



## UvA-DARE (Digital Academic Repository)

### The omnivorous predator *Macrolophus pygmaeus* induces production of plant volatiles that attract a specialist predator

Zhang, N.X.; Andringa, J.; Brouwer, J.; Alba, J.M.; Kortbeek, R.W.J.; Messelink, G.J.; Janssen, A.

**DOI**

[10.1007/s10340-021-01463-3](https://doi.org/10.1007/s10340-021-01463-3)

**Publication date**

2022

**Document Version**

Final published version

**Published in**

Journal of Pest Science

**License**

Article 25fa Dutch Copyright Act

[Link to publication](#)

**Citation for published version (APA):**

Zhang, N. X., Andringa, J., Brouwer, J., Alba, J. M., Kortbeek, R. W. J., Messelink, G. J., & Janssen, A. (2022). The omnivorous predator *Macrolophus pygmaeus* induces production of plant volatiles that attract a specialist predator. *Journal of Pest Science*, 95(3), 1343-1355. <https://doi.org/10.1007/s10340-021-01463-3>

**General rights**

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

**Disclaimer/Complaints regulations**

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

*UvA-DARE is a service provided by the library of the University of Amsterdam (<https://dare.uva.nl>)*



# The omnivorous predator *Macrolophus pygmaeus* induces production of plant volatiles that attract a specialist predator

Nina Xiaoning Zhang<sup>1,4</sup> · Joke Andringa<sup>1</sup> · Jitske Brouwer<sup>1</sup> · Juan M. Alba<sup>1</sup> · Ruy W. J. Kortbeek<sup>3</sup> · Gerben J. Messelink<sup>2</sup> · Arne Janssen<sup>1,5</sup>

Received: 15 July 2021 / Revised: 28 October 2021 / Accepted: 25 November 2021 / Published online: 12 January 2022  
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

## Abstract

It has become clear that omnivorous predators can induce plant defences that affect the performance and host plant choice of herbivores. They are also known to induce the production of plant volatiles that can affect the behaviour of herbivores searching for plants. These volatiles may also affect the searching behaviour of other predators, which was investigated here. The predatory mite *Phytoseiulus persimilis* preferred plants previously exposed to the omnivorous mirid *Macrolophus pygmaeus* over clean plants. The mites were equally attracted to plants previously exposed to the omnivore and subsequently infested by spider mites (*Tetranychus urticae*, prey of the predatory mite and the omnivore) and plants infested with spider mites alone. Moreover, the mites were more attracted to plants infested with prey and subsequently exposed to the omnivore than plants infested with prey but not exposed to the omnivore. The predatory mites were also significantly more attracted to plants on which the omnivores were still present. Experience of the predatory mites with volatiles from plants previously exposed to the omnivore and without prey resulted in a loss of the preference for volatiles emitted by plants exposed to the omnivore. Analysis of the volatiles showed that plant exposure to omnivores induced qualitative and quantitative changes in the volatile blend. Together, these results suggest that omnivorous predators induce the production of plant volatiles that can interfere with the searching behaviour of other predators. The consequences of such interference for biological pest control remain to be investigated.

**Keywords** Olfactometer · Omnivore · Learning · *Phytoseiulus persimilis* · *Macrolophus pygmaeus*

## Introduction

To protect themselves from herbivory, plants produce specific compounds that can directly reduce the survival, development and oviposition of herbivores. These secondary metabolites are part of direct plant defences against herbivores (Karban and Baldwin 1997; Kant et al. 2015). Furthermore, plants can defend themselves indirectly by promoting the action of natural enemies of the herbivores, for example, by providing supplemental food, e.g. extrafloral nectar and pollen (Pemberton and Lee 1996; Heil et al. 2001; Wäckers 2001), shelter such as domatia (Walter 1996), and by producing herbivore-induced volatiles that are attractive to natural enemies of the herbivores (Dicke et al. 1990; Turlings et al. 1990; Sabelis et al. 1999). These volatiles do not only emanate from the damaged plant tissue but also systemically from non-damaged tissue (Turlings and Tumlinson 1992; Dicke 1994; Röse et al. 1996; Guerrieri et al. 1999). The quality and quantity of these volatiles differ among plant

---

Editorial Responsibility: Alberto Urbaneja.

✉ Juan M. Alba  
j.m.albacano@uva.nl

✉ Arne Janssen  
arne.janssen@uva.nl

<sup>1</sup> IBED, Evolutionary and Population Biology, University of Amsterdam, Science Park 904, 1098XH Amsterdam, The Netherlands

<sup>2</sup> Wageningen UR Greenhouse Horticulture, PO Box 20, 2265 ZG Bleiswijk, The Netherlands

<sup>3</sup> Department of Plant Physiology, Swammerdam Institute for Life Sciences, University of Amsterdam, P.O. Box 94215, 1090 GE Amsterdam, the Netherlands

<sup>4</sup> Present Address: Department of Ecology, Swedish University of Agricultural Sciences, Box 7044, 75007 Uppsala, Sweden

<sup>5</sup> Department of Entomology, Federal University of Viçosa, Viçosa, MG, Brazil

species (van den Boom et al. 2004), with herbivore species (De Moraes et al. 1998; Birkett et al. 2003; Dicke et al. 2009), with the duration of the feeding of the herbivores (Takabayashi et al. 1994b; Turlings et al. 1998; Kant et al. 2004), and they can change depending on abiotic factors (Takabayashi et al. 1994a; Dicke and van Loon 2000).

Predatory arthropods and parasitoids can discriminate between volatiles from plants attacked by their prey or host and those of plants attacked by non-prey or non-host herbivores (Sabelis and van de Baan 1983; De Moraes et al. 1998), and they can also discriminate between quantitative differences in the composition of major volatile blends from different plants attacked by the same herbivore species (Du et al. 1998; Guerrieri et al. 1999; Birkett et al. 2003). For example, the predatory mite *Phytoseiulus persimilis* was attracted by volatiles from bean leaves infested by its prey *Tetranychus urticae* and not by those of apple leaves infested by *Panonychus ulmi*, which is not a prey of this predator (Sabelis and van de Baan 1983). However, it has also been reported that predatory arthropods and parasitoids respond to volatiles from plants attacked by non-prey or non-host herbivores (Shimoda and Dicke 2000; Sabelis et al. 2007).

Like herbivores, predatory omnivores are also known to induce direct plant defences when feeding on their host plant, and this can affect the performance of herbivores (De Puyseleir et al. 2011; Pappas et al. 2015; Pérez-Hedo et al. 2015b, a; Naselli et al. 2016; Zhang et al. 2018). Less is known, however, on the induction of plant volatiles by omnivores feeding on their host plant (Moayeri et al. 2007; Pérez-Hedo et al. 2015a; Bouagga et al. 2018a, b; Zhang et al. 2019b). Here, we investigate the induction of volatile production by the omnivorous predatory bug *Macrolophus pygmaeus* and we assess the response of another predator, the predatory mite *P. persimilis* to volatiles emanating from plants exposed to the omnivore. We also identify the volatiles produced and induced by the omnivore.

*Macrolophus pygmaeus* is commercially used for biological control of several pests (van Lenteren 2012). Besides feeding on plant tissue (Perdikis and Lykouressis 2000), it attacks a wide range of arthropod pests such as whiteflies (Montserrat et al. 2000), thrips (Riudavets and Castañé 1998), aphids (Alvarado et al. 1997), spider mites (Hansen et al. 1999), leaf miners (Arnó et al. 2003) and Lepidoptera species including *Tuta absoluta* (Urbaneja et al. 2009). Earlier, others and we have shown that *M. pygmaeus* affected the performance of the herbivorous pests *T. urticae*, *Frankliniella occidentalis* and *Bemisia tabaci* through induced direct plant defences (Pappas et al. 2015; Bouagga et al. 2018b; Zhang et al. 2018). Feeding by *M. pygmaeus* also affected plant phenology and reproduction, and these changes also altered the performance of the omnivore itself (Zhang et al. 2019a). Moayeri et al. (2007) showed that feeding by *M. pygmaeus* induced the production of 11 additional volatile

compounds in bean plants. We previously showed that females of *T. urticae* and *F. occidentalis* avoided plants previously exposed to *M. pygmaeus* (Zhang et al. 2019b), which was possibly mediated by volatiles. We therefore sought to confirm the induction of the production of volatiles by performing olfactometer experiments and by analysing the headspace of plants exposed to the omnivore. Because these volatiles are often used by natural enemies when searching for their herbivorous prey, we were particularly interested in the effects of these volatiles on the behaviour of other predators. In particular, we studied the behaviour of the predatory mite *P. persimilis*, which relies on olfactory cues to locate plants infested with its prey, the two-spotted spider mite, from a distance (Sabelis and van de Baan 1983; Sabelis et al. 1984; Dicke et al. 1990). Because both the omnivore and the predatory mite are commercially used in biological control, these two species often co-occur in greenhouse crops, but not much is known about their interactions and how this would potentially affect biological control. Potentially, *M. pygmaeus* can attack and will compete for prey with *P. persimilis* and we therefore expected that *P. persimilis* would avoid plants with *M. pygmaeus*.

