volatiles was positively correlated with the density of *T. absoluta* infestation, unlike *T. vaporariorum* infestation. The predator was also attracted to volatiles of *T. absoluta* larval frass, but not to *T. vaporariorum* honeydew or *T. absoluta* sex pheromone. Among the herbivore-induced plant volatiles (HIPVs) that characterised the attractive plants infested with 20 *T. absoluta* larvae, olfactometer bioassays revealed that *N. tenuis* is attracted to the monoterpenes  $\alpha$ -pinene,  $\alpha$ -phellandrene, 3-carene,  $\beta$ -phellandrene and  $\beta$ -ocimene, whereas (*E*)- $\beta$ -caryophyllene was found to repel the predator. In dose-response bioassays, the five-component blend of the attractants elicited a relatively low attraction in the predator, and removal of  $\beta$ -phellandrene from the blend enhanced the attraction of the predator to the resulting four-component blend, suggesting synergism among four monoterpenes.

**CONCLUSION:** These findings suggest that a four-component blend of  $\alpha$ -pinene,  $\alpha$ -phellandrene, 3-carene and  $\beta$ -ocimene could be used as a kairomone-based lure to recruit the predator for the biological control of *T. absoluta* and *T. vaporariorum*.

© 2021 The Authors. *Pest Management Science* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

**Keywords:** biological control; kairomones; HIPVs; leafminer *Tuta absoluta*; whitefly *Trialeurodes vaporariorum*

## 1 INTRODUCTION

Biological control is based on the use of natural enemies to reduce the population density of pest organisms and their damage to field crops to below economic threshold levels.<sup>1</sup> Biological control is one of the most promising alternatives to spraying synthetic pesticides widely for the management of insect pests.<sup>2</sup> However, the use of pesticides has turned to be unsustainable for pest control due to development of resistance in the pest and negative impacts on the environment and on human health.<sup>2</sup> Four main biological control strategies are used to control insect pests: classical, augmentation, conservation and natural.<sup>3</sup> Classical biological control involves controlling an exotic pest by introducing a natural enemy from the pest's aboriginal home into the invaded area for long-term pest control.<sup>1,3</sup> Augmentation biological control is the periodic release of a natural enemy to enable rapid control of the pest.<sup>4</sup> Conservation biological control, on the other hand, does not require release of natural enemies, but habitat manipulation by human actions to provide resources to natural enemies, especially resident ones, to build up their populations in the target crop.<sup>5</sup> Natural biological control is pest control by

resident natural enemies without human intervention.<sup>3</sup> However, all these control strategies face challenges in term of retaining the populations of natural enemies in the target crop and limiting their emigration to keep them within the target crop to achieve a satisfactory level of pest control. These challenges may be overcome using semiochemical-based lures to attract and/or retain

\* Correspondence to: E Deletre, International Centre of Insect Physiology and Ecology, P.O. Box 30772-00100, Nairobi, Kenya, E-mail: emilie.deletre@cirad.fr

a International Centre of Insect Physiology and Ecology, Nairobi, Kenya

b Department of Zoology and Entomology, University of Pretoria, Hatfield, South Africa

c UPR HORTSYS, University of Montpellier, CIRAD, Montpellier, France

d Biopass2, Cirad-IRD-ISRA-UGB – Centre de coopération internationale en recherche agronomique pour le développement, Institut de Recherche pour le Développement-Institut Sénégalais de Recherches Agricoles, Université Gaston Berger, Dakar, Senegal

natural enemies in crop fields, thereby enhancing the biological control of insect pests.<sup>6–8</sup>

To locate their hosts or prey, natural enemies rely on specific kairomones contained in the chemical blends released by herbivore-infested plants or the infesting herbivores.<sup>8,9</sup> Herbivore-induced plant volatiles (HIPVs) act primarily as long-range kairomones for natural enemies in locating the target feeding and oviposition sites.<sup>8,10,11</sup> For instance, methyl salicylate, an HIPV released by rose plants infested by the potato aphid *Macrosiphum euphorbiae* (Thom.), enhanced the attraction of the predator *Chrysoperla rufilabris* Burmeister to field crops.<sup>12</sup> Similarly, natural enemies eavesdrop on the pheromones and other chemical cues derived from eggs, larvae/nymphs and adults or by-products (frass, honeydew, oral secretions) of their host or prey species to find them.<sup>9</sup>

The South American tomato pinworm or leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), and the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), are major pests of tomato worldwide.<sup>13–16</sup> Since the invasion of *T. absoluta* in Europe and sub-Saharan Africa (SSA), this pest has caused 80–100% yield losses to tomato when no control measures are applied.<sup>13,15</sup> Adults of the whiteflies *T. vaporariorum* and *Bemisia tabaci* (Gennadius) are vectors of viruses which in combination with the sap-sucking feeding behaviour of these pests cause 30–100% yield losses in the absence of control measures, posing a serious threat to tomato production in Europe and SSA.<sup>14,16,17</sup>

The use of zoophytophagous hemipteran predators for the biological control of insect pests has received considerable attention in recent years.<sup>18,19</sup> These predators can also feed on plants in the absence of prey, allowing them to survive.<sup>20</sup> The generalist predatory bug *N. tenuis* (Reuter) (Hemiptera: Miridae) plays a key role in suppressing the leafminer *T. absoluta* and the whitefly *T. vaporariorum* in tomato (*Solanum lycopersicum* L.) plants, preying heavily on eggs of the leaf miner, and on the third and fourth nymphal instars of the whitefly.<sup>21–23</sup> *N. tenuis* is native in Europe and SSA<sup>24,25</sup> where *T. absoluta* and *T. vaporariorum* are invasive pests.<sup>14,26</sup> Despite the huge potential of *N. tenuis* to suppress these pests,<sup>19,22,23</sup> countries that host the predator are still experiencing substantial tomato yield losses,<sup>13,15</sup> suggesting that the populations of this predator in the crop fields are too small to satisfactorily control the pests. The development of kairomone-based lures could help recruit and retain the predator on tomato crops, thereby improving biological control of *T. absoluta* and whiteflies. *N. tenuis* is known to be attracted to volatiles of tomato plants infested by its prey, *T. absoluta* and *B. tabaci*,<sup>27</sup> but the specific attractive compounds have not been identified. Terpenes are the dominant volatiles commonly found in plant secretions,<sup>28</sup> as well as in tomato plants infested by *T. absoluta* and whiteflies,<sup>29,30</sup> and these volatiles are known to play a key role in attracting natural enemies.<sup>31</sup> We thus hypothesised that terpenes are responsible for the attraction of the mirid predator *N. tenuis* to volatiles of tomato plants infested by *T. absoluta* and whiteflies. Volatiles from prey or their by-products such as frass and honeydew also play a kairomonal role in the attraction of predatory insects.<sup>32,33</sup> Except for pheromones reported to be unattractive to the predator *N. tenuis*,<sup>27</sup> the response of this predator to the kairomones from its prey is not known. We thus assessed the attractiveness of plant and prey semiochemicals to the predator *N. tenuis* in relation to infestation densities of *T. absoluta* and *T. vaporariorum*, compared the choices made by the predator between volatiles of tomato plants infested with

*T. absoluta* and those of plants infested with *T. vaporariorum*, and then analysed the volatiles emitted by the most attractant odour source and identified the specific compounds that are attractive to *N. tenuis*. We discuss our findings in relation to the potential use of these attractants to formulate a kairomone-based lure to recruit and retain *N. tenuis* in the vicinity of tomato crop fields to enhance augmentation and conservation biological control of leafminers and whiteflies.

## 2 MATERIALS AND METHODS

### 2.1 Plants

Tomato (*S. lycopersicum* L. cv. Kilele F1 Hybrid) plants were grown in a screen greenhouse at the International Centre of Insect Physiology and Ecology (icipe, Nairobi, Kenya) with provision of water and fertilizer, but without application of pesticides, as described by Ayelo et al.<sup>30</sup>

### 2.2 Insects

All insect species were reared in the laboratory at icipe, under 23–28 °C, 50–65% relative humidity (RH) and a 12:12 h light:dark (L:D) photoperiod regime.

*T. absoluta* were reared on 6- to 8-week-old tomato plants placed in Plexiglass cages (60 × 60 × 80 cm). Plants were exposed to oviposition by adult insects for 1 week and renewed weekly. The infested plants were then placed in an empty Plexiglass cage and kept for 2 weeks. All emerged adults were transferred into another rearing cage for plant infestation. *T. absoluta* adults were fed on 80% honey solution.

*T. vaporariorum* were reared on 6-week-old tomato plants in Plexiglass cages (40 × 40 × 50 cm). The adults were allowed to oviposit for 3 days, after which the infested plants were transferred to a screen house (25–30 °C, 50–70% RH). From 15–18 days postinfestation, the leaves were checked every day and those with the fourth instar nymphs were cut off and their petioles inserted into water-soaked floral foam. These nymph-infested leaves were thereafter returned to the rearing Plexiglass cages, where the adults emerged.

*N. tenuis* were reared on 6- to 8-week-old tomato plants in Plexiglass cages (40 × 40 × 50 cm). The insects were provided with 80% honey solution (Icipe, Nairobi, Kenya), nonviable eggs of *Ephestia kuehniella* Zeuler (Biotop, Livron, France) and commercial pollen (M. Lacarte, Terce, France) twice a week. To avoid cannibalism on the youngest nymphal instars, each week the emerging adults were collected and transferred to another Plexiglass cage. Male and female adults of *N. tenuis* (1:1 ratio) between 2 and 7 days old were used in the experiments. Since what adults of *N. tenuis* experience during rearing affects their subsequent choice preference,<sup>34</sup> the experimental insects were starved for 48 h (deprived of their host plant and prey but provided with water and 80% honey solution).

### 2.3 Y-tube olfactometer bioassays

Dual-choice tests were performed to evaluate the olfactory response of *N. tenuis* to plant and prey odours using a Y-tube olfactometer setup. The Y-shaped glass tube (2.5 cm internal diameter) consisted of a 12-cm stem and two 6-cm arms forming a 60° angle, and was oriented vertically as suggested for Miridae species.<sup>35</sup> The Y-tube was mounted inside a cardboard box (35 × 35 × 55 cm) used as observation chamber which was uniformly lit using a 220–240 V cool white fluorescent tube placed above the Y-tube. A vacuum pump (KNF Laboport type

N86KT.18, France) was used to suck the air which then was filtered by an active carbon-based filter and passed through the odour source container, which was a 10-L cylindrical glass jar, at a constant flow rate of 150 mL min<sup>-1</sup> to enter the olfactometer arms, making an air flow rate of 300 mL min<sup>-1</sup> in the Y-tube stem or control arm of the olfactometer. A single *N. tenuis* was placed at the base of the Y-tube stem, and its first choice was recorded over a 10 min observation period. The insect was considered to have made a choice when it climbed and penetrated 3 cm inside a given arm. Eighty insects were tested per choice test and 10 insects were tested per day. Insects which did not choose either arm within the observation period (i.e. nonresponsive insects) accounted for 1–10% of all the insects tested and were not included in the data analysis. One plant was used for 10 insects, and after five insects the Y-tube was replaced by another one, and the positions of the volatile sources were switched between the olfactometer arms to account for positional bias. Between the choice assays, the Y-tubes were cleaned with dichloromethane, and before they were used again on another day they were cleaned with Teepol odourless detergent and hot water, rinsed with acetone and distilled water, then oven-dried at 150 °C overnight.

### 2.3.1 Response of *N. tenuis* to plant volatiles

The olfactory response of *N. tenuis* to volatiles emitted by healthy and prey-infested tomato plants was investigated. Infested plants were obtained by exposing a single 4-week-old plant to 50, 100 or 200 *T. vaporariorum* adults or to 5, 10 or 20 *T. absoluta* second- and third-instar larvae for 4 days. The pot containing the plant was wrapped in aluminum foil to prevent volatile contamination from the soil, and the tested plant was thereafter placed in the glass jar container. The following combinations were tested: (i) air versus air (control), (ii) air versus healthy plant, (iii) air versus *T. absoluta*-infested plant, (iv) air versus *T. vaporariorum*-infested plant, (v) healthy plant versus *T. absoluta*-infested plant, (vi) healthy plant versus *T. vaporariorum*-infested plant, (vii) *T. vaporariorum*-infested plant versus *T. absoluta*-infested plant and (viii) plant with high infestation of *T. absoluta* versus plant with low infestation of *T. absoluta*.