## Materials and methods

### Cultures

Sweet pepper plants (*Capsicum annuum* L. Spider F1, Enza Zaden Beheer B.V., The Netherlands) were grown from seeds in pots ( $\varnothing = 14$  cm) with soil in a climate room dedicated to growing clean plants ( $25 \pm 1$  °C, 60–70% RH, 16: 8 L: D). Water was supplied twice a week. Four-week-old plants with six-eight true leaves (about 20 cm high) were used for experiments. Plants of 5 to 8 weeks old were used for the rearing of spider mites.

A culture of *M. pygmaeus* was established with fifth instar nymphs from a commercial rearing (Koppert Biological Systems BV, Berkel en Rodenrijs, The Netherlands). They were reared in insect-proof cages (BugDorm-44545F,  $47.5 \times 47.5 \times 47.5$  cm, mesh size: 160  $\mu$ m) in a separate climate room (conditions as above) with *Ephestia kuehniella* eggs as food and 4- to 5-week-old sweet pepper plants as both food supply and oviposition substrate. New *E. kuehniella* eggs were added twice a week and new plants were added every 2 weeks. Adults from the culture were used for experiments.

The culture of two-spotted spider mites was started with individuals that were obtained from a cucumber culture in our laboratory (Janssen 1999) and was reared on intact sweet pepper plants in a separate climate room (conditions as above). New plants were provided twice a week. The mites were cultivated for 10 months on sweet pepper plants before

being used for experiments, allowing them to adapt to this host plant (Magalhães et al. 2007).

Predatory mites, originating from a culture (Koppert strain) reared with spider mites as food on bean plants (van Wijk et al. 2008), were transferred to sweet pepper leaves infested with spider mites. These leaves were placed on a plastic platform in a tray filled with water with soap, preventing the mites from escaping. New sweet pepper leaves infested with spider mites were provided five to six times per week. Predatory mites were reared in this way for more than three generations before being used in the experiments.

### Olfactometer experiments

Responses of predatory mites to different plant volatiles were tested using a Y-tube olfactometer (Sabelis and van de Baan 1983). It consisted of a Y-shaped glass tube ( $\varnothing$  4 cm, length of arms and base 13 cm) with a metal wire in the middle on which the predators could walk freely. A transparent hose connected each arm of the Y-tube to a separate glass container ( $50 \times 40 \times 40$  cm) that had an air inlet and outlet covered with fine mesh (80  $\mu$ m). A tray with three sweet pepper plants that had received one treatment was placed inside one container 30 min prior to the experiment and served as one source of volatiles; the other container received a tray with three plants of another treatment. The base of the Y-tube was connected to a vacuum pump, creating an air flow in the olfactometer ( $0.45 \pm 0.05$  m/s). Anemometers (VelociCalc® Air Velocity Meter 9545-A, TSI Incorporated, USA) were used to measure the flow rate in both arms of the olfactometer, which were calibrated with valves inserted into the transparent hoses. With equal flow rates in both arms, two separate volatile plumes were formed in the base of the Y-tube, with their interface coinciding with the metal wire (Sabelis and van de Baan 1983; Janssen et al. 1997).

After disconnecting the vacuum pump from the base of the Y-tube, a starved (1 h) gravid female of *P. persimilis* was introduced onto the beginning of the metal wire in the Y-tube with a fine brush. Immediately afterwards, the vacuum pump was connected. The mite was observed until it had reached the end of one of the arms, which was then noted as a choice, or until five minutes had passed (no choice). Subsequently, it was removed and the next female was introduced. After five females that made a choice, the volatile sources were switched by connecting the hoses to the other arm or to the other container and the wind speed was measured and calibrated. This was done to correct for unforeseen asymmetries in the experimental set-up that could influence the choice of the predatory mites (Janssen et al. 1997). Each replicate of an olfactometer experiment consisted of 20 female predatory mites that made a choice for one of the two volatile sources. Individuals that did not make a choice within five minutes were not included, so a

replicate was continued until 20 predatory mites had made a choice. On average, 1.92 mites per replicate did not make a choice within 5 min. The olfactometer and the hoses were washed with odourless detergent, rinsed with demineralized water to remove the detergent and left to dry between replicates. Plant containers were cleaned with alcohol (60%) and air-dried overnight. All female predatory mites were only used once for an experiment and new plants were used for each replicate.

To make sure that females of *P. persimilis* from our culture responded to volatiles from sweet pepper plants, a preliminary olfactometer test was conducted. Three sweet pepper plants (4–5 weeks old) were infested for 2 days with 240 adult spider mites per plant with an average of 30 adults per leaf. Subsequently, an olfactometer experiment was conducted with these plants as one volatile source and another group of plants of the same age and size, but not attacked by spider mites as the other volatile source. This test was replicated two times with two different sets of plants and two groups of predators. *Phytoseiulus persimilis* significantly preferred treated plants to clean plants in both replicates (plants with spider mites attracted  $82.5 \pm 2.5\%$  of the predators; GLM:  $\text{Chi}^2 = 18.35$ , *d. f.* = 1,  $P < 0.001$ ), showing that they can recognize volatiles from infested sweet pepper plants.

### Response to plants exposed to *M. pygmaeus*

Six sweet pepper plants (4 weeks old with six–eight true leaves) were transferred each into a separate insect-proof cage (as above) in a separate climate room (conditions as above). Five adult females and five adult males of *M. pygmaeus* were released in three cages. Plants in the other three cages were kept clean, serving as control. Four days later, which is sufficient time to induce volatile production in plants (Kant et al. 2004), all *M. pygmaeus* (alive and dead) were removed from the three treated plants. On average, 60% of *M. pygmaeus* survived these 4 days, and plants without alive *M. pygmaeus* at the end of this treatment were not used for further experiments. The three treated plants were grouped together and served as one volatile source, the group of three control plants served as the other volatile source in an olfactometer experiment. The response of *P. persimilis* to these two groups of plants was tested as described above. Five replicates were conducted in total, with five different groups of plants (three plants per treatment per replicate) and predatory mites. The preference of the predatory mites for a particular volatile source was tested with a log-linear model for contingency tables with a generalized linear model (GLM) with treatment and replicate and their interaction as factors and the numbers of predators choosing for the volatile sources as response variable with a Poisson error distribution (log link) (Crawley 2013). All

statistical analyses were performed with R (R Core Team 2019).

### Response to plants exposed to *M. pygmaeus* and infested with *T. urticae*

To check whether previous infestation by omnivores would change the response of predatory mites towards plants with their spider mite prey, the groups of plants tested in four out of the five replicates of the above experiment were transferred back to their respective cages, and 240 female spider mites (on average 30 per leaf) were subsequently released on each plant. Each plant was placed in a tray filled with water to prevent mites from escaping. Two days later, another olfactometer experiment was conducted with these three plants previously exposed to *M. pygmaeus* and subsequently infested with spider mites as one volatile source and the three plants only infested with spider mites as the other volatile source. The spider mites were present on the plants during the test.

Other groups of plants were also treated with spider mites and *M. pygmaeus*, but the order of the treatment was changed compared to the experiment above. Six sweet pepper plants were each infested with 240 adult spider mites per plant for 2 days. Subsequently, an olfactometer experiment was conducted as described above, with two groups of three spider mite-infested plants to make sure that these two groups of plants had similar attractiveness to the predatory mites before they were exposed to *M. pygmaeus*. Thereafter, all plants were transferred back to their cages, and the group of three plants that attracted a (non-significantly) lower number of predatory mites subsequently received five adult males and five adult female *M. pygmaeus*. The other three plants were kept without mirids but with spider mites. After 4 days, all mirids were removed from the three plants, and their attractiveness of this group of plants was compared with that of the other group of three plants in the olfactometer. Four replicates were conducted, each with new groups of three plants.

The preference of the predatory mites for a particular volatile source in each experiment was compared using the same method as above. Differences in preference between the two experiments were tested by comparing the proportions of predators choosing for a volatile source with a GLM with a binomial error distribution (logit link) with the experimental treatment as factor.

### Response of *P. persimilis* to plants with *M. pygmaeus*

The following experiment was conducted to check the response of *P. persimilis* to *M. pygmaeus* present on the plants. Two groups of plants, one group treated with *M. pygmaeus*, the other group consisting of clean plants, were

prepared as above. All *M. pygmaeus* remaining in the cages with the treated plants were collected after the 4 days of treatment and kept in 1.5 ml Eppendorf Safe-Lock tubes and the plants were transferred to the container of the olfactometer. The collected *M. pygmaeus* were subsequently released in this container and their number was supplemented with mirids from the culture to an average of five males and five females per plant. The untreated plants served as the alternative volatile source. Four replicates were conducted, each with a new set of plants.

We subsequently removed the mirids from containers after the olfactometer test, all plants were left in the containers overnight with an airflow of about 0.20 m/s to prevent condensation of water on plants and container walls, and the response of the predatory mites to the volatiles of these plants and the control plants was measured the next day. This was done with two of the four groups of plants of the previous test.

The preference of the predatory mites for a particular volatile source in each experiment was compared using the same method as above. Differences in preference between different two experiments were tested with GLM in R as above.

### Response of *P. persimilis* with experience with volatiles from plants previously exposed to *M. pygmaeus*

Predatory mites were given an experience with the volatiles of plants with *M. pygmaeus* and without food as follows. Four plants treated with *M. pygmaeus* and four clean plants were prepared as described above. From one of the treated plants, leaf discs ( $\varnothing = 15$  mm) were made and placed in plastic cups (odourless medicine cups,  $\varnothing = 20$  mm, height = 3 cm) filled with water to support the leaf discs and prevent mites from escaping. Leaf discs of such plants have induced direct defences (Zhang et al. 2018). Subsequently, gravid females of *P. persimilis* collected from the culture were each transferred to one of these leaf discs, which did not contain food for the predatory mites. The cups were closed with lids with a ventilation hole covered with fine mesh (80  $\mu\text{m}$ ), thus volatiles could enter and leave the cups. Thereafter, all the cups were placed on top of an upside-down plastic tray (30  $\times$  24 cm) in a cage (same type as above) with one of the *M. pygmaeus*-treated plants. Numbers of alive *M. pygmaeus* in all three cages were counted and new individuals were added to a total of five adult females and five adult males per cage. Thus, *P. persimilis* were able to perceive cues from the treated leaf disc, volatiles from the treated plant and from *M. pygmaeus*. A second group of gravid females of *P. persimilis* were treated similarly, but were incubated on leaf discs from one clean plant and placed in a cage with one clean plant without *M. pygmaeus*.



Twenty-four hours later, these predatory mites were used in an olfactometer test. All *M. pygmaeus* were removed from the three plants, and these plants were used as one volatile source; the three clean plants as the other. A third group of *P. persimilis* was collected from the culture and starved for one hour and was subsequently also tested in the olfactometer with the same plants. Five replicates with five different groups of plants were conducted in total; in the last replicate, we did not test predatory mites from the culture.

The preference of the predatory mites for a particular volatile source in each experiment was compared using the same method as above. Differences in preference between different experiments were tested with a GLM as above.

### Volatile collection and analysis

Three groups of plants with different treatments (as above) were prepared for volatile analysis: (1) clean plants as control; (2) plants exposed to five females and five males of *M. pygmaeus* for 4 days, after which all *M. pygmaeus* were removed before volatiles were collected (*M. pygmaeus* removed); (3) plants exposed to five females and five males of *M. pygmaeus* for 4 days, with the omnivores present during volatile collection (*M. pygmaeus* present). To ensure that all plants in this last treatment had the same number of *M. pygmaeus*, mirids from the culture were added until there were five males and five females per plant. Three plants from the same treatment were used as one replicate, in total 21 clean plants, 27 plants from which *M. pygmaeus* was removed, and 15 plants on which *M. pygmaeus* was present were used. The volatile collection was performed in three blocks over time. The three plants were placed in a 40 L glass desiccator, and volatile sampling was done according to Kant et al. (2004). Briefly, desiccators were ventilated with carbon-filtered pressured air at a flow rate of 400 ml per minute. Air from the desiccator was sampled during 24 h by trapping it on 50 mg of Porapak type Q 80–100 Mesh (Supelco) enclosed in a 5 mm wide glass tube. We also collected volatiles from males plus females of *M. pygmaeus* without plants as above. One hundred adult omnivores were placed in a 100 ml glass desiccator, volatiles were collected for 12 h, 14 h and 48 h.

Volatiles were eluted from the adsorbent using 2 mL pentane:diethyl ether (4:1) spiked with 2.5 ng/ $\mu$ l of benzyl acetate (BA) as internal standard. One microliter of the eluate was injected (splitless) in the injector port of an Agilent 7890A gas chromatograph and immediately heated to 275 °C. Compounds were separated on a HP-5 ms column (30 m  $\times$  250  $\mu$ m, 0.25  $\mu$ m film thickness; Agilent) with a temperature program set to 40 °C for 5 min, increasing to 250 °C at a rate of 15 °C per min and an additional 5 min at 250 °C. Helium was used as the carrier gas with the transfer flow set to 3 mL/min and a column flow rate of 1 mL/min

thereafter. The chromatograph was coupled to an Agilent 7200 accurate-mass quadrupole time-of-flight mass spectrometer to generate the mass spectra, operating in electron ionization mode (70 eV) at 230 °C and collected with an acquisition rate of 20 scans per second acquiring ions at a 30–500 m/z range.

Peaks were detected by chromatogram deconvolution using Agilent MassHunter Qualitative Analysis software using the following settings: signal-to-noise ratio = 2; RT window size factor = 100; minimal peak area = 500 counts; m/z accuracy = 50 ppm. Identified peaks were integrated over the acquired samples using Agilent MassHunter Quantitative Analysis software. Here, the area of the base peak was taken for quantification with an accuracy of 50 ppm and corrected with the internal standard. For compound identification, the Kovats Index was calculated and in combination with the mass spectrum compared to Adams and NIST libraries. Compound identification was confirmed using authentic standards when available.

Volatiles emanating from the empty set-up were considered contaminations and their peak values, averaged per block were subtracted from the values found for the biological sources within the same block. Subsequently, negative values and zeros were replaced with half of the minimum detection value. We also removed compounds that occurred only in one of the samples per treatment. We calculated the fold changes of the volatiles from plants exposed to *M. pygmaeus* relative to control plants per block and then averaged these values. For further analyses, peaks were log-transformed and either normalized with the Pareto method (Grace and Hudson 2017), or not normalized, and analysed with a linear discriminant analysis (LDA) in R (Venables and Ripley 2002). To further confirm the most important volatiles separating the three treatments, we performed a tree analysis (Ripley 2016) and a Partial Least Squares Discriminant Analysis (see Supplemental Methods). Peaks that had a relevant weight in both PLS-DA and LDA analyses were further identified. This resulted in 21 plant volatiles. We also identified two volatile compounds specific of *M. pygmaeus*.