### 2.3.2 Response of *N. tenuis* to prey semiochemicals

*N. tenuis* olfactory response to semiochemicals emitted by prey (second and third larval instars of *T. absoluta*, and third and fourth nymphal instars of *T. vaporariorum*) and the prey's by-products (larval frass and commercial lure of the sex pheromone of *T. absoluta* and *T. vaporariorum* nymph honeydew) was evaluated. *T. absoluta* sex pheromone lure is made of (*E,Z,Z*)-3,8,11-tetradecatrien-1-yl acetate and (*E,Z*)-3,8-tetradecadien-1-yl acetate (both 98.5% purity) in a ratio of 90:10 loaded inside a rubber septum dispenser (Biological Systems Ltd, Nairobi, Kenya), and the lure was purchased from Koppert (Biological Systems Ltd, Nairobi, Kenya). The larval frass was collected from *T. absoluta* larvae-infested tomato leaves, while the droplets of honeydew produced by the nymphs were collected from tomato plant leaves heavily infested by *T. vaporariorum* nymphs (about 500–700 nymphs per leaf) using a fine camel hairbrush, as in the study by Hung et al.<sup>36</sup> Bioassays were conducted by testing the clean air (blank, control) against (i) a piece of the commercial sex pheromone lure, (ii) three *T. absoluta* larva densities (5, 10 and 20), (iii) three doses of fresh *T. absoluta* larval frass (15, 30 and 60 mg), (iv) three *T. vaporariorum* nymph densities (50, 100 and 200) and (v) three doses of honeydew (15, 30 and 60 mg). Each test sample was

placed in an Eppendorf tube and tested against an empty Eppendorf tube (control). The Eppendorf tubes serving as odour containers were connected to the edge of the olfactometer arms and linked to the air flow meter using Teflon tube (PTFE, 4 mm internal diameter × 6 mm external diameter). The charcoal-filtered air flow of 150 mL min<sup>-1</sup> passed through the Eppendorf tubes, where it carried the test odours to enter the olfactometer arms. The insects were tested and their choices recorded as described above.

## 2.4 Collection and analysis of headspace plant volatiles

The headspace tomato plant volatiles were collected using a dynamic push-pull system and then analysed by coupled gas chromatography–mass spectrometry (GC–MS), as described in Ayelo et al.<sup>30</sup> A charcoal-filtered airflow was pulled at a rate of 200 mL min<sup>-1</sup> into the odour source containers, which were the same as the 10-L glass jars used in the behavioural assays. Volatiles were collected only from healthy and *T. absoluta*-infested tomato plants since volatiles of plants infested with *T. absoluta* were more attractive to the predator than volatiles of plants infested with *T. vaporariorum* (see section 3). Headspace volatiles were trapped onto precleaned 30-mg Super-Q adsorbent traps (Gainesville, FL, USA) for 24 h with four replicates. The volatiles were eluted with 150 µL of dichloromethane (DCM) after which the eluent was concentrated to 50 µL under a gentle nitrogen flow and 5 µL of biphenyl (99% purity) solution (20 ng µL<sup>-1</sup>) was added as internal standard. The solution was stored at –80 °C until analysis.

One microlitre of volatile extract was analysed on a Shimadzu QP2010 Ultra GC–MS equipped with an Inert Cap 5MS/NP nonpolar and low bleed capillary column made of 5% diphenyl and 95% dimethylpolysiloxane, 30 m × 0.25 mm × 0.25 µm film thickness (GL Sciences, Tokyo, Japan). Analysis was performed in the splitless mode using helium as carrier gas at a constant flow rate of 1 mL min<sup>-1</sup>. The oven temperature was set at 35 °C for 5 min and then increased at 10 °C min<sup>-1</sup> to reach a final temperature of 280 °C, which was held for 10.5 min. The retention time, library mass spectra (NIST11 and Wiley9), electron ionization spectrum and Kovats retention indices (RIs) were used to tentatively identify the compounds and comparison with published mass spectra and Kovats RIs from online NIST library was done. RIs of the identified compounds were determined using retention times of a mixture of straight-chain alkane (C<sub>8</sub>–C<sub>23</sub>) standards. Synthetic standards, where available, were run to confirm the identification of compounds by comparison of the expected retention time and the MS spectra. The compounds were quantified (ng plant<sup>-1</sup> h<sup>-1</sup>) relative to the peak area and the concentration of the internal standard using formula adapted from Wang et al.,<sup>37</sup> as follows:

$$Rr = \frac{C_a \times V}{24} \text{ and } C_a = \frac{PA_a}{PA_{is}} \times C_{is} \times 5 \mu\text{L}$$

where *Rr* is the release rate (ng plant<sup>-1</sup> h<sup>-1</sup>), which is equal to the concentration *C<sub>a</sub>* (ng µL<sup>-1</sup>) of the analyte in the volatile eluent multiplied by the volume (*V*, µL) of the volatile eluent in which the aliquot (5 µL) of internal standard has been applied, and then divided by the volatile collection period (24 h). *PA<sub>a</sub>* is the peak area of the identified analyte, *PA<sub>is</sub>* is the peak area of the internal standard and *C<sub>is</sub>* is the concentration (ng µL<sup>-1</sup>) of internal standard.

## 2.5 Chemicals

All synthetic standards used to confirm the identified compounds were purchased from Merck (France) and included (*Z*)-3-hexen-

1-ol, *p*-xylene,  $\alpha$ -pinene, sabinene,  $\beta$ -pinene, 6-methyl-5-hepten-2-one,  $\beta$ -myrcene, 2-carene,  $\alpha$ -phellandrene, 3-carene,  $\alpha$ -terpinene, *p*-cymene,  $\beta$ -phellandrene,  $\beta$ -ocimene,  $\alpha$ -terpineol, linalool, allo-ocimene, methyl salicylate,  $\gamma$ -terpinene, terpinolene,  $\gamma$ -elemene,  $\beta$ -elemene, (*E*)- $\beta$ -caryophyllene, geranyl acetone,  $\alpha$ -humulene and  $\beta$ -ionone. Chemical purity was between 90% and 99%, except for  $\alpha$ -phellandrene (85%) and sabinene (75%). DCM (99.9% purity) was purchased from Merck (Germany).

## 2.6 Bioassays with synthetic compounds

The attractiveness of synthetic  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -myrcene, 2-carene,  $\alpha$ -phellandrene, 3-carene,  $\alpha$ -terpinene,  $\beta$ -phellandrene,  $\beta$ -ocimene, terpinolene,  $\beta$ -elemene,  $\gamma$ -terpinene, (*E*)- $\beta$ -caryophyllene and  $\alpha$ -humulene to the predator *N. tenuis* was tested using the previously described Y-tube olfactometer setup. Each compound was tested at three doses: its natural release rate per plant (dose in ng plant<sup>-1</sup> h<sup>-1</sup> from plants infested with 20 *T. absoluta* larvae) (Table 1), then 10- and 100-fold higher doses, which correspond to the release rates of 10 and 100 equivalent plants in an hour. Thereafter, the five compounds ( $\alpha$ -pinene,  $\alpha$ -phellandrene, 3-carene,  $\beta$ -phellandrene and  $\beta$ -ocimene) found to be attractive to the predator were tested in a five-component blend of the five attractants and in a four-component blend (mixture of  $\alpha$ -pinene,  $\alpha$ -phellandrene, 3-carene and  $\beta$ -ocimene blend without the most abundant compound,  $\beta$ -phellandrene). Each blend was tested at four doses: a mixture of the attractive doses (i.e. 230 ng 3-carene, 320 ng  $\beta$ -ocimene, 1310 ng  $\alpha$ -pinene, 1640 ng  $\alpha$ -phellandrene and 1823 ng  $\beta$ -phellandrene), blend B1, which was subsequently diluted to one-half (blend B2), one-fourth (blend B3) and one-tenth (blend B4). DCM (solvent) was used to dilute the compounds, then a 10- $\mu$ L aliquot of the test solution was loaded onto a 2  $\times$  2-cm filter paper and tested against a filter paper loaded with 10- $\mu$ L DCM (control). After 30 s to allow for the evaporation of the solvent, the impregnated filter papers were placed at the edge of the olfactometer arms and renewed for every insect. Eighty insects were tested per choice test as described above.

## 2.7 Statistical analyses

The frequencies of odours chosen by *N. tenuis* were compared using a chi-squared test. The data of the Volatile Organic Compounds (VOC) release rates (doses in ng plant<sup>-1</sup> h<sup>-1</sup>) (Table 1) were tested for normality using Shapiro–Wilk's test, and homogeneity of variance using Bartlett's test. These data were not normally distributed, and their variance was not homogenous, hence a nonparametric Kruskal–Wallis ANOVA test was applied for their comparison between healthy and *T. absoluta*-infested plants, followed by a Dunn's test with Bonferroni's adjustment as *post hoc* test to separate means.<sup>38</sup> A random forest (RF) analysis<sup>39</sup> was performed to select the VOCs that best distinguished *T. absoluta*-infested tomato plants from healthy plants, based on the mean decrease in accuracy (MDA) obtained using the RF 'importance' function and the out of bag (OOB) error, which allowed measuring the prediction error of random forests (100%, OOB error).<sup>40,41</sup> Using the function 'MDSplot' of the RF package, a multidimensional scaling (MDS) plot<sup>42</sup> was performed to visualise the similarity among healthy plant and *T. absoluta*-infested plant categories. A sparse partial least square discriminant analysis (sPLS-DA) biplot was performed in the mixOmics package<sup>43</sup> to illustrate how the discriminating VOCs were correlated with healthy and *T. absoluta*-infested plants. The function 'perf' and the 'leave-one-group-out' cross-validation method in the mixOmics package, as well as the sPLS-DA parameters (R2X, R2Y and Q2) served to validate the sPLS-DA model.<sup>44</sup> A clustering heatmap,

using the function 'cim' in the mixOmics package,<sup>44</sup> was performed to illustrate variations in the emission of the most discriminating VOCs across replicates of healthy and *T. absoluta* larvae-infested plants. For bioassays with synthetic compounds, we used compounds that were commercially available among the top discriminating VOCs (i.e. VOCs with MDA  $\geq$  60), which were highly correlated with the 20 *T. absoluta* larvae-infested plants since these plants were more attractive to the predator than each of the other attractant plants (i.e. plants infested with 10 *T. absoluta* larvae or 100 *T. vaporariorum* adults) (see section 3). All statistical analyses were performed using R, version 4.0.2.<sup>45</sup>

## 3 RESULTS

### 3.1 Response of *N. tenuis* to plant volatiles

*N. tenuis* was attracted to *T. absoluta*- and *T. vaporariorum*-induced tomato plant volatiles and this olfactory response was dependent on prey infestation density (Fig. 1). The predator was attracted to volatiles released by plants infested with 10 or 20 *T. absoluta* larvae when compared to clean air ( $\chi^2 = 10.45$ ,  $P = 0.001$  and  $\chi^2 = 14.63$ ,  $P < 0.001$ , respectively) or to volatiles of healthy plants ( $\chi^2 = 7.48$ ,  $P = 0.006$  and  $\chi^2 = 9.35$ ,  $P = 0.002$ , respectively) (Fig. 1). Additionally, volatiles of plants infested with 20 *T. absoluta* larvae were more attractive to the predator than volatiles of plants infested with 10 or five *T. absoluta* larvae ( $\chi^2 = 8.56$ ,  $P = 0.003$  and  $\chi^2 = 9.35$ ,  $P = 0.002$ , respectively). On the other hand, *N. tenuis* displayed no preference for volatiles of plants infested with five *T. absoluta* larvae compared to volatiles of healthy plants ( $\chi^2 = 0.68$ ,  $P = 0.409$ ) or clean air ( $\chi^2 = 2.88$ ,  $P = 0.089$ ) (Fig. 1). Similarly, the predator was not attracted to volatiles of healthy plants compared to clean air ( $\chi^2 = 0.36$ ,  $P = 0.831$ ) (Fig. 1).

*T. vaporariorum*-infested tomato plant volatiles were more attractive to *N. tenuis* than clean air only when the plants were infested with 100 whiteflies ( $\chi^2 = 8.22$ ,  $P = 0.004$ ) (Fig. 1). The predator did not discriminate between clean air and volatiles of plants infested with 50 ( $\chi^2 = 0.47$ ,  $P = 0.494$ ) or 200 *T. vaporariorum* adults ( $\chi^2 = 1.87$ ,  $P = 0.171$ ). No significant differences were observed in the frequencies of predators that chose volatiles of healthy plants when compared to volatiles of plants infested with 50 ( $\chi^2 = 0.33$ ,  $P = 0.566$ ), 100 ( $\chi^2 = 2.22$ ,  $P = 0.136$ ) or 200 *T. vaporariorum* adults ( $\chi^2 = 0.85$ ,  $P = 0.356$ ) (Fig. 1).

Comparison of the predator choice between volatiles of the attractant *T. vaporariorum*- and *T. absoluta*-infested tomato plants revealed that volatiles of plants infested with *T. absoluta* larvae were more attractive to *N. tenuis* than volatiles of plants infested with 100 *T. vaporariorum* adults ( $\chi^2 = 4.21$ ,  $P = 0.04$  and  $\chi^2 = 8.01$ ,  $P = 0.005$  for 10 and 20 *T. absoluta* larval densities, respectively) (Fig. 1).

### 3.2 Response of *N. tenuis* to prey-associated semiochemicals

*N. tenuis* was significantly attracted to volatiles from *T. absoluta* larvae frass at a dose of 60 mg compared to clean air ( $\chi^2 = 9.01$ ,  $P = 0.003$ ), but volatiles from lower frass doses of 30 mg ( $\chi^2 = 2.54$ ,  $P = 0.11$ ) or 15 mg ( $\chi^2 = 0.01$ ,  $P = 0.91$ ) did not attract the predator (Fig. 2). Compared with clean air, the predator was not attracted to *T. absoluta* sex pheromone lure ( $\chi^2 = 0.22$ ,  $P = 0.61$ ) or to volatiles of *T. vaporariorum* nymph honeydew tested at 15, 30 or 60 mg doses ( $\chi^2 = 0.02$ ,  $P = 0.91$ ;  $\chi^2 = 0.09$ ,  $P = 0.77$ ;  $\chi^2 = 0$ ,  $P = 1$ , respectively). Similarly, *N. tenuis* was not attracted to odours from *T. absoluta* larvae or *T. vaporariorum* nymphs at any of the densities tested compared to clean air (Fig. 2).

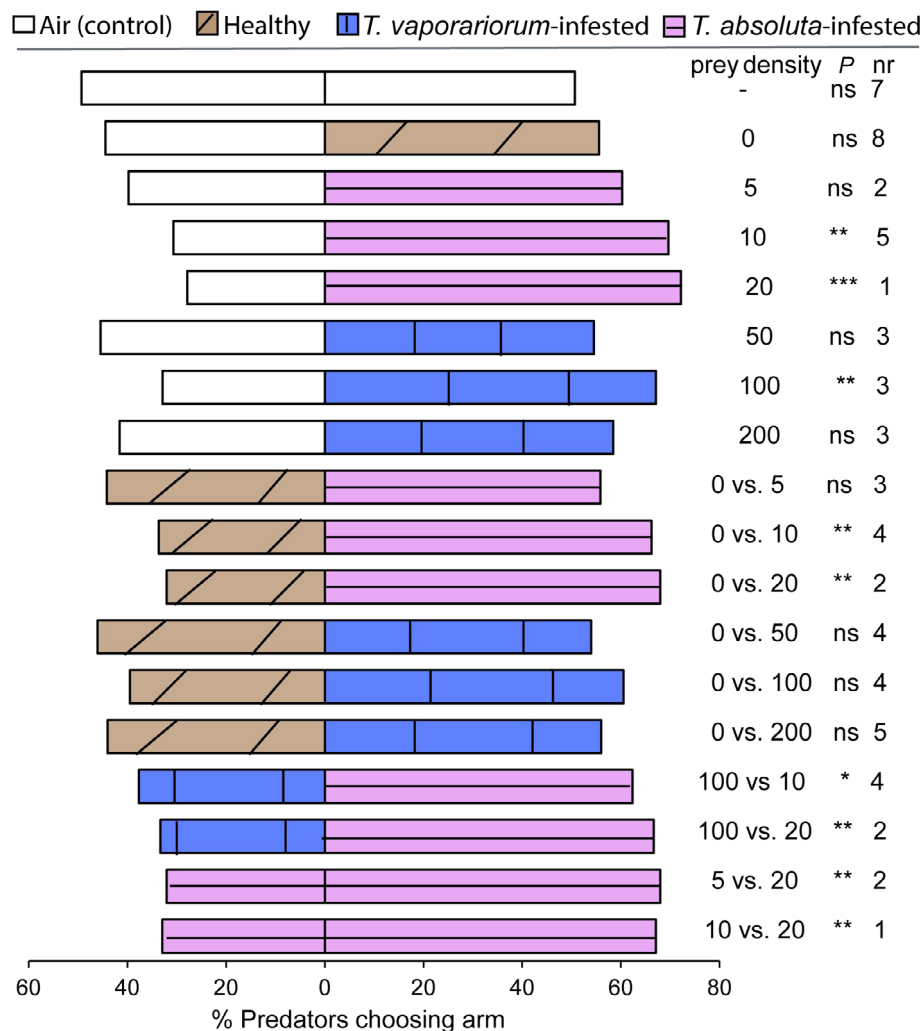


**Table 1.** Mean amount ( $\pm$  SE) (ng plant<sup>-1</sup> h<sup>-1</sup>) of the volatiles in the headspace of healthy tomato plants and plants infested with 5, 10 and 20 *Tuta absoluta* larvae ( $n = 4$ )

Peak no. <sup>†</sup>	RT (min)	K <sub>f</sub> <sup>‡</sup> alk.	K <sub>f</sub> <sup>§</sup> lit.	Compound <sup>¶</sup>	Chemical Class	Healthy plant	Plants infested with 5, 10 and 20 <i>T. absoluta</i>			P value <sup>  </sup>
							5Ta-inf	10Ta-inf	20Ta-inf	
1	8.15	856	858	(Z)-3-hexenol <sup>‡</sup>	Alcohol	nd	nd	0.14 $\pm$ 0.05 <sup>a</sup>	0.22 $\pm$ 0.06 <sup>b</sup>	<b>0.011</b>
2	8.34	865	865	<i>p</i> -xylene <sup>‡</sup>	Benzenoid	0.08 $\pm$ 0.05	0.30 $\pm$ 0.02	0.41 $\pm$ 0.14	0.33 $\pm$ 0.01	0.092
3	9.83	936	939	$\alpha$ -pinene <sup>‡</sup>	Monoterpene	1.26 $\pm$ 0.20 <sup>a</sup>	2.32 $\pm$ 0.31 <sup>ab</sup>	5.22 $\pm$ 0.30 <sup>bc</sup>	13.11 $\pm$ 1.38 <sup>c</sup>	<b>0.004</b>
4	10.42	963	963	Benzaldehyde	Aldehyde	nd	0.08 $\pm$ 0.05	0.21 $\pm$ 0.08	0.20 $\pm$ 0.08	0.112
5	10.62	972	972	3,7,7-trimethyl-1,3,5-cycloheptatriene	Monoterpene	1.41 $\pm$ 0.68 <sup>a</sup>	2.14 $\pm$ 0.52 <sup>ab</sup>	6.96 $\pm$ 0.78 <sup>bc</sup>	13.37 $\pm$ 0.40 <sup>c</sup>	<b>0.004</b>
6	10.72	978	978	$\beta$ -pinene <sup>‡</sup>	Monoterpene	nd	0.05 $\pm$ 0.03 <sup>a</sup>	0.15 $\pm$ 0.04 <sup>ab</sup>	0.68 $\pm$ 0.06 <sup>b</sup>	<b>0.005</b>
7	10.82	983	983	trans-isolimonene	Monoterpene	nd	0.16 $\pm$ 0.10 <sup>a</sup>	0.70 $\pm$ 0.12 <sup>ab</sup>	1.71 $\pm$ 0.14 <sup>b</sup>	<b>0.003</b>
8	10.99	989	987	6-methyl-5-hepten-2-one <sup>‡</sup>	Ketone	nd	0.15 $\pm$ 0.05	0.17 $\pm$ 0.10	0.18 $\pm$ 0.06	0.144
9	11.03	993	992	$\beta$ -myrcene <sup>‡</sup>	Monoterpene	0.40 $\pm$ 0.03 <sup>a</sup>	1.25 $\pm$ 0.25 <sup>a</sup>	1.69 $\pm$ 0.43 <sup>ab</sup>	4.14 $\pm$ 0.32 <sup>b</sup>	<b>0.005</b>
10	11.21	1002	1001	2-carene <sup>‡</sup>	Monoterpene	1.345 $\pm$ 1.96 <sup>a</sup>	24.30 $\pm$ 3.02 <sup>ab</sup>	47.50 $\pm$ 3.72 <sup>bc</sup>	86.93 $\pm$ 6.22 <sup>c</sup>	<b>0.003</b>
11	11.30	1006	1006	$\alpha$ -phellandrene <sup>‡</sup>	Monoterpene	1.21 $\pm$ 0.19 <sup>a</sup>	2.32 $\pm$ 0.30 <sup>ab</sup>	0.91 $\pm$ 0.32 <sup>ab</sup>	16.41 $\pm$ 2.97 <sup>c</sup>	<b>0.004</b>
12	11.40	1011	1011	3-carene <sup>‡</sup>	Monoterpene	nd	0.21 $\pm$ 0.02 <sup>a</sup>	3.12 $\pm$ 0.09 <sup>ab</sup>	2.47 $\pm$ 0.13 <sup>b</sup>	<b>0.002</b>
13	11.51	1016	1017	$\alpha$ -terpinene <sup>‡</sup>	Monoterpene	0.89 $\pm$ 0.18 <sup>a</sup>	1.59 $\pm$ 0.24 <sup>a</sup>	0.51 $\pm$ 0.13 <sup>b</sup>	7.91 $\pm$ 1.23 <sup>b</sup>	<b>0.004</b>
14	11.67	1028	1027	<i>p</i> -cymene <sup>‡</sup>	Monoterpene	0.17 $\pm$ 0.03 <sup>a</sup>	0.39 $\pm$ 0.08 <sup>b</sup>	0.72 $\pm$ 0.34 <sup>b</sup>	182.26 $\pm$ 8.23 <sup>c</sup>	<b>0.033</b>
15	11.78	1034	1032	$\beta$ -phellandrene <sup>‡</sup>	Monoterpene	39.00 $\pm$ 4.74 <sup>a</sup>	66.69 $\pm$ 7.20 <sup>ab</sup>	120.35 $\pm$ 6.82 <sup>bc</sup>	110 $\pm$ 0.13	0.061
16	11.92	1042	1041	(Z)- $\beta$ -ocimene <sup>‡</sup>	Monoterpene	0.36 $\pm$ 0.04	0.39 $\pm$ 0.15	0.53 $\pm$ 0.23	0.41 $\pm$ 0.23 <sup>b</sup>	<b>0.029</b>
17	12.02	1046	1044	Benzene acetaldehyde	Aldehyde	nd	nd	0.08 $\pm$ 0.03 <sup>a</sup>	3.51 $\pm$ 0.19 <sup>c</sup>	<b>0.022</b>
18	12.09	1051	1051	(E)- $\beta$ -ocimene <sup>‡</sup>	Monoterpene	0.49 $\pm$ 0.11 <sup>a</sup>	0.61 $\pm$ 0.04 <sup>ab</sup>	1.13 $\pm$ 0.37 <sup>bc</sup>	1.44 $\pm$ 0.17 <sup>b</sup>	<b>0.007</b>
19	12.25	1060	1060	$\gamma$ -terpinene <sup>‡</sup>	Monoterpene	nd	0.19 $\pm$ 0.05 <sup>a</sup>	0.90 $\pm$ 0.30 <sup>a</sup>	2.16 $\pm$ 0.29 <sup>c</sup>	<b>0.003</b>
20	12.81	1092	1090	Terpinolene <sup>‡</sup>	Monoterpene	0.26 $\pm$ 0.04 <sup>a</sup>	0.41 $\pm$ 0.05 <sup>ab</sup>	0.88 $\pm$ 0.08 <sup>bc</sup>	0.26 $\pm$ 0.15	0.367
21	12.92	1098	1097	Linalool <sup>‡</sup>	Monoterpene	nd	0.09 $\pm$ 0.16	0.12 $\pm$ 0.07	0.63 $\pm$ 0.11 <sup>b</sup>	<b>0.005</b>
22	13.33	1121	1128	Allo-ocimene <sup>‡</sup>	Monoterpene	nd	0.06 $\pm$ 0.02 <sup>a</sup>	0.63 $\pm$ 0.35 <sup>b</sup>	0.32 $\pm$ 0.10 <sup>b</sup>	<b>0.006</b>
23	13.47	1129	1131	Neo-allo ocimene	Monoterpene	0.08 $\pm$ 0.03 <sup>ab</sup>	0.02 $\pm$ 0.01 <sup>a</sup>	0.06 $\pm$ 0.03 <sup>ab</sup>	0.24 $\pm$ 0.03 <sup>c</sup>	<b>0.022</b>
24	14.26	1174	—	Unidentified	Ester	nd	nd	0.22 $\pm$ 0.13	0.69 $\pm$ 0.30	<b>0.003</b>
25	14.35	1179	1182	(Z)-3-hexenyl Butanoate	Monoterpene	nd	0.14 $\pm$ 0.10 <sup>a</sup>	0.30 $\pm$ 0.11 <sup>ab</sup>	0.72 $\pm$ 0.17 <sup>b</sup>	<b>0.025</b>
26	14.42	1183	1183	Dill ether	Monoterpene	nd	nd	0.04 $\pm$ 0.03	0.04 $\pm$ 0.02	0.083
27	14.49	1186	1186	$\alpha$ -terpineol <sup>‡</sup>	Monoterpene	nd	nd	1.88 $\pm$ 0.56 <sup>b</sup>	2.23 $\pm$ 0.78 <sup>b</sup>	<b>0.033</b>
28	14.55	1200	1197	Methyl salicylate <sup>‡</sup>	Ester	nd	0.28 $\pm$ 0.30 <sup>a</sup>	2.44 $\pm$ 0.38 <sup>bc</sup>	7.73 $\pm$ 0.82 <sup>c</sup>	<b>0.004</b>
29	16.65	1344	1342	$\delta$ -elemene	Sesquiterpene	0.69 $\pm$ 0.11 <sup>a</sup>	1.47 $\pm$ 0.30 <sup>ab</sup>	0.35 $\pm$ 0.05 <sup>a</sup>	1.55 $\pm$ 0.27 <sup>b</sup>	<b>0.005</b>
30	17.40	1402	1400	$\beta$ -elemene <sup>‡</sup>	Sesquiterpene	nd	0.37 $\pm$ 0.12 <sup>a</sup>	0.18 $\pm$ 0.08 <sup>a</sup>	0.75 $\pm$ 0.15 <sup>b</sup>	<b>0.004</b>
31	17.46	1407	1406	(Z)-jasnone	Ketone	nd	nd	0.06 $\pm$ 0.01	0.08 $\pm$ 0.01	0.565
32	17.71	1425	1414	$\alpha$ -cedrene	Sesquiterpene	0.03 $\pm$ 0.02	0.04 $\pm$ 0.02	5.70 $\pm$ 1.24 <sup>bc</sup>	14.65 $\pm$ 1.79 <sup>c</sup>	<b>0.004</b>
33	17.82	1432	1428	(E)- $\beta$ -caryophyllene <sup>‡</sup>	Sesquiterpene	1.32 $\pm$ 0.11 <sup>a</sup>	2.82 $\pm$ 0.64 <sup>ab</sup>	0.22 $\pm$ 0.03 <sup>a</sup>	0.59 $\pm$ 0.06 <sup>b</sup>	<b>0.019</b>
34	17.93	1442	1440	$\gamma$ -elemene <sup>‡</sup>	Sesquiterpene	0.10 $\pm$ 0.04 <sup>a</sup>	0.19 $\pm$ 0.11 <sup>a</sup>	0.56 $\pm$ 0.10 <sup>ab</sup>	1.31 $\pm$ 0.35 <sup>b</sup>	<b>0.015</b>
35	18.09	1453	1454	Geranyl acetone <sup>‡</sup>	Ketone	0.49 $\pm$ 0.06 <sup>a</sup>	0.30 $\pm$ 0.06 <sup>a</sup>	1.19 $\pm$ 0.25 <sup>b</sup>	3.51 $\pm$ 0.37 <sup>c</sup>	<b>0.006</b>
36	18.26	1466	1460	$\alpha$ -humulene <sup>‡</sup>	Sesquiterpene	0.50 $\pm$ 0.05 <sup>a</sup>	0.75 $\pm$ 0.13 <sup>ab</sup>	213.57 $\pm$ 13.69 <sup>bc</sup>	374.32 $\pm$ 17.99 <sup>c</sup>	<b>0.003</b>
—	—	—	—	Total (ng plant <sup>-1</sup> h <sup>-1</sup> )	—	62.21 $\pm$ 7.81 <sup>a</sup>	110.10 $\pm$ 11.88 <sup>ab</sup>	213.57 $\pm$ 13.69 <sup>bc</sup>	374.32 $\pm$ 17.99 <sup>c</sup>	<b>0.003</b>