We generated a list of 23 volatile compounds combining these plant volatiles and the two *M. pygmaeus* volatiles (Table 1). We performed a multivariate analysis of variance (MANOVA) using data of the 23 compounds to check the overall effects of treatments on volatile levels. Because the MANOVA showed a significant effect, we subsequently analysed each compound separately. We compared peaks of each treatment using linear mixed-effect models, with treatment as fixed factors and individual plant as a random factor. The distribution of the residuals was checked for normality. From all volatile peaks, N-hexyl acetate was  $\log(x + 0.1)$  transformed, 6-methyl-5-hepten-2-one  $\log(x + 1)$  transformed, one unidentified compound was not transformed,

**Table 1** Levels (fold changes, mean  $\pm$  SE) of volatiles from plants exposed to the omnivore *M. pygmaeus* with either the omnivore absent or present during volatile collection relative to clean, unexposed plants

Compound	Kovats Index	<i>M. pygmaeus</i> removed	<i>M. pygmaeus</i> present
<b>Z-jasmone<sup>a*</sup></b>	1412	87 $\pm$ 20 (b)	482 $\pm$ 257 (b)
<b>E-<math>\beta</math>-ocimene<sup>a***</sup></b>	1151	80 $\pm$ 30 (b)	937 $\pm$ 450 (b)
<b>linalool<sup>a</sup></b>	1102	18 $\pm$ 10 (ab)	136 $\pm$ 100 (b)
<b>eucarvone</b>	1156	27 $\pm$ 20 (ab)	93 $\pm$ 56 (b)
<i>E</i> - $\beta$ -guaiene <sup>a</sup>	1512	0.4 $\pm$ 0.1 (a)	0.6 $\pm$ 0.4(a)
<i>E</i> -nerolidol <sup>a**</sup>	1574	19 $\pm$ 10 (a)	172 $\pm$ 77 (b)
9-eicosyne <sup>*</sup>	1628	0.3 $\pm$ 0.1 (a)	0.5 $\pm$ 0.2 (a)
methyl salicylate <sup>a*</sup>	1205	21 $\pm$ 13 (ab)	100 $\pm$ 45 (b)
<i>Z</i> - $\beta$ -guaiene <sup>a*</sup>	1520	37 $\pm$ 23 (ab)	152 $\pm$ 67 (b)
phenylpropene	1466	2 $\pm$ 1.7 (a)	0.8 $\pm$ 0.1 (a)
selina-3,11-dien-6- $\alpha$ -ol	1639	5.4 $\pm$ 3.3 (ab)	66 $\pm$ 51 (a)
<b>Z-nerolidol<sup>a</sup></b>	1537	4 $\pm$ 1.6 (a)	7 $\pm$ 6 (a)
Unknown oxygenated monoterpene <sup>**</sup>	1185	1.3 $\pm$ 0.2 (a)	2.4 $\pm$ 0.4 (b)
Unknown sesquiterpene <sup>***</sup>	1479	1.3 $\pm$ 0.4 (a)	10 $\pm$ 1.9 (b)
Unknown terpene	1570	1 $\pm$ 0.5 (a)	1.4 $\pm$ 0.8 (a)
Unknown benzene ester <sup>*</sup>	1644	1.2 $\pm$ 0.2 (ab)	1.7 $\pm$ 0.2 (b)
Unknown alkane	1701	0.5 $\pm$ 0.1 (a)	0.7 $\pm$ 0.2 (a)
Unknown	1796	1.3 $\pm$ 0.2 (a)	1.2 $\pm$ 0.2 (a)
Unknown sesquiterpene	1791	9 $\pm$ 6 (a)	2.4 $\pm$ 1.2 (a)
2, 3-dimethylbenzaldehyde	1193	0.7 $\pm$ 0.1 (a)	1.9 $\pm$ 0.9 (a)
n-hexyl acetate <sup>b</sup>	1010	1.6 $\pm$ 0.3 (b)	13 $\pm$ 2 (c)
6-methyl-5-hepten-2-one <sup>b</sup>	1210	1 $\pm$ 0.01 (a)	1303 $\pm$ 320(b)

<sup>a</sup>: Compounds identified with standards. <sup>b</sup>: volatile compounds specific for *M. pygmaeus*. Compounds in bold letters contribute significantly to the separation of the three treatment groups with both PLSDA and LDA analyses. Significant effects of treatment on the relative levels of volatiles from three groups of plants are indicated by asterisks. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ . Letters indicate differences among treatments: a: not different from the control (clean plants); treatments labelled with b differ significantly from those with c and both differ from the control

and the other peaks were all log ( $x + 0.01$ ) transformed, based on the distribution of the residuals.

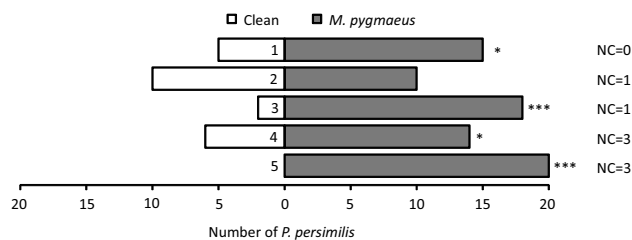
## Results

### Response to plants exposed to *M. pygmaeus*

*Phytoseiulus persimilis* significantly preferred plants previously exposed to *M. pygmaeus* over clean plants in four out of the five replicates (Fig. 1). No significant preference was found in one replicate, resulting in a significant interaction between treatment and replicate (GLM:  $\text{Chi}^2 = 20.20$ ,  $d. f. = 4$ ,  $P < 0.001$ ). Overall, the predatory mites showed a clear preference for *M. pygmaeus*-treated plants to clean plants.

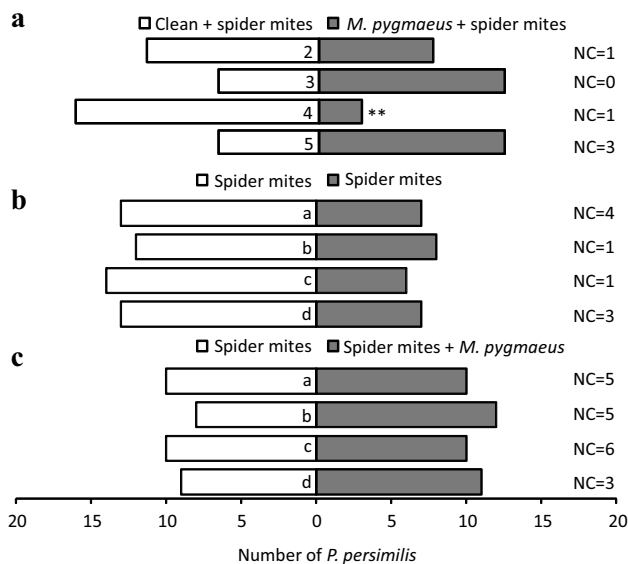
### Response to plants exposed to *M. pygmaeus* and infested with *T. urticae*

When both groups of plants of the previous experiment were subsequently infested with spider mites, the



**Fig. 1** Preference of *P. persimilis* for the volatiles from clean plants (white bars) or plants previously exposed to *M. pygmaeus* (grey bars); Each bar represents the result of a separate replicate, each with new groups of plants and predatory mites. Numbers along the vertical axis give the identity of these groups of plants, most of which groups were also used for the experiments which results are shown in Fig. 2. Each bar represents the response of 20 predatory mites. NC indicates the number of predatory mites that did not respond. Per replicate, significant preference for one of the two volatiles is indicated by asterisks (\*:  $P < 0.05$ ; \*\*\*:  $P < 0.001$ )

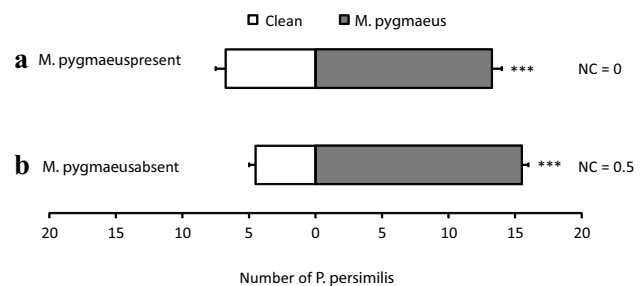
preference for *M. pygmaeus*-treated plants disappeared (Fig. 2a). *Phytoseiulus persimilis* showed no significant preference in three out of four replicates and showed a preference for plants that had not been exposed to *M.*



**Fig. 2** Preference of *P. persimilis* for the volatiles emanating from two groups of plants: **a** plants previously exposed to *M. pygmaeus* for 4 days and subsequently infested with spider mites for 2 days (grey bars) vs. unexposed plants subsequently infested with spider mites for 2 days (white bars); **b** plants infested with spider mites for 2 days (white bars) vs plants infested with spider mites for 2 days (grey bars); **c** plants infested with spider mites for 6 days (white bars) vs plants infested with spider mites for 6 days and exposed to *M. pygmaeus* for the last 4 days (grey bars). Within each panel, each bar represents the result of a different replicate, each with new groups of plants and predatory mites. Results presented in **a** were obtained with the same groups of plants as in Fig. 1; they were first tested after exposure to the omnivore (Fig. 1), then infested with spider mites and tested again (**a**). Numbers along the vertical axis give the identity of these groups of plants. Results presented in **b** and **c** were also obtained with the same plants; they were first tested after infestation with spider mites (**b**), then exposed to the omnivore and tested again (**c**). Letters along the vertical axis give the identity of these groups of plants. Each bar represents the response of 20 predatory mites. NC indicates the number of predatory mites that did not respond

*pygmaeus* in the fourth replicate, resulting in a significant interaction between treatment and replicate (GLM:  $\text{Chi}^2 = 14.83$ ,  $d. f. = 3$ ,  $P = 0.002$ ). The difference in preference between the previous experiment and the current one was highly significant (c.f. Figures 1 and 2a, GLM:  $\text{Chi}^2 = 16.9$ ,  $d. f. = 1$ ,  $P < 0.0001$ ), showing that the preference for plants exposed to *M. pygmaeus* disappeared when these plants plus the clean plants were subsequently attacked by spider mites.