<sup>†</sup> Peak numbers correspond to the peaks shown in Fig. 3.<sup>‡</sup> Retention index relative to C<sub>8</sub>–C<sub>23</sub> n-alkanes of an Inert Cap 5MS/NP capillary column.<sup>§</sup> Retention index obtained from the literature.<sup>¶</sup> Identification of compounds based on retention time (RT), retention indices (KI) and mass spectra using NIST11 and Wiley9 libraries, and comparison with published mass spectra and retention indices from online NIST library.<sup>||</sup> P-values of the nonparametric Kruskal–Wallis ANOVA for comparison of amounts of volatile compounds between healthy plants and *Tuta absoluta* larvae-infested plants (Ta-inf.). Significant values are in bold and means with different letters are significantly different at  $\alpha = 0.05$ .<sup>‡</sup> Compounds confirmed using authentic standards.

nd, not detected.



**Figure 1.** Responses (%) of *Nesidiocoris tenuis* to volatiles of healthy tomato plants or plants infested with *Tuta absoluta* or *Trialeurodes vaporariorum* in a Y-tube olfactometer choice test. nr, number of nonresponsive insects (i.e. insects that made no choice) out of 80 insects tested per choice test. *P* indicates statistical significance levels with ns = no significant difference ( $P > 0.05$ ); \*, \*\*, \*\*\* = significant differences, respectively, at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  from  $\chi^2$  test at  $\alpha = 0.05$ .

### 3.3 Analysis of tomato volatiles

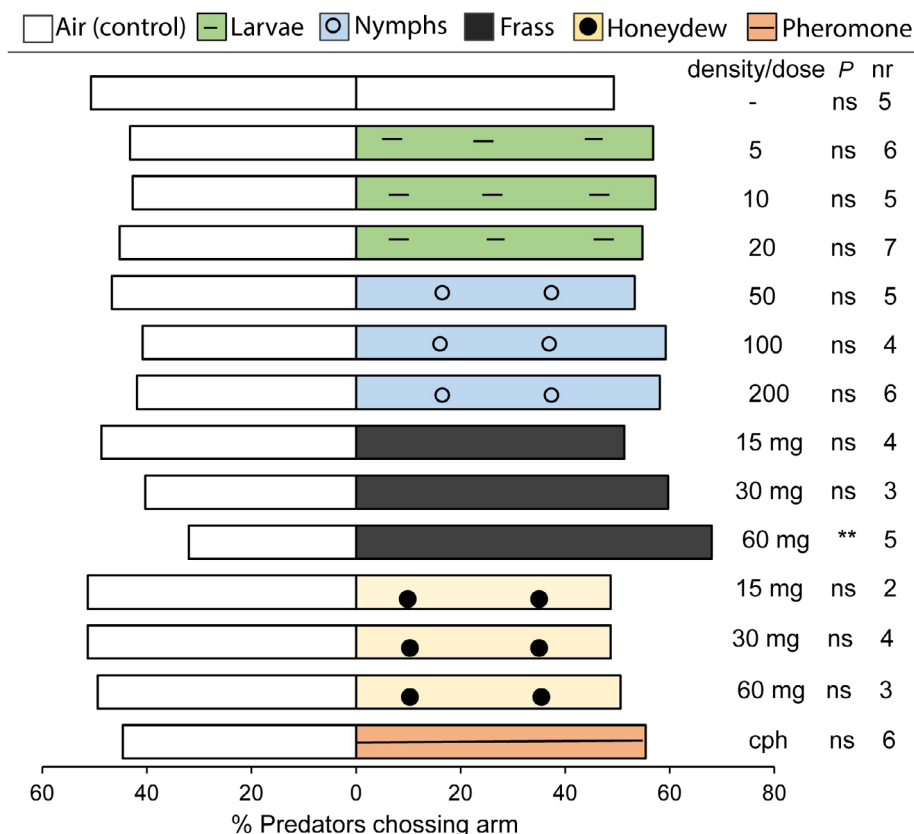
A total of 36 volatile organic compounds (VOCs) were detected in the volatile profiles of *T. absoluta*-infested tomato plants (Table 1 and Fig. 3). The VOCs belonged to seven chemical classes dominated by monoterpenes and sesquiterpenes (Table 1). The monoterpenes were dominated by 2-carene and  $\beta$ -phellandrene, and represented 92.6%, 92%, 90.7% and 87.2% of the total amount of volatiles released from healthy plants and plants infested with 5, 10 and 20 *T. absoluta* larvae, respectively (Table 1 and Fig. 3). Sesquiterpenes accounted for 4.3%, 5.1%, 4.2% and 7.5% of the total amount of volatiles released from healthy plants and plants infested with 5, 10 and 20 *T. absoluta* larvae, respectively, (*E*)- $\beta$ -caryophyllene,  $\delta$ -elemene and  $\alpha$ -humulene being the most abundant sesquiterpenes (Table 1).

The composition of volatiles varied quantitatively and qualitatively between constitutive and *T. absoluta*-induced tomato plant volatiles (Table 1 and Fig. 3). Herbivory by *T. absoluta* larvae induced 17 specific VOCs, including (*Z*)-3-hexenol, allo-ocimene, (*Z*)-3-hexenyl butanoate,  $\alpha$ -terpineol, methyl salicylate and (*Z*)-jasmonone. Quantitative differences were mainly observed between plants infested with 10 or 20 *T. absoluta* larvae and healthy plants. This was reflected in the VOCs for which emission rates increased

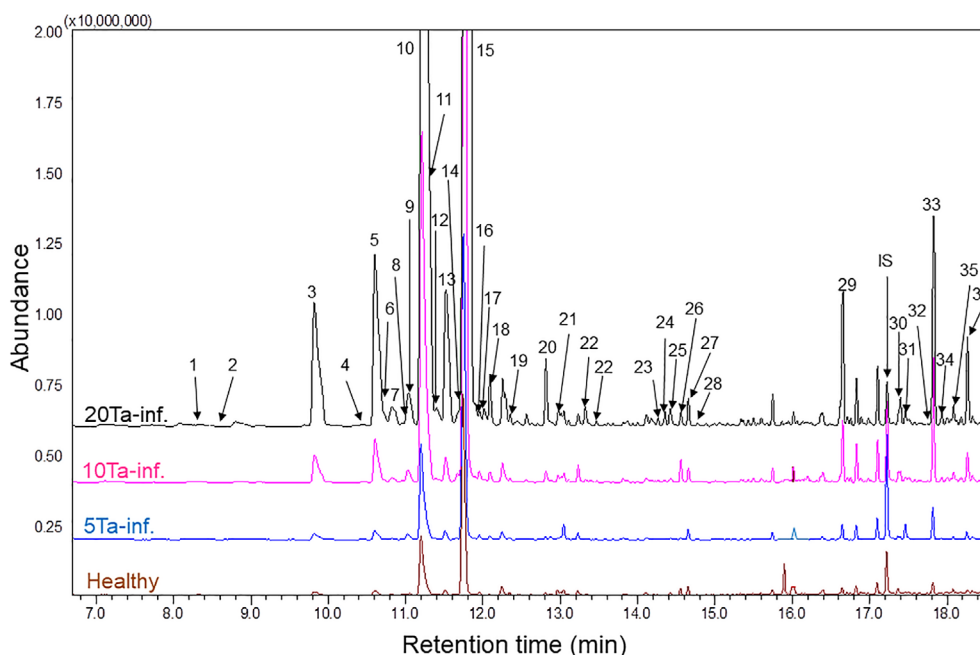
with an increase in the infestation density, as seen for  $\alpha$ -pinene, 3,7,7-trimethyl-1,3,5-cycloheptatriene, 2-carene,  $\alpha$ -phellandrene,  $\alpha$ -terpinene,  $\beta$ -phellandrene, (*E*)- $\beta$ -caryophyllene,  $\delta$ -elemene and  $\alpha$ -humulene. On the other hand, emission rates of some VOCs, e.g. *p*-xylene, (*Z*)- $\beta$ -ocimene, linalool and  $\alpha$ -cedrene, did not vary between healthy and *T. absoluta*-infested plants (Table 1).

### 3.4 Selection of volatile compounds for bioassays with authentic standards

The 25 VOCs that best distinguished healthy plants from plants infested with 5, 10 and 20 *T. absoluta* larvae were highlighted by the MDA of the RF analysis (Fig. 4(A)). Using the top discriminating VOCs (MDA  $\geq 60$ ), the MDS plot grouped the plant categories into four distinct clusters: one composed of healthy plants, the second composed of five *T. absoluta* larvae-infested plants, the third composed of plants infested with 10 *T. absoluta* larvae and the fourth group composed of plants infested with 20 *T. absoluta* larvae (Fig. 4(B)). The sPLS-DA biplot revealed that most of the discriminating VOCs were associated with plants infested with 20 *T. absoluta* larvae (Fig. 4(C)). The first two dimensions of the sPLS-DA explained 92.9% of total variation, with dimension 1 accounting for 90.6% and mainly correlated with  $\alpha$ -pinene,



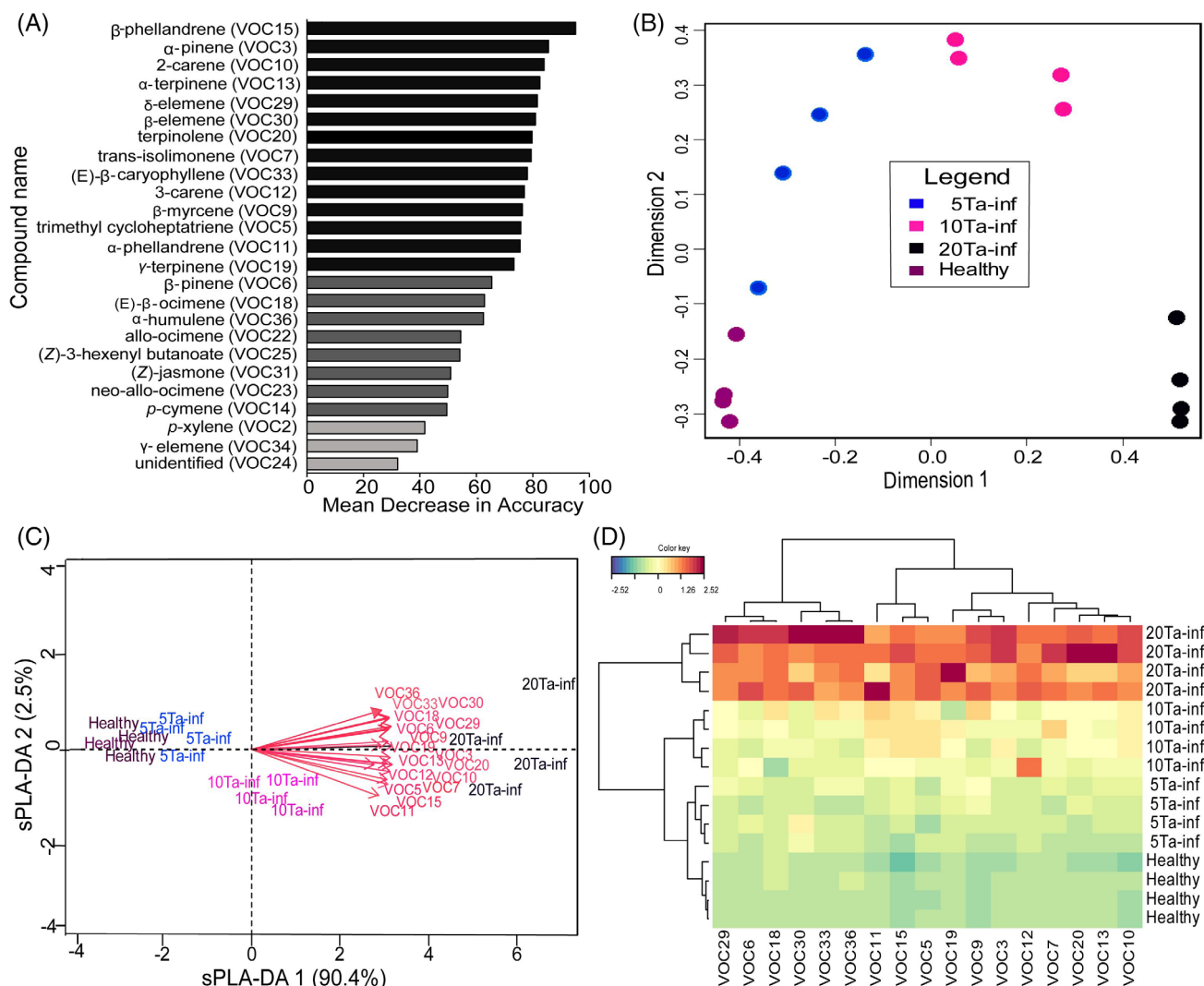
**Figure 2.** Responses (%) of *Nesidiocoris tenuis* to volatiles of prey and prey by-products in a Y-tube olfactometer choice test. nr, number of nonresponsive insects (i.e. insects that made no choice) out of 80 insects tested per choice test; cph, one piece of the commercial sex pheromone of *Tuta absoluta*. *P* indicates statistical significance levels with ns = no significant difference ( $P > 0.05$ ) and \*\* = significant difference at  $P < 0.01$  from  $\chi^2$  test at  $\alpha = 0.05$ .



**Figure 3.** GC-MS profiles of headspace volatiles from healthy tomato plants and plants infested with 5, 10 and 20 *Tuta absoluta* larvae (Ta-inf.). Numbers correspond to the volatile compounds listed in Table 1. IS, internal standard (biphenyl).

2-carene, *trans*-isolimonene, 3,7,7-trimethyl-1,3,5-cycloheptatriene,  $\beta$ -pinene,  $\delta$ -elemene,  $\beta$ -phellandrene, (*E*)- $\beta$ -ocimene,  $\alpha$ -humulene, terpinolene,  $\beta$ -myrcene and (*E*)- $\beta$ -caryophyllene. Dimension

2 explained only 2.5% of total variation and was highly correlated with  $\alpha$ -phellandrene and  $\beta$ -elemene. Heatmap clustering showed that most of the discriminating VOCs were abundant in



**Figure 4.** Determination of the most discriminating volatiles and their correlation with healthy tomato plants and plants infested with 5, 10 and 20 *Tuta absoluta* larvae (5Ta-inf, 10Ta-inf and 20Ta-inf, respectively). (A) The 25 volatiles that best distinguished between healthy and infested plants are ranked in decreasing order based on mean decrease in accuracy of the random forest analysis. (B) Multidimensional scaling (MDS) plot showing the distribution of healthy and infested plants using the top 17 discriminating VOCs (MDA  $\geq 60$ ) ( $R^2X = 0.993$ ,  $R^2Y = 0.949$ ,  $Q^2 = 0.768$ ). (C) sPLS-DA biplot showing the correlation of the top discriminating volatiles with healthy and infested plants ( $R^2X = 0.993$ ,  $R^2Y = 0.949$ ,  $Q^2 = 0.768$ ). (D) Clustering heatmap showing abundance of the top discriminating VOCs across replicates of healthy and infested plants.

20 *T. absoluta* larvae-infested plants (Fig. 4(D)). Among the 17 top discriminating VOCs highly correlated with the attractant 20 *T. absoluta* larvae-infested plants, we tested the 14 compounds that were commercially available, including β-phellandrene, α-pinene, 2-carene, α-terpinene, β-elemene, terpinolene, (E)-β-caryophyllene, β-myrcene, 3-carene, α-phellandrene, γ-terpinene, β-pinene, β-ocimene and α-humulene.

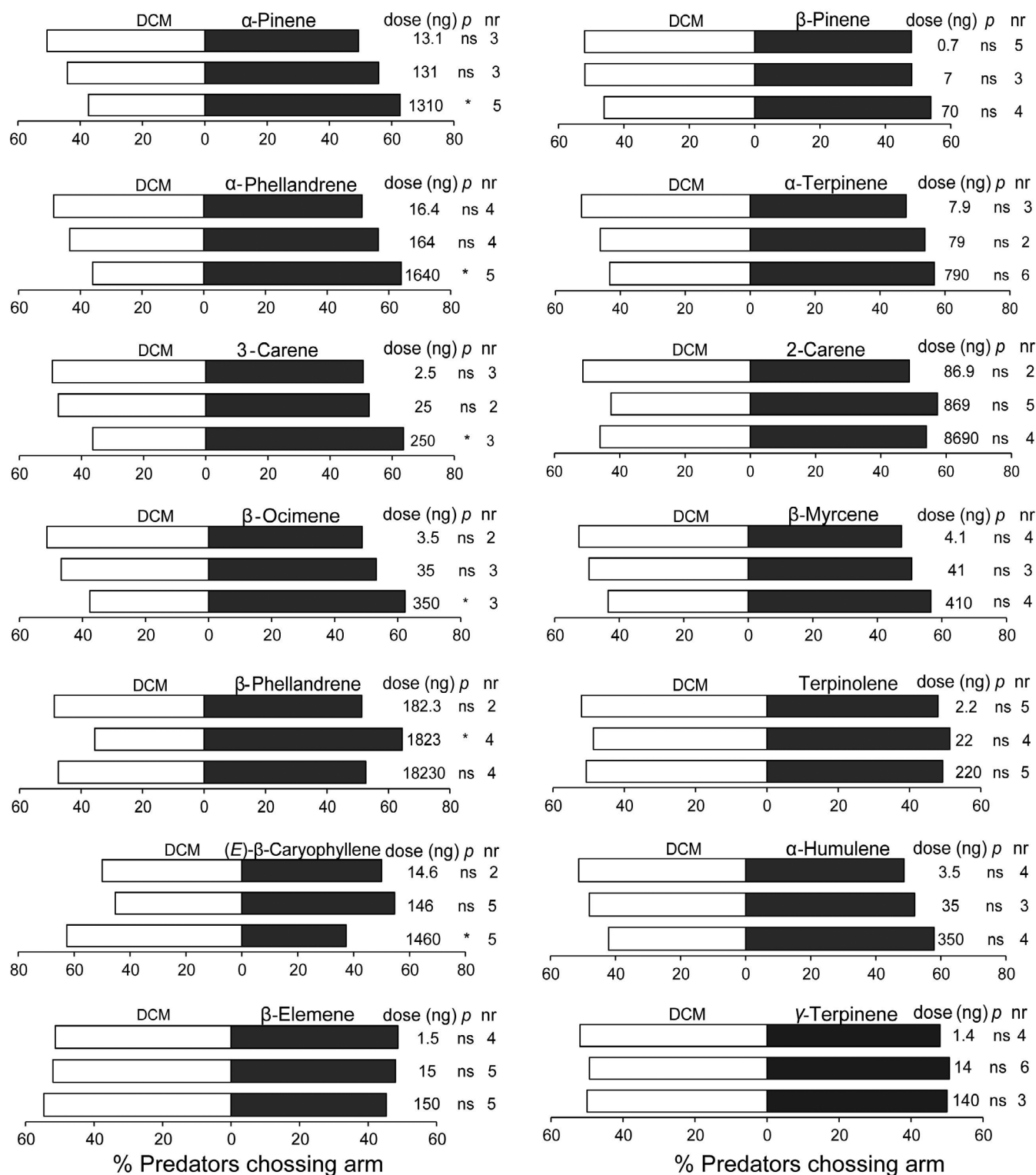
### 3.5 Bioassays with synthetic compounds

The predator *N. tenuis* was attracted to the highest of the doses tested for 3-carene (250 ng) ( $\chi^2 = 6.28$ ,  $P = 0.012$ ), β-ocimene (350 ng) ( $\chi^2 = 4.21$ ,  $P = 0.04$ ), α-pinene (1310 ng) ( $\chi^2 = 4.32$ ,  $P = 0.037$ ) and α-phellandrene (1640 ng) ( $\chi^2 = 5.33$ ,  $P = 0.021$ ) compared to the control (DCM) (Fig. 5). However, lower doses of one-tenth and one-hundredth of these attractant doses were not attractive to the predator compared to the control (Fig. 5). β-phellandrene at a dose of 1823 ng attracted *N. tenuis* ( $\chi^2 = 5.8$ ,  $P = 0.016$ ) compared to the control, whereas 10-fold lower (182.3 ng) ( $\chi^2 = 0.01$ ,  $P = 0.091$ ) or higher (18 230 ng) ( $\chi^2 = 0.73$ ,

$P = 0.118$ ) doses did not. On the other hand, 1460 ng of (E)-β-caryophyllene was repellent to *N. tenuis* ( $\chi^2 = 4.32$ ,  $P = 0.037$ ), and the predator was not sensitive to the lower doses of 1.46 ng ( $\chi^2 = 0$ ,  $P = 1$ ) and 146 ng ( $\chi^2 = 0.48$ ,  $P = 0.49$ ) compared to the control. The other compounds, β-pinene, β-myrcene, 2-carene, α-terpinene, γ-terpinene, terpinolene, β-elemene and α-humulene, were not attractive to the predator at the doses tested in our experiments (Fig. 5).