When plants were first infested with spider mites, none of the two groups of plants attracted significantly more *P. persimilis* (Fig. 2b). However, because we assigned the groups of plants that were slightly more attractive to one treatment (Fig. 2b, left-hand bars) and the other groups to the other treatment (Fig. 2b, right-hand bars), there was an overall significant preference for the groups of plants that were more attractive (GLM:  $\text{Chi}^2 = 7.32$ ,  $d. f. = 1$ ,  $P = 0.007$ ).



**Fig. 3** Preference of *P. persimilis* for the volatiles emanating from clean plants (white bars) and plants exposed to *M. pygmaeus* (grey bars), with **a** and without **b** *M. pygmaeus* present on the plants. Shown are average numbers (+ SE) of predators of 4 replicates (*M. pygmaeus* present) or 2 replicates (*M. pygmaeus* absent), each with new groups of plants and predators. See legend to Fig. 1 for more explanation

When the least attractive groups of plants were subsequently exposed to *M. pygmaeus*, they became equally attractive as the groups of plants not exposed to *M. pygmaeus* (Fig. 2c, GLM:  $\text{Chi}^2 = 0.45$ ,  $d. f. = 1$ ,  $P = 0.50$ ). The change in preference due to exposure to *M. pygmaeus* was significant (cf. Figure 2b, c, GLM:  $\text{Chi}^2 = 5.77$ ,  $d. f. = 1$ ,  $P = 0.0163$ ).

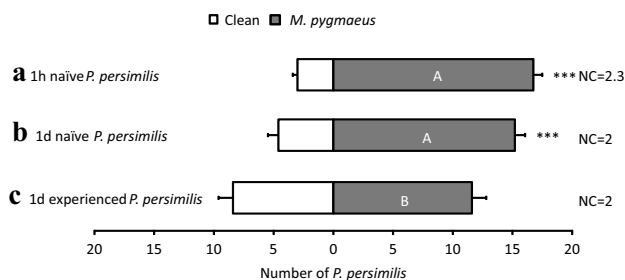
### Response of *P. persimilis* to plants with *M. pygmaeus*

*Phytoseiulus persimilis* preferred plants on which *M. pygmaeus* were present to clean plants (Fig. 3a, GLM:  $\text{Chi}^2 = 8.61$ ,  $d. f. = 1$ ,  $P = 0.0034$ ). When the omnivores were subsequently removed from the plants and attraction was tested again the following day (Fig. 3b, GLM:  $\text{Chi}^2 = 12.8$ ,  $d. f. = 1$ ,  $P = 0.0003$ ), the preference for *M. pygmaeus*-exposed plants was somewhat more pronounced (Fig. 3b), but the difference between preference for plants with the omnivore present or removed was not significant (cf. 3a, 3b, GLM:  $\text{Chi}^2 = 1.66$ ,  $d. f. = 1$ ,  $P = 0.198$ ). The results presented in Fig. 3b further confirmed earlier results showing the attraction of *P. persimilis* to plants previously exposed to *M. pygmaeus* without these mirids being present on the plants (Fig. 3b, cf. Fig. 1).

### Effect of experience of *P. persimilis*

The attraction of *P. persimilis* to plants exposed to *M. pygmaeus* was somewhat surprising because the omnivores can feed on eggs of *P. persimilis*, hence, the predatory mites were expected to avoid plants with the omnivore. We therefore investigated whether experience of *P. persimilis* with the omnivore would change its response towards volatiles of plants exposed to the omnivore. *Phytoseiulus persimilis* with and without experience with the volatiles from plants exposed to *M. pygmaeus* showed different attractiveness to plants previously exposed to the omnivore and clean plants



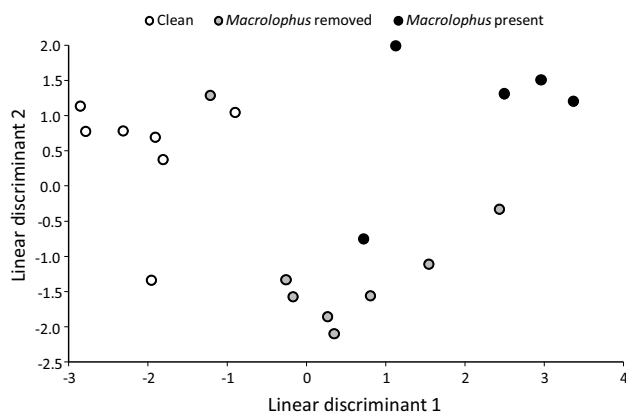


**Fig. 4** Preference of *P. persimilis* (mean+SE) for volatiles from clean plants (white bars) and from plants exposed to *M. pygmaeus* (grey bars). Shown are results of three groups of predatory mites with different experiences with volatile cues from plants exposed to *M. pygmaeus*: **a** *P. persimilis* without experience, starved for 1 h; **b** *P. persimilis* without experience, starved for 1 day; **c** *P. persimilis* experienced with the volatiles from plants with *M. pygmaeus* plus volatiles from *M. pygmaeus* in the absence of food for 1 day. Each bar represents the average of 4 (1 h naïve) or 5 replicates (1d naïve and 1d experienced), each with new groups of plants and predators. Different capital letters indicate significant differences among the three groups of *P. persimilis* (contrasts after GLM:  $P < 0.05$ ). See legend to Fig. 1 for more explanation

(Fig. 4, GLM:  $\text{Chi}^2 = 17.40$ ,  $d. f. = 2$ ,  $P = 0.00017$ ). Predatory mites without experience with *M. pygmaeus*-treated plants, starved for 1 h prior to the test, showed a significant preference for plants exposed to *M. pygmaeus* (Fig. 4a, GLM:  $\text{Chi}^2 = 42.2$ ,  $d. f. = 1$ ,  $P < 0.0001$ ). This is consistent with previous results (Figs. 1, 3b), again confirming that *P. persimilis* preferred plants exposed to *M. pygmaeus* over clean plants. When naïve *P. persimilis* were starved for 24 h in the presence of volatiles of clean plants, they were also attracted to plants exposed to *M. pygmaeus* (Fig. 4b, GLM:  $\text{Chi}^2 = 29.9$ ,  $d. f. = 1$ ,  $P < 0.0001$ ). The two groups of naïve mites showed similar attractiveness to plants exposed to *M. pygmaeus* (Fig. 4a, b, contrasts with glht function of package lsmeans). When given 1 day of experience with the volatiles from plants exposed to *M. pygmaeus* in the absence of food, the preference of *P. persimilis* for these plants disappeared (Fig. 4c, GLM:  $\text{Chi}^2 = 2.57$ ,  $d. f. = 1$ ,  $P = 0.109$ ). The response of the experienced predatory mites differed significantly from the two groups of naïve mites (Fig. 4, contrasts as above).

### Volatile analysis

A linear discriminant analysis resulted in a good separation of the three groups (Fig. 5), with the first linear discriminant explaining 77% of the variance. A tree analysis separated the clean and plants exposed to the omnivore (Fig. S1). The PLSDA analysis separated the three treatments, and the two first components explained more than 50% of the variance (Fig. S2). The 21 plant volatiles with the largest differences between the clean plants and the exposed plants and the



**Fig. 5** Grouping of the three treatments as a function of two linear discriminants, based on the 16 selected volatile compounds (see text)

two *M. pygmaeus*-specific volatiles are presented in Table 1. The compounds (*Z*)-jasmone, (*E*)- $\beta$ -ocimene, linalool, (*E*)-nerolidol and eucarvone were identified in both the LDA and PLSDA analysis. In general, plants with the omnivore present produced higher amounts of the selected volatiles than plants with the omnivore removed prior to the measurements, although differences were not always significant (Table 1). Compounds that were significantly released in higher amounts by the omnivore-exposed plants compared to clean plants include (*Z*)-jasmone, (*E*)- $\beta$ -ocimene, linalool, (*E*)-nerolidol, methyl salicylate (MeSA), an oxygenated monoterpene, a sesquiterpene and a benzene ester (Table 1). Exposed plants also produced lower amounts of other volatiles than clean plants (e.g. (*E*)- $\beta$ -guaiene and 9-eicosyne), though the differences were not significant (Table 1, averages < 1). Two volatiles specific of *M. pygmaeus* were identified, namely n-hexyl acetate and 6-methyl-5-hepten-2-one.