*N. tenuis* was attracted to the blend of the five attractants (α-pinene, α-phellandrene, 3-carene, β-phellandrene and β-ocimene) only when mixed at one-quarter of their attractive doses compared to the control ( $\chi^2 = 4.32$ ,  $P = 0.037$ ) (Fig. 6). The predator was not attracted to the five-component blend of the attractive compounds mixed at their attractive doses ( $\chi^2 = 0.01$ ,  $P = 0.91$ ) or when the doses were diluted to one-half ( $\chi^2 = 0.33$ ,  $P = 0.57$ ) or one-tenth ( $\chi^2 = 0.05$ ,  $P = 0.82$ ) compared to the control. However, when the most abundant compound (β-phellandrene) was left out of the five-component blend, the predator was more attracted to the resulting four-component blend of



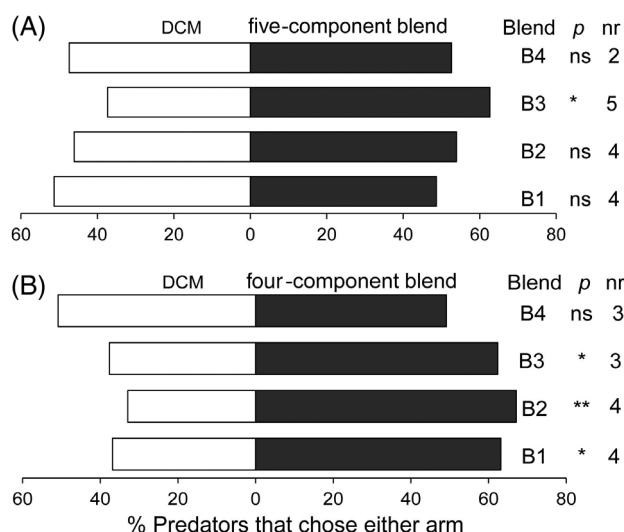


**Figure 5.** Behavioural responses of *Nesidiocoris tenuis* to synthetic compounds tested at three doses corresponding to release rates by 1, 10 and 100 equivalent plants in 1 h. nr, number of nonresponsive insects (i.e. insects that made no choice) out of 80 insects tested per choice test. DCM, dichloromethane. *P*, statistical significance levels with ns = no significant difference ( $P > 0.05$ ); \* = significant difference ( $P < 0.05$ ) from  $\chi^2$  test at  $\alpha = 0.05$ .

$\alpha$ -pinene,  $\alpha$ -phellandrene, 3-carene and  $\beta$ -ocimene mixed at their attractive doses ( $\chi^2 = 3.91$ ,  $P = 0.048$ ), as well as to the blends containing one-half ( $\chi^2 = 8.22$ ,  $P = 0.004$ ) and one-quarter ( $\chi^2 = 4.21$ ,  $P = 0.04$ ) of the compound attractive doses compared to the control (Fig. 6).

## 4 DISCUSSION

The olfactory responses of the generalist predator *N. tenuis* to semiochemicals emitted by tomato plants and the prey *T. absoluta* and *T. vaporariorum* were investigated. Our findings revealed that *N. tenuis* is attracted to tomato volatiles induced



**Figure 6.** Behavioural responses of *Nesidiocoris tenuis* to (A) the five-component blend of the attractive compounds,  $\alpha$ -pinene,  $\alpha$ -phellandrene, 3-carene,  $\beta$ -phellandrene and  $\beta$ -ocimene, and (B) the four-component blend containing  $\alpha$ -pinene,  $\alpha$ -phellandrene, 3-carene and  $\beta$ -ocimene. The compounds were mixed at their attractive doses to make the blend B1, which was subsequently diluted to one-half (B2), one-fourth (B3) and one-tenth (B4). nr, number of nonresponsive insects (i.e. insects that made no choice) out of 80 insects tested per choice test. DCM, dichloromethane. P, statistical significance levels with ns = no significant difference ( $P > 0.05$ ); \*, \*\* = significant differences, respectively, at  $P < 0.05$  and  $P < 0.01$  from  $\chi^2$  test at  $\alpha = 0.05$ .

by the feeding of its prey, and that the predator prefers volatiles of plants infested with *T. absoluta* over those of plants infested with *T. vaporariorum*. Rim et al.<sup>46</sup> reported that *N. tenuis* was more attracted to volatiles of eggplant, *Solanum melongena* L. infested with the cotton leafworm, *Spodoptera litura* Fabricius compared to volatiles of eggplant infested with the two spotted spider mite, *Tetranychus urticae* Koch. In our study, the preference of *N. tenuis* may be explained by the differences in the composition of the volatiles induced by either prey species or by the predator's innate preference for prey species. Relative to volatiles released by tomato plants infested with *T. vaporariorum*,<sup>30</sup> we found that the plants released larger amounts of volatiles when infested with *T. absoluta*, as previously reported for volatiles of *T. absoluta*-infested tomato plants compared to those of *B. tabaci*-infested plants.<sup>29</sup> *T. absoluta* and *T. vaporariorum* have different feeding modes (leaf mining versus phloem sap sucking), and the variation in volatile signature is consequently attributed to differences in the plant defence pathways induced by these herbivores.<sup>47</sup> Leaf mining or chewing larvae activate the jasmonic acid pathway in host plants and trigger the emission of large amounts of volatiles compared to phloem sap-sucking whiteflies, which are known to activate the salicylic acid pathway.<sup>47,48</sup>

Since *N. tenuis* preys more on *T. absoluta* than on whiteflies,<sup>22,23</sup> it is possible that the predator has an innate preference for plant odours induced by its preferred prey species. The generalist anthocorid predator *Orius majusculus* (Reuter) was reported to be more attracted to volatiles of maize plants infested with its preferred prey, the leafhopper *Zyginidia scutellaris* (Herrich-Schäffer), than to those of plants infested with *Dalbulus maidis* (DeLong & Wolcott).<sup>49,50</sup> On the contrary, the mirid predator *Orius laevigatus* (Fieber) fed more on thrips *Frankliniella occidentalis* (Pergande) than on spider mites *Tetranychus urticae* Koch but preferred volatiles of spider mite-infested cucumber

plants to those of thrip-infested plants.<sup>51</sup> However, it might be hard to separate the effect of the prey preference from that of the HIPV emission, since in our study and that of Ardanuy et al.,<sup>49</sup> the preferred prey induced larger levels of volatiles than the less accepted prey. It is likely that the combination of prey and plant species determines the attraction of *N. tenuis*. The efficacy of this predator for long-term control of *T. absoluta* could depend on the performance of the predator on its preferred prey, *T. absoluta*. However, the preference-performance hypothesis does not necessarily correlate with the choice of foraging patch.<sup>52</sup> *T. absoluta* eggs were found to be of poor quality, i.e. lower fertility and longer developmental time, for the performance of the mirid predator *M. pygmaeus* compared to other prey, including nymphs of the whitefly *B. tabaci*.<sup>53</sup> If the same effect applies to the predator *N. tenuis*, the provision of suitable alternative prey to increase the population of the predator could provide good long-term control of *T. absoluta*.<sup>53,54</sup>

Considering the infesting prey species separately, our results showed that the pattern of *N. tenuis* response to volatiles of tomato plants infested by *T. absoluta* differed from that to volatiles of plants infested with *T. vaporariorum*. The findings indicated that *N. tenuis* displayed a density-dependent olfactory response to volatiles induced by *T. absoluta*, whereby the predator was shown to be highly attracted to volatiles of plants infested with high prey density, i.e. 20 *T. absoluta* larvae compared to those of plants infested with lower prey densities, i.e. five or 10 *T. absoluta* larvae. Similarly, the generalist mirid predator *Macrolophus pygmaeus* (Rambur) (Hemiptera: Miridae) has been reported to prefer volatiles of tomato plants infested with 20 *T. absoluta* larvae over those of plants infested with five larvae.<sup>55</sup> Generalist mirid predators searching for prey are known to exploit quantitative and qualitative differences in volatile compositions between healthy plants and prey-infested plants.<sup>56,57</sup> We found that volatiles from healthy and *T. absoluta* larvae-infested tomato plants were dominated by the monoterpenes 2-carene and  $\beta$ -phellandrene, as previously reported in other tomato cultivars, particularly Money-maker and Semiramis by De-Backer et al.,<sup>56</sup> and Anastasaki et al.,<sup>58</sup> Surprisingly, Silva et al.,<sup>29</sup> found the monoterpenes  $\alpha$ -terpinene and limonene to be the most dominant volatiles, although these authors used the Money-maker cultivar. Also, upon herbivory by *T. absoluta*, we found an increase in the emission rates of the monoterpenes  $\alpha$ -pinene,  $\beta$ -myrcene,  $\alpha$ -phellandrene, (*E*)- $\beta$ -ocimene and the sesquiterpene (*E*)- $\beta$ -caryophyllene, as reported in previous studies.<sup>29,55</sup> On the other hand, differences among these studies were more pronounced in the compositions of the novel compounds released upon herbivory. The herbivore-induced specific volatiles in our study included (Z)-3-hexenol, methyl salicylate, linalool, (Z)-3-hexenyl butanoate and (Z)-jasmonone previously reported in the Semiramis tomato cultivar,<sup>58</sup> as well as sabinene, allo-ocimene and  $\beta$ -elemene, which were not found to be specific to the volatiles of Money-maker tomato cultivar infested with *T. absoluta*.<sup>29,55</sup>