### Discussion

We show that volatiles of sweet pepper plants exposed to *M. pygmaeus* were attractive to the predatory mite *P. persimilis*, although we observed differences among replicates in the attractiveness (Fig. 1a). These differences might have been caused by differences in survival of *M. pygmaeus* during the plant treatments in different replicates, although on average 60% of the mirids survived. Lower numbers of *M. pygmaeus* feeding on the plants may induce lower amounts of volatiles, which might be less attractive to predatory mites. Overall, predatory mites preferred the *M. pygmaeus*-infested plants over clean plants, and this preference was confirmed in subsequent experiments (Figs. 3b, 4a). Thus, we conclude that *P. persimilis* was more attracted by the volatiles emanating from plants previously exposed to *M. pygmaeus* than to volatiles from clean plants. In any case, these data suggest

that the presence of *M. pygmaeus* could potentially interfere with the long-distance searching behaviour of the predatory mite, which will often be attracted to plants with the omnivore, but without its prey. If predators would stay on these plants, they would consequently starve, but they can learn the association between the volatiles and the lack of food, and subsequently disperse.

This is not the first study showing that omnivores can induce the production of plant volatiles. A study by Moayeri et al. (2007) showed that, compared to clean plants, 11 additional compounds were produced when *M. pygmaeus* was feeding on bean plants. More recently, Bouagga et al. (2018a, b) found that another omnivorous predator (*Orius laevigatus*) induced the production of volatiles by plants. Together with this study, these results confirm that volatile production was induced by plant feeding of the omnivorous predators. Earlier, we also found that the spider mites and thrips preferred clean plants over plants previously exposed to *M. pygmaeus* (Zhang et al. 2019b), and these responses may have been partly or entirely based on the volatile produced by these plants. We conclude that plant volatiles induced by *M. pygmaeus* play a role in plant–herbivore–predator interactions. In this study, lower, more realistic, numbers of omnivores were used than in the study by Pérez-Hedo et al. (2015a, 5 females and 5 males here vs 25 individuals/plant), which still resulted in strong effects on the behaviour of other predators. This is remarkable, because these low densities of omnivores hardly cause any damage to the plants (Castañé et al. 2011), and much higher numbers of herbivores are often used to induce direct plant defences or the induction of volatile production (Maeda and Takabayashi 2001).

When plants that had been exposed to the omnivore were subsequently attacked by spider mites, the prey of the predatory mites, the predatory mites were equally attracted by these plants and plants exposed to the omnivore. When plants were first attacked by spider mites and were subsequently exposed to *M. pygmaeus*, they became somewhat more attractive than before exposure. All in all, these results show that *P. persimilis* remains attracted to plants with spider mite prey, despite the effects of the omnivore on plant attractiveness. We observed differences among replicates in the attractiveness of volatiles from plants exposed to *M. pygmaeus* and subsequently attacked by spider mites (Fig. 2a). This might have been caused by differences in numbers of spider mites during the treatment in different replicates, and lower densities of spider mites resulting in lower amounts of volatiles (Maeda and Takabayashi 2001), making the plants less attractive for predatory mites. We indeed observed lower numbers of spider mites on plants exposed to *M. pygmaeus* than on unexposed plants ( $126 \pm 12.9$  vs.  $171 \pm 7.4$ , respectively), which was consistent with earlier results showing that spider mites performed less well on plants previously

exposed to *M. pygmaeus* than on clean plants (Zhang et al. 2018).

When the omnivores were present on the plants, predatory mites could also perceive cues from the omnivores themselves. Plants with the omnivores were still significantly attractive to the predatory mites, but this attractiveness was slightly less pronounced than that of predator-induced plants without the omnivore (Fig. 3); however, this difference was not significant. We therefore conclude that cues from the omnivores themselves did not interfere significantly with the choice of the predatory mites.

Plants exposed to the omnivore produced different amounts of volatiles than clean plants. The headspace of plants exposed to the omnivore contained higher amounts of Z-jasmone, (*E*)- $\beta$ -ocimene, (*E*)-nerolidol, methyl salicylate (MeSA) and (*Z*)- $\beta$ -guaiene than that of clean plants, which are known to be produced by plants under attack by herbivores, including sweet pepper plants. Another omnivorous predator, *Orius laevigatus*, was found to induce the production of linalool, MeSA and nerolidol in sweet pepper plants (Bouagga et al. 2018a). Linalool and (*E*)- $\beta$ -ocimene are known to be attractive to *P. persimilis* (Dicke et al. 1990; van den Boom et al. 2004; van Wijk et al. 2008). However, the attractiveness or repellence of mixtures of volatiles cannot be inferred from that of the single compounds because *P. persimilis* perceives these mixtures as synthetic entities and not as a collection of individual compounds (van Wijk et al. 2008, 2010). The exposed plants in our study produced several volatiles in lower amounts than clean plants, meaning that plant feeding by the omnivore changed ratios of the volatile blends. During volatile collection, both plant volatiles and volatiles from the omnivore were collected, and we found 2 volatiles specific for *M. pygmaeus*, which were also found in another study (Moayeri et al. 2007).

An open question is why predatory mites were attracted by volatiles emanating from plants that had been exposed to the omnivores. Because of natural variation in volatile blends, depending on host plant species, herbivore species, plant age and other factors (Takabayashi et al. 1994a; De Moraes et al. 1998; van den Boom et al. 2004), natural enemies face the difficult task to discriminate among many different blends (Sabelis et al. 1999; Takabayashi et al. 2006). It is often suggested that the ability to learn to respond to volatile cues associated with their prey or host increases their searching efficiency, which is very important for many predatory arthropods and parasitoids (Lewis and Tumlinson 1988; Turlings et al. 1993; Drukker et al. 2000a, b; Takabayashi et al. 2006; Hilker and McNeil 2008; Janssen et al. 2014). This was the case in our study: after experience with volatiles from plants with *M. pygmaeus* in the absence of prey, the preference for the volatiles produced by these plants disappeared (Fig. 4c). Such reduction of attraction after an experience of the association between a volatile

blend and the absence of suitable prey has been reported before (Ardanuy et al. 2016). This shows that the predatory mites probably associated the volatiles with the absence of food, suggesting that predatory mites that would initially be naïve and attracted to plants with the omnivore but without spider mites, would subsequently no longer be attracted to such plants. The question then is whether they would still be attracted to plants with spider mites; this remains to be investigated.

A further open question is whether volatiles of plants attacked by spider mites are more attractive to *P. persimilis* than plants induced by *M. pygmaeus* (non-prey). A problem with testing this is, that it is not clear how many *M. pygmaeus* and how many spider mites should be used to infest the plants. The two species are rather different in size, cause different types of damage and are therefore difficult to compare on a per-individual basis. Hence, if an experiment would show that plants with spider mites are more or less attractive than plants with *M. pygmaeus*, this could be because of quantitative differences in the volatiles rather than qualitative differences. Hence, an answer to this question would require a whole series of experiments with various densities of the two species plus the accompanying volatile analyses, and this was beyond the main research question of the study.

Cues from prey and plants with prey give important information for prey plant choice, however, cues associated with the presence of competitors also affect plant selection. Avoidance of patches occupied by competitors based on volatiles occurs in parasitoids (Janssen et al. 1995a, b; Tamó et al. 2006) and predatory mites (Janssen et al. 1997; Magalhães et al. 2005), and prey patches with cues associated with spider mite eggs killed by competitors were less attractive for *P. persimilis* (Choh et al. 2017). In our study, spider mite-infested plants were subsequently exposed to *M. pygmaeus*, thus cues associated with killed spider mite eggs were present on these plants. Moreover, other cues associated with the presence of the mirids, e.g. volatiles, chemical marks and faeces from *M. pygmaeus* (Moayeri et al. 2007) may also serve as cues for predatory mites. We observed that *M. pygmaeus* can prey on all stages of *P. persimilis* and also does so in the presence of spider mites. We therefore expected that the predatory mites would avoid plants with the omnivores, but instead, plants with the omnivores were more attractive for *P. persimilis* than clean plants. However, we also observed that adult predatory mites could escape from the omnivores (NX. Zhang and J. Brouwer pers. obs.); thus, *M. pygmaeus* is perhaps not a dangerous predator for adult female *P. persimilis*. Furthermore, the predatory mites oviposit inside the web produced by spider mites and use it as a refuge to prevent predation from other predators (Sabelis and Bakker 1992; Cloutier and Johnson 1993; Roda et al. 2000; Lemos et al. 2015); hence, adult but also juvenile

*P. persimilis* may be able to escape from predation by the omnivore.

Another question that remains to be answered is how the induction of volatile production would benefit the omnivore. Interestingly, Moayeri et al. (2007) showed that *M. caliginosus* (an earlier synonym of *M. pygmaeus*) males were attracted by plants previously exposed to their female conspecifics, and gender-specific responses to omnivore-induced plant volatiles were described for *Nesidiocoris tenuis* (Rim et al. 2018). Perhaps omnivores induce plants to produce volatiles to attract mates, thus economizing on producing large quantities of sex pheromones. Clearly, further studies are needed for understanding the role of omnivore-induced plant responses in multitrophic interactions.

It is also not clear what is the advantage of plants to produce volatiles when omnivorous predators are feeding on them. One suggested function of herbivore-induced plant volatiles is to attract natural enemies, and one may argue that the plants guarded by the omnivorous predators do not need to produce volatiles to attract more natural enemies. A mechanical explanation for the production of volatiles is that plant volatiles act as signal compounds between different plant parts (Frost et al. 2007), through which unattacked and undefended parts can become primed against future attacks, but it is unclear why these distant parts should be primed when omnivores are present on the plant. Possibly, plants do not produce volatiles to attract natural enemies, but to signal to herbivores that the plant is defended. This was confirmed by our earlier study that spider mites and thrips preferred clean plants over plants previously exposed to *M. pygmaeus* (Zhang et al. 2019b). The repellence of herbivores would not only reduce herbivore damage to the plant, but also prevent the transmission of plant pathogens by herbivores. However, without experience, some predators may take this information as indication of the presence of herbivores, which might be the case for *P. persimilis*.

*Macrolophus pygmaeus* is used for biocontrol of various pests, such as whiteflies, aphids and spider mites in various crops, including sweet pepper. *Phytoseiulus persimilis* is a specialist natural enemy of spider mites and is used in many crops, hence, there is a real possibility that these two species will be released in the same crop. Under such conditions, the plant feeding by *M. pygmaeus* will interfere with the searching behaviour of *P. persimilis*, and it remains to be investigated to what extent this will affect spider mite control. However, we suspect that the volatiles induced by *M. pygmaeus* will also interfere with the searching behaviour of other natural enemies. Indeed, Pérez-Hedo et al. (2015a) found indications that the whitefly parasitoid *Encarsia formosa* was attracted by tomato plants exposed by each of three species of mirid bugs. So plant feeding by omnivores can interfere with the searching behaviour of other natural enemies. Depending on the

capacity of these other enemies to learn to discriminate between the volatiles of plants induced by omnivores and plants induced by their prey, this may interfere with biological pest control. There are several possible scenarios. First, the other natural enemies may be attracted to the volatiles induced by omnivores, as was found here, and this may distract them from foraging for their prey. It may also result in increased intraguild predation between the omnivore and the other natural enemies. Second, the other natural enemies may be repelled by the volatiles induced by the omnivore, thus avoiding intraguild predation. However, this can potentially result in enemy-free space of the pest of the other natural enemy if it is no prey of the omnivore. Hence, future research should not just focus on the production of plant volatiles as a consequence of phytophagy by omnivores, and on the induction of plant defences in general, but also on their effects on other enemies, considering also their capacity to change their behaviour as a result of experience.

We previously showed that omnivore feeding on plants interfered with the performance of herbivores through induced direct defences (Zhang et al. 2018, 2019b) and affected the phenology and reproduction of plants and the performance of the omnivore (Zhang et al. 2019a). The results of the current study suggest that feeding by these omnivores also induces the production of plant volatiles, which can affect the behaviour of a specialist predator of a plant pest and, consequently, can result in disruption of biological control (Zhang et al. 2021). Clearly, further studies on the effects of omnivores on dynamics of plant-associated food webs are needed.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s10340-021-01463-3>.

**Acknowledgements** We thank Enza Zaden Beheer B.V. for the supply of sweet pepper seeds, Koppert Biological Systems for the supply of *Macrolophus* and *Ephestia* eggs, Alexandra Revynthi for spider mites and predatory mites, Peter de Ruiter, Michel Haring, Louise Vet, Josep Jaques, Isabel Smallegange and Martijn Egas for comments on the manuscript and the population biology group for discussions. Two anonymous reviewers are thanked for constructive comments.

**Author contributions** NXZ, GJM, AJ and JMA conceived and designed the experiments. NXZ, JA and JB performed behavioural experiments. NXZ, JMA and RWJK collected and analysed plant volatiles. NXZ, JMA and AJ analysed the data and wrote the manuscript. All authors commented and corrected it.

**Funding** JMA was supported as researcher associated with NWO STW-VIDI-13492. NXZ received a scholarship from the China Scholarship Council (CSC).

**Availability of data and material** The datasets generated during the current study are available from the corresponding author on reasonable request.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The authors declare no conflict of interest that is relevant to the content of this article.

**Ethical approval** Plants and insects were used in this study. All applicable international, national and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

## References

- Alvarado P, Baltà O, Alomar O (1997) Efficiency of four heteroptera as predators of *Aphis gossypii* and *Macrosiphum euphorbiae* (Hom.: Aphididae). *Entomophaga* 42:215–226. <https://doi.org/10.1007/BF02769899>
- Ardanuy A, Albajes R, Turlings TCJ (2016) Innate and learned prey-searching behavior in a generalist predator. *J Chem Ecol* 42:497–508. <https://doi.org/10.1007/s10886-016-0716-9>
- Arnó J, Alonso E, Gabarra R (2003) Role of the parasitoid *Diglyphus isaea* and the predator *Macrolophus caliginosus* in the control of leafminers. *IOBC WPRS Bull* 26:79–84
- Birkett MA, Chamberlain K, Guerrieri E et al (2003) Volatiles from whitefly-infested plants elicit a host-locating response in the parasitoid, *Encarsia formosa*. *J Chem Ecol* 29:1589–1600. <https://doi.org/10.1023/A:1024218729423>
- Bouagga S, Urbaneja A, Rambla JL et al (2018a) *Orius laevigatus* strengthens its role as a biological control agent by inducing plant defenses. *J Pest Sci* 91:55–64. <https://doi.org/10.1007/s10340-017-0886-4>
- Bouagga S, Urbaneja A, Rambla JL et al (2018b) Zoophytophagous mirids provide pest control by inducing direct defences, antixenosis and attraction to parasitoids in sweet pepper plants. *Pest Manag Sci* 74:1286–1296. <https://doi.org/10.1002/ps.4838>
- Castañé C, Arno J, Gabarra R, Alomar O (2011) Plant damage to vegetable crops by zoophytophagous mirid predators. *Biol Control* 59:22–29. <https://doi.org/10.1016/j.biocontrol.2011.03.007>
- Choh Y, Sabelis MW, Janssen A (2017) Predatory interactions between prey affect patch selection by predators. *Behav Ecol Sociobiol* 71:66. <https://doi.org/10.1007/s00265-017-2288-2>
- Cloutier C, Johnson SG (1993) Predation by *Orius tristicolor* (Hemiptera: Anthocoridae) on *Phytoseiulus persimilis* (Acarina: Phytoseiidae): testing for compatibility between biocontrol agents. *Environ Entomol* 22:477–482. <https://doi.org/10.1093/ee/22.2.477>
- Crawley MJ (2013) *The R Book*. Wiley, Chichester, UK
- De Moraes CM, Lewis WJ, Pare PW et al (1998) Herbivore-infested plants selectively attract parasitoids. *Nature* 393:570–573. <https://doi.org/10.1038/31219>
- De Puyssseleyr V, Höfte M, De Clercq P (2011) Ovipositing *Orius laevigatus* increase tomato resistance against *Frankliniella occidentalis* feeding by inducing the wound response. *Arthropod-Plant Interact* 5:71–80. <https://doi.org/10.1007/s11829-010-9117-0>
- Dicke M (1994) Local and systemic production of volatile herbivore-induced terpenoids - Their role in plant-carnivore mutualism.