Herbivore-induced plant volatiles are exploited by natural enemies in the location of plants infested by their hosts or prey.<sup>8,31</sup> Our findings showed that the monoterpene HIPVs  $\alpha$ -pinene, 3-carene,  $\alpha$ -phellandrene,  $\beta$ -phellandrene and  $\beta$ -ocimene are among the compounds responsible for the attraction of the predator *N. tenuis* to volatiles of *T. absoluta*-infested tomato plants. A previous study reported that phytophagy of tomato plants by *N. tenuis* induces the emission of volatiles, including the alcohol (Z)-3-hexenol, the benzenoid ester methyl salicylate and the green leaf volatiles hexenyl butyrate, (Z)-3-hexenyl propanoate and (Z)-3-hexenyl butanoate,<sup>57</sup> which attracted conspecific individuals.<sup>59</sup> In our study, the attractive compounds were identified by testing a range of concentrations in relation to what we could

expect under conditions of tomato fields where thousands of plants continuously release volatiles to remotely attract natural enemies. It is known that the concentration of odour plumes is diluted by the wind over distances in the field, suggesting that doses of kairomones to be applied to attract natural enemies in the field would be higher than doses that elicited attraction when tested in laboratory assays.<sup>60</sup> In the dose–response assays, we thus increased the volatile concentrations from the release rate of a plant (dose in  $\text{ng plant}^{-1} \text{h}^{-1}$ ) to doses released by 10 and 100 equivalent plants in an hour to get insights into the threshold concentrations detectable by and attractive to the predator *N. tenuis* for future field application purposes. Some of the monoterpenes shown here to be attractive to *N. tenuis* have been reported to attract other mirid predators, such as *M. pygmaeus*, which was attracted to  $\alpha$ -pinene and  $\beta$ -phellandrene.<sup>56</sup> Contrary to our expectation, the blend of the five attractants,  $\alpha$ -pinene,  $\alpha$ -phellandrene, 3-carene,  $\beta$ -phellandrene and  $\beta$ -ocimene (mixed at their attractive doses), elicited a relatively low attraction in *N. tenuis*. It is known that in a blend, some compounds might inhibit the activity of another compound depending on their concentrations and ratios, thereby reducing overall detection and the excitatory activity of the blend on the insect's olfactory neurons and behavioural responses.<sup>61,62</sup> We found that removing  $\beta$ -phellandrene from the blend enhanced the attraction of the predator to the resulting four-component blend containing  $\alpha$ -pinene,  $\alpha$ -phellandrene, 3-carene and  $\beta$ -ocimene, and the four-component blend was attractive to the predator in a range of concentrations. This finding suggests that the use of this blend as a kairomone in the field crop is promising for the recruitment of the predator. The repellent effect of (*E*)- $\beta$ -caryophyllene on *N. tenuis* response and the relatively low attraction of the predator to the blend containing  $\beta$ -phellandrene suggest that high concentrations of these compounds in the plant background odour could interfere with the effectiveness of the four-component blend in attracting the predator. Such negative interference could be reduced by using the kairomone blend in crops for which the background odour contains no or low amounts of the above compounds, and by improving its formulation in terms of concentrations and ratios of the attractants involved, as well as the solvent and the dispenser to be used to ensure convenient diffusion of the lure in field crops.<sup>8</sup>

*N. tenuis* response to volatiles of *T. vaporariorum*-infested tomato plant did not correlate with the infestation density. Volatiles of plants infested with 100 whiteflies attracted *N. tenuis* compared to clean air, but the predator did not distinguish volatiles of whitefly-infested plants from those of healthy plants. There are two possible explanations for these findings: the insect previous experience (naïve versus odour learning) and the volatile signature. It is known that previous experience of host plant volatile affects subsequent preference of zoophytophagous predators, including *N. tenuis*.<sup>33</sup> Lins et al.,<sup>27</sup> reported that naïve *N. tenuis* were not attracted to volatiles of *B. tabaci*-infested tomato plants compared to those of healthy plants, whereas experienced predators, i.e. those that had been exposed to prey or prey-infested plants for feeding and habituation to prey and plant odours, were found to prefer volatiles of prey-infested plants over volatiles of healthy plants. Although tomato plants infested with 100 *T. vaporariorum* adults released volatiles that are quantitatively and qualitatively different from volatiles of healthy plants, it is possible that the low levels of the attractive compounds ( $\alpha$ -pinene,  $\alpha$ -phellandrene, 3-carene,  $\beta$ -phellandrene and  $\beta$ -ocimene) in *T. vaporariorum*-infested plant volatiles<sup>30</sup> did not enable the predator to distinguish volatiles of the two

plant categories. Phloem sap sucking by whiteflies induces a moderate increase in tomato plant volatile emission, whereas whiteflies at high densities suppress the emission of volatiles in tomato plants.<sup>30</sup> The varying responses of *N. tenuis* to whitefly-infested plants have been reported in other predatory insects of phloem feeding herbivores, as seen in the predator *Harmonia axyridis* (Pallas), which was attracted to volatiles of cabbage plants infested with 60 adults of the green peach aphid *Myzus persicae* (Sülzer), but not to volatiles of plants infested with lower (10 and 30) or higher (90) prey densities.<sup>63</sup> In our study, the attraction of *N. tenuis* to volatiles of plants infested with 100 *T. vaporariorum* adults is explained by the increase of the emission level of the monoterpenes  $\alpha$ -pinene, 3-carene,  $\alpha$ -phellandrene,  $\beta$ -phellandrene and  $\beta$ -ocimene,<sup>30</sup> which attracted the predator. Although we used naïve *N. tenuis* in our study, we do not think that this will affect the application of these attractants for the recruitment of the predators in the field. Lins et al.,<sup>27</sup> found both naïve and experienced *N. tenuis* to be attracted to volatiles of *B. tabaci*-infested plants but only experienced *N. tenuis* preferred volatiles of infested plants to those of healthy plants. These findings imply that naïve and experienced *N. tenuis* can detect the same plant odours, here the kairomone blend, but they may be differently sensitive to the concentration of the kairomone. In our study, naïve *N. tenuis* were attracted to a range of concentrations of the four-component blend of the attractants ( $\alpha$ -pinene, 3-carene,  $\alpha$ -phellandrene and  $\beta$ -ocimene). Hence, it is likely that the kairomone blend will still be attractive to wild *N. tenuis* which may have experienced/learned the individual attractants in nature.

Natural enemies also exploit kairomones emitted by prey and hosts to locate them.<sup>9,32,33</sup> However, our results revealed that prey-associated kairomones play a minor role in the attraction of the generalist predator *N. tenuis*. Volatiles of *T. absoluta* larval frass attracted the predator *N. tenuis*, but volatiles of *T. vaporariorum* nymph honeydew did not. The chrysopid predator *Chrysoperla carnea* (Stephens) was attracted to volatiles from frass of the diamondback moth, *Plutella xylostella* (L.), larvae that fed on cabbage plants.<sup>64</sup> In a previous study, we observed that the volatile compounds emitted by *T. absoluta* larval frass are mainly composed of the volatiles of plants infested with *T. absoluta* (Ayelo et al., unpublished). Unlike *N. tenuis*, the mirid predator *Cyrtorhinus lividipennis* Reuter was attracted to volatiles of the honeydew of its prey, the brown leafhopper *Nilaparvata lugens* (Stål).<sup>33</sup> The nonattraction of *N. tenuis* to honeydew volatiles may be explained by the fact that it is a zoophytophagous generalist predator which would prefer to feed on plants rather than on honeydew, or that the concentrations of volatiles in the honeydew are too low to attract the predator. It is known that honeydews emit low levels of volatiles<sup>65</sup> and mainly supplement the dietary need of predatory insects for carbohydrates.<sup>66</sup> *N. tenuis* was not attracted to the commercial sex pheromone of *T. absoluta*, confirming previous findings by Lins et al.,<sup>27</sup> who reported that *N. tenuis* was not attracted to the blend of synthetics of the two compounds found in *T. absoluta* sex pheromone or to the pheromone naturally produced by *T. absoluta* females. The nonattraction to the sex pheromones may be explained by the fact that the predator is a generalist and may not have coevolved with the prey species, as reported for the generalist predators of bark beetles, some of which were shown to be attracted to their prey's pheromone, while others were unable to eavesdrop on the pheromonal cues.<sup>67</sup> Our findings imply that the pheromone-based mass trapping technique for controlling *T. absoluta*<sup>68</sup> will not interfere with the foraging behaviour of the predator nor reduce its population dynamics in the field. Similarly, *N. tenuis* was not attracted to odours of the prey themselves, as also reported in the hemipteran

predator *Geocoris* spp., which was not attracted to the headspace body volatiles of its prey, the larvae of the tobacco hawkmoth, *Manduca sexta* (L.), reared on *Nicotiana attenuata* Torr. ex *S. Watson* plants.<sup>69</sup> However, the prey kairomones may serve as contact signals for prey recognition and acceptance by predatory insects,<sup>70</sup> which was not investigated in the present study.

## 5 CONCLUSION

Our study reveals that the densities of *T. absoluta* larvae and *T. vaporariorum* adults on tomato plants influence the response of the generalist mirid predator *N. tenuis* to volatiles produced by the prey-infested tomato plants. The predator preferred volatiles of *T. absoluta*-infested plants over those of *T. vaporariorum*-infested plants, mainly due to quantitative differences in the plant HIPVs. Our findings show that the four-component blend of the monoterpenes  $\alpha$ -pinene, 3-carene,  $\alpha$ -phellandrene and  $\beta$ -ocimene elicits a strong attraction in the predator, suggesting synergism among these four monoterpenes. These attractive compounds could thus be used to formulate a kairomone-based lure to enhance biological control and to complement other integrated pest management approaches against *T. absoluta* and *T. vaporariorum*. Specifically, the kairomone lure could be used to recruit and retain *N. tenuis* for conservation biological control or to trap the predators to be released in greenhouses for augmentation biological control of these pests in tomato crops.

## ACKNOWLEDGEMENTS

We acknowledge the financial support provided by the French National Research Agency (ANR) through CIRAD (Award no. ANR-16-CE32-0010-01), the University of Pretoria and the National Research Foundation through the NRF grants for C.W.W.P. (Grant no. CPRR160502163617) and A.A.Y. (IFRR Grant no. 109380 and Y-rated Researchers Grant no. RDYR180504326262). The authors also gratefully acknowledge financial support provided by the following *icipe* core donors: the UK Foreign, Commonwealth & Development Office, the Swedish International Development Cooperation Agency, the Swiss Agency for Development and Cooperation, the Federal Democratic Republic of Ethiopia and the Kenyan Government. P.M.A. was supported by the University of Pretoria and the German Academic Exchange Service In-Region Postgraduate Scholarship (Personal Grant no. 91672680). The views expressed herein do not necessarily reflect the official opinion of the donors.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

- Eilenberg J, Hajek A and Lomer C, Suggestions for unifying the terminology in biological control. *BioControl* **46**:387–400 (2001).
- Gay H, Before and after silent spring: from chemical pesticides to biological control and integrated pest management — Britain, 1945–1980. *Ambix* **59**:88–108 (2012).
- Seehausen ML, Afonso C, Jactel H and Kenis M, Classical biological control against insect pests in Europe, North Africa, and the Middle East: what influences its success? *NeoBiota* **65**:169–191 (2021).
- van Lenteren JC, The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl* **57**:1–20 (2012).
- Zhu P, Lu Z, Heong K, Chen G, Zheng X, Xu H et al., Selection of nectar plants for use in ecological engineering to promote biological control of rice pests by the predatory bug, *Cyrtorhinus lividipennis* (Heteroptera: Miridae). *PLoS One* **9**:e108669 (2014).
- Kelly JL, Hagler JR and Kaplan I, Semiochemical lures reduce emigration and enhance pest control services in open-field predator augmentation. *Biol Control* **71**:70–77 (2014).
- Peri E, Moujahed R, Wajnberg E and Colazza S, Applied chemical ecology to enhance insect parasitoid efficacy in the biological control of crop pests, in *Chemical Ecology of Insects: Applications and Associations with Plants and Microbes*, ed. by Tabata J. Taylor & Francis, New York, London, pp. 234–267 (2018).
- Ayelo PM, Pirk CWW, Yusuf AA, Chailleux A, Mohamed SA and Deletre E, Exploring the kairomone-based foraging behaviour of natural enemies to enhance biological control: a review. *Front Ecol Evol* **9**:641974 (2021).
- Afsheen S, Wang X, Li R, Zhu C-S and Lou Y-G, Differential attraction of parasitoids in relation to specificity of kairomones from herbivores and their by-products. *Insect Sci* **15**:381–397 (2008).
- Kessler A and Baldwin IT, Defensive function of herbivore-induced plant volatile emissions in nature. *Science* **291**:2141–2144 (2001).
- Heil M and Ton J, Long-distance signalling in plant defence. *Trends Plant Sci* **13**:264–272 (2008).
- Salamanca J, Souza B, Lundgren JG and Rodriguez-Saona C, From laboratory to field: electro-antennographic and behavioral responsiveness of two insect predators to methyl salicylate. *Chemoecology* **27**:51–63 (2017).
- Desneux N, Wajnberg E, Wyckhuys KAG, Burgio G, Arpaia S, Narváez-Vasquez CA et al., Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. *J Pest Sci* **83**:197–215 (2010).
- Gamarra H, Carhuapoma P, Mujica M, Kreuze J and Kroschel J, Greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood 1956), in *Pest Distribution and Risk Atlas for Africa: Potential, Global and Regional Distribution and Abundance of Agricultural and Horticultural Pests and Associated Biocontrol Agents under Current and Future Climates*, ed. by Kroschel J, Mujica N, Carhuapoma P and Sporleder M. International Potato Center (CIP), Lima, pp. 154–168 (2016).
- Mansour R, Brévault T, Chailleux A, Cherif A, Grissa-Lebdi K, Haddi K et al., Occurrence, biology, natural enemies and management of *Tuta absoluta* in Africa. *Entomol Gen* **38**:83–112 (2018).
- Perring TM, Stansly PA, Liu TX, Smith HA and Andreason SA, Whiteflies: biology, ecology, and management, in *Sustainable Management of Arthropod Pests of Tomato*, ed. by Wakil W, Brust GE and Perring T. Netherlands: Elsevier Inc. Academic Press, pp. 73–110 (2018).
- Hanssen IM and Lapidot M, Major tomato viruses in the mediterranean basin. *Adv Virus Res* **84**:31–66 (2012).
- Dumont F, Aubry O and Lucas E, From evolutionary aspects of zoophytophagy to biological control. *Front Ecol Evol* **6**:221 (2018).
- Pérez-Hedo M, Riahi C and Urbaneja A, Use of zoophytophagous mirid bugs in horticultural crops: current challenges and future perspectives. *Pest Manag Sci* **77**:33–42 (2020).
- Pérez-Hedo M and Urbaneja A, The zoophytophagous predator *Nesidiocoris tenuis*: a successful but controversial biological control agent in tomato crops, in *Advances in Insect Control and Resistance Management*, ed. by Horowitz AR and Ishaaya I. Springer, Cham, pp. 121–138 (2016).
- Urbaneja A, Montón H and Mollá O, Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. *J Appl Entomol* **133**:292–296 (2009).
- Calvo FJ, Lorente MJ, Stansly PA and Belda JE, Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisia tabaci* in greenhouse tomato. *Entomol Exp Appl* **143**:111–119 (2012).
- Shaltiel-Harpaz LA, Gerling D, Graph S, Kedoshim H, Azola L, Rozenberg T et al., Control of the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), in open-field tomatoes by indigenous natural enemies occurring in Israel. *J Econ Entomol* **109**:120–131 (2015).
- Zappalà L, Biondi A, Alma A, Al-Jboory IJ, Arnò J, Bayram A et al., Natural enemies of the south American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. *J Pest Sci* **86**:635–647 (2013).
- Pineda S, Henry TJ, Corrales-Madrid JL, Martínez AM and Figueroa JI, First records of the dicyphine plant bug *Nesidiocoris tenuis* (Hemiptera: Heteroptera: Miridae: Bryocorinae) in Mexico. *Proc Entomol Soc Wash* **119**:290–295 (2017).
- Desneux N, Luna MG, Guillemaud T and Urbaneja A, The invasive South American tomato pinworm, *Tuta absoluta*, continues to spread in Afro-Eurasia and beyond: the new threat to tomato world production. *J Pest Sci* **84**:403–408 (2011).



- 27 Lins JC, van Loon JJA, Bueno VHP, Lucas-Barbosa D, Dicke M and van Lenteren JC, Response of the zoophytophagous predators *Macrolophus pygmaeus* and *Nesidiocoris tenuis* to volatiles of uninfested plants and to plants infested by prey or conspecifics. *BioControl* **59**:707–718 (2014).
- 28 Pichersky E and Raguso RA, Why do plants produce so many terpenoid compounds? *New Phytol* **220**:692–702 (2018).
- 29 Silva DB, Weldegergis BT, van Loon JJA and Bueno VHP, Qualitative and quantitative differences in herbivore-induced plant volatile blends from tomato plants infested by either *Tuta absoluta* or *Bemisia tabaci*. *J Chem Ecol* **43**:53–65 (2017).
- 30 Ayelo PM, Yusuf AA, Pirk CWW, Chailleux A, Mohamed SA and Deletre E, The role of *Trialeurodes vaporariorum*-infested tomato plant volatiles in the attraction of *Encarsia formosa* (Hymenoptera: Aphelinidae). *J Chem Ecol* **47**:192–203 (2021).
- 31 Turlings TCJ and Erb M, Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, application potential. *Annu Rev Entomol* **63**:433–452 (2018).
- 32 Royer L and Boivin G, Infochemicals mediating the foraging behaviour of *Aleochara bilineata* gyllenhal adults: sources of attractants. *Entomol Exp Appl* **90**:199–205 (1999).
- 33 Lakshmi VJ, Krishnaiah K, Lingaiah T and Pasalu IC, Rice leafhopper and planthopper honeydew as a source of host searching kairomone for the mirid bug predator, *Cyrtorhinus lividipennis* (Reuter) (Hemiptera: Miridae). *Biol Control* **14**:7–13 (2000).
- 34 Rim H, Uefune M, Ozawa R, Yoneya K and Takabayashi J, Experience of plant infestation by the omnivorous arthropod *Nesidiocoris tenuis* affects its subsequent responses to prey-infested plant volatiles. *BioControl* **62**:233–242 (2017).
- 35 Ingegno BL, Pansa MG and Tavella L, Plant preference in the zoophytophagous generalist predator *Macrolophus pygmaeus* (Heteroptera: Miridae). *Biol Control* **58**:174–181 (2011).
- 36 Hung KY, McElfresh JS, Zou Y, Wayadande A and Gerry AC, Identification of volatiles from plants infested with honeydew-producing insects, and attraction of house flies (Diptera: Muscidae) to these volatiles. *J Med Entomol* **57**:667–676 (2019).
- 37 Wang C, Zhang W, Li H, Mao J, Guo C, Ding R et al., Analysis of volatile compounds in pears by HS-SPME-GC×GC-TOFMS. *Molecules* **24**:1775 (2019).
- 38 Dinno A, Nonparametric pairwise multiple comparisons in independent groups using Dunn's test. *Stata J* **15**:292–300 (2015).
- 39 Breiman L, Random forests. *Mach Learn* **45**:5–32 (2001).
- 40 Liaw A and Wiener M, Classification and regression by random Forest. *R News* **2**:18–22 (2002).
- 41 Ranganathan Y and Borges RM, Reducing the babel in plant volatile communication: using the forest to see the trees. *Plant Biol* **12**:735–742 (2010).
- 42 Hout MC, Megan HP and Goldinger SD, Multidimensional Scaling. *Cognit Sci* **4**:93–103 (2013).
- 43 Lê Cao K-A, Boitard S, Besse P and Sparse PLS, Discriminant analysis: biologically relevant feature selection and graphical displays for multiclass problems. *BMC Bioinform* **12**:253 (2011).
- 44 Rohart F, Gautier B, Singh A and Lê Cao K-A, MixOmics: an R package for 'Omics feature selection and multiple data integration. *PLoS Comput Biol* **13**:e1005752 (2017).
- 45 R Core Team, *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. <https://www.r-project.org> (2020).
- 46 Rim H, Uefune M, Ozawa R and Takabayashi J, Olfactory response of the omnivorous mirid bug *Nesidiocoris tenuis* to eggplants infested by prey: specificity in prey developmental stages and prey species. *Biol Control* **91**:47–54 (2015).
- 47 Danner H, Desurmont GA, Cristescu SM and van Dam NM, Herbivore-induced plant volatiles accurately predict history of coexistence, diet breadth, and feeding mode of herbivores. *New Phytol* **220**:726–738 (2018).
- 48 Yang J-N, Wei J-N and Kang L, Feeding of pea leafminer larvae simultaneously activates jasmonic and salicylic acid pathways in plants to release a terpenoid for indirect defense. *Insect Sci*. **28**:811–824 (2021). <https://doi.org/10.1111/1744-7917.12820>.
- 49 Ardanuy A, Albajes R and Turlings TCJ, Innate and learned prey-searching behavior in a generalist predator. *J Chem Ecol* **42**:497–507 (2016).
- 50 Ardanuy A, Lee MS and Albajes R, Landscape context influences leaf-hopper and predatory *Orius* spp. abundance in maize fields. *Agric For Entomol* **20**:81–92 (2018).
- 51 Venzon M, Janssen A and Sabelis MW, Prey preference and reproductive success of the generalist predator *Orius laevigatus*. *Oikos* **97**:116–124 (2002).
- 52 Gripenberg S, Mayhew PJ, Parnell M and Roslin T, A meta-analysis of preference-performance relationships in phytophagous insects. *Ecol Lett* **13**:383–393 (2010).
- 53 Sylla S, Brévault T, Diarra K, Bearez P and Desneux N, Life-history traits of *Macrolophus pygmaeus* with different prey foods. *PLoS One* **11**:e0166610 (2016).
- 54 Mollá O, Biondi A, Alonso-Valiente M and Urbaneja A, A comparative life history study of two mirid bugs preying on *Tuta absoluta* and *Ephesia kuehniella* eggs on tomato crops: implications for biological control. *BioControl* **59**:175–183 (2014).
- 55 De-Backer L, Megido RC, Fauconnier ML, Brostaux Y, Francis F and Verheggen F, *Tuta absoluta*-induced plant volatiles: attractiveness towards the generalist predator *Macrolophus pygmaeus*. *Arthropod-Plant Interact* **9**:465–476 (2015).
- 56 De-Backer L, Bawin T, Schott M, Gillard L, Marko IE, Francis F et al., Betraying its presence: identification of the chemical signal released by *Tuta absoluta*-infested tomato plants that guide generalist predators toward their prey. *Arthropod-Plant Interact* **11**:111–120 (2017).
- 57 Perez-Hedo M, Rambla LJ, Graneel A and Urbaneja A, Biological activity and specificity of miridae-induced plant volatiles. *BioControl* **63**:203–213 (2018).
- 58 Anastasaki E, Drizou F and Milonas PG, Electrophysiological and oviposition responses of *Tuta absoluta* females to herbivore-induced volatiles in tomato plants. *J Chem Ecol* **44**:288–298 (2018).
- 59 Silva DB, Urbaneja A and Perez-Hedo M, Response of mirid predators to synthetic herbivore-induced plant volatiles. *Entomol Exp Appl* **169**:125–132 (2020).
- 60 Takemoto H and Takabayashi J, Parasitic wasps *Aphidius ervi* are more attracted to a blend of host-induced plant volatiles than to the independent compounds. *J Chem Ecol* **41**:801–807 (2015).
- 61 Hatano E, Saveer AM, Borrero-Echeverry F, Strauch M, Zakir A, Bengtsson M et al., A herbivore-induced plant volatile interferes with host plant and mate location in moths through suppression of olfactory signalling pathways. *BMC Biol* **13**:75 (2015).
- 62 Borrero-Echeverry F, Becher PG, Birgersson G, Bengtsson M, Witzgall P and Saveer AM, Flight attraction of *Spodoptera littoralis* (Lepidoptera, Noctuidae) to cotton headspace and synthetic volatile blends. *Front Ecol Evol* **3**:56 (2015).
- 63 Yoon C, Seo D-K, Yang J-O, Kang S-H and Kim G-H, Attraction of the predator, *Harmonia axyridis* (Coleoptera: Coccinellidae), to its prey, *Myzus persicae* (Hemiptera: Aphididae), feeding on chinese cabbage. *J Asia-Pacific Entomol* **13**:255–260 (2010).
- 64 Reddy GVP, Holopainen JK and Guerrero A, Olfactory responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. *J Chem Ecol* **28**:131–143 (2002).
- 65 Leroy PD, Heuskin S, Sabri A, Verheggen FJ, Farmakidis J, Lognag G et al., Honeydew volatile emission acts as a kairomonal message for the asian lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). *Insect Sci* **19**:498–506 (2012).
- 66 Ge Y, Pingping L, Zhang L, Snyder WE, Smith M and Shi W, A sticky situation: honeydew of the pear psylla disrupts feeding by its predator *Orius sauteri*. *Pest Manag Sci* **76**:75–84 (2019).
- 67 Erbilgin N and Raffa KF, Kairomonal range of generalist predators in specialized habitats: responses to multiple phloeophagous species emitting pheromones vs. host odors. *Entomol Exp Appl* **99**:205–210 (2001).
- 68 Megido RC, Haubruge E and Verheggen FJ, Pheromone-based management strategies to control the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). A review. *Biotechnol Agron Soc Environ* **17**:475–482 (2013).
- 69 Weinhold A and Baldwin IT, Trichome-derived o-acyl sugars are a first meal for caterpillars that tags them for predation. *PNAS* **108**:7855–7859 (2011).
- 70 Sigsgaard L, Oviposition preference of *Anthocoris nemoralis* and *A. nemorum* (Heteroptera: Anthocoridae) on pear leaves affected by leaf damage, honeydew and prey. *Biocontrol Sci Technol* **15**:139–151 (2005).