- J Plant Physiol 143:465–472. [https://doi.org/10.1016/S0176-1617\(11\)81808-0](https://doi.org/10.1016/S0176-1617(11)81808-0)
- Dicke M, van Beek TA, Posthumus MA et al (1990) Isolation and identification of volatile kairomone that affects acarine predator-prey interactions - Involvement of host plant in its production. J Chem Ecol 16:381–396. <https://doi.org/10.1007/BF01021772>
- Dicke M, van Loon JJ, Soler R (2009) Chemical complexity of volatiles from plants induced by multiple attack. Nat Chem Biol 5:317–324. <https://doi.org/10.1038/nchembio.169>
- Dicke M, van Loon JJA (2000) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. Entomol Exp Appl 97:237–249. <https://doi.org/10.1046/j.1570-7458.2000.00736.x>
- Drukker B, Bruin J, Jacobs G et al (2000a) How predatory mites learn to cope with variability in volatile plant signals in the environment of their herbivorous prey. Exp Appl Acarol 24:881–895. <https://doi.org/10.1023/A:1010645720829>
- Drukker B, Bruin J, Sabelis MW (2000b) Anthocorid predators learn to associate herbivore-induced plant volatiles with presence or absence of prey. Physiol Entomol 25:260–265. <https://doi.org/10.1046/j.1365-3032.2000.00190.x>
- Du Y, Poppy GM, Powell W et al (1998) Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. J Chem Ecol 24:1355–1368. <https://doi.org/10.1023/A:1021278816970>
- Frost CJ, Appel M, Carlson JE et al (2007) Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. Ecol Lett 10:490–498. <https://doi.org/10.1111/j.1461-0248.2007.01043.x>
- Grace SC, Hudson DA (2017) Processing and visualization of metabolomics data using R. In: Prasain JK (ed) Metabolomics: fundamentals and applications. InTech, Croatia, pp 63–94
- Guerrieri E, Poppy GM, Powell W et al (1999) Induction and systemic release of herbivore-induced plant volatiles mediating in-flight orientation of *Aphidius ervi*. J Chem Ecol 25:1247–1261. <https://doi.org/10.1023/A:1020914506782>
- Hansen DL, Brødsgaard HF, Enkegaard A (1999) Life table characteristics of *Macrolophus caliginosus* preying upon *Tetranychus urticae*. Entomol Exp Appl 93:267–273. <https://doi.org/10.1046/j.1570-7458.1999.00587.x>
- Heil M, Koch T, Hilpert A et al (2001) Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius*, is induced, indirect, defensive response elicited by jasmonic acid. Proc Natl Acad Sci U S A 98:1083–1088. <https://doi.org/10.1073/pnas.98.3.1083>
- Hilker M, McNeil J (2008) Chemical and behavioral ecology in insect parasitoids. In: Wajnberg E, Bernstein C, van Alphen JJM (eds) Behavioral ecology of insect parasitoids: how to behave optimally in a complex odorous environment. Blackwell, New York, USA, pp 693–705
- Janssen A (1999) Plants with spider-mite prey attract more predatory mites than clean plants under greenhouse conditions. Entomol Exp Appl 90:191–198. <https://doi.org/10.1046/j.1570-7458.1999.00438.x>
- Janssen A, Bruin J, Jacobs G et al (1997) Predators use volatiles to avoid prey patches with conspecifics. J Anim Ecol 66:223–232. <https://doi.org/10.2307/6024>
- Janssen A, Fonseca JO, Colares F et al (2014) Time scales of associating food and odor by predator communities in the field. Behav Ecol 25:1123–1130. <https://doi.org/10.1093/beheco/aru094>
- Janssen A, van Alphen JJM, Sabelis MW, Bakker K (1995a) Odour-mediated avoidance of competition in *Drosophila* parasitoids - the ghost of competition. Oikos 73:356–366. <https://doi.org/10.2307/3545959>
- Janssen A, van Alphen JJM, Sabelis MW, Bakker K (1995b) Specificity of odour-mediated avoidance of competition in *Drosophila* parasitoids. Behav Ecol Sociobiol 36:229–235. <https://doi.org/10.1007/BF00165831>
- Kant MR, Ament K, Sabelis MW et al (2004) Differential timing of spider mite-induced direct and indirect defenses in tomato plants. Plant Physiol 135:483–495. <https://doi.org/10.1104/pp.103.038315>
- Kant MR, Jonckheere W, Knecht B et al (2015) Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. Ann Bot 115:1015–1051. <https://doi.org/10.1093/aob/mcv054>
- Karban R, Baldwin IT (1997) Induced responses to herbivory. University of Chicago Press, Chicago. <https://doi.org/10.7208/chicago/9780226424972.001.0001>
- Lemos F, Bernardo A, Dias C et al (2015) Breaking and entering: predators invade the shelter of their prey and gain protection. Exp Appl Acarol 67:247–257. <https://doi.org/10.1007/s10493-015-9951-y>
- Lewis WJ, Tumlinson JH (1988) Host detection by chemically-mediated associative learning in a parasitic wasp. Nature 331:257–259. <https://doi.org/10.1038/331257a0>
- Maeda T, Takabayashi J (2001) Production of herbivore-induced plant volatiles and their attractiveness to *Phytoseius persimilis* (Acari: Phytoseiidae) with changes of *Tetranychus urticae* (Acari: Tetranychidae) density on a plant. Appl Entomol Zool 36:47–52. <https://doi.org/10.1303/aez.2001.47>
- Magalhães S, Fayard J, Janssen A et al (2007) Adaptation in a spider mite population after long-term evolution on a single host plant. J Evol Biol 20:2016–2027. <https://doi.org/10.1111/j.1420-9101.2007.01365.x>
- Magalhães S, Tudorache C, Montserrat M et al (2005) Diet of intraguild predators affects antipredator behavior in intraguild prey. Behav Ecol 16:364–370. <https://doi.org/10.1093/beheco/arh171>
- Moayeri HRS, Ashouri A, Poll L, Enkegaard A (2007) Olfactory response of a predatory mite to herbivore induced plant volatiles: multiple herbivory vs. single herbivory. J Appl Entomol 131:326–332. <https://doi.org/10.1111/j.1439-0418.2007.01177.x>
- Montserrat M, Albajes R, Castane C (2000) Functional response of four Heteropteran predators preying on greenhouse whitefly (Homoptera : Aleyrodidae) and western flower thrips (Thysanoptera : Thripidae). Env Entomol 29:1075–1082. <https://doi.org/10.1603/0046-225X-29.5.1075>
- Naselli M, Urbaneja A, Siscaro G et al (2016) Stage-related defense response induction in tomato plants by *Nesidiocoris tenuis*. Int J Mol Sci 17:1210. <https://doi.org/10.3390/ijms17081210>
- Pappas ML, Steppuhn A, Geuss D et al (2015) Beyond predation: the zoophytophagous predator *Macrolophus pygmaeus* induces tomato resistance against spider mites. PLoS ONE 10:e0127251. <https://doi.org/10.1371/journal.pone.0127251>
- Pemberton RW, Lee JH (1996) The influence of extrafloral nectaries on parasitism of an insect herbivore. Am J Bot 83:1187–1194. <https://doi.org/10.1002/j.1537-2197.1996.tb13900>
- Perdikis D, Lykouressis D (2000) Effects of various items, host plants, and temperatures on the development and survival of *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae). Biol Control 17:55–60. <https://doi.org/10.1006/bcon.1999.0774>
- Pérez-Hedo M, Bouagga S, Jaques JA et al (2015a) Tomato plant responses to feeding behavior of three zoophytophagous predators (Hemiptera: Miridae). Biol Control 86:46–51. <https://doi.org/10.1016/j.biocontrol.2015.04.006>
- Pérez-Hedo M, Urbaneja-Bernat P, Jaques JA et al (2015b) Defensive plant responses induced by *Nesidiocoris tenuis* (Hemiptera: Miridae) on tomato plants. J Pest Sci 88:65–73. <https://doi.org/10.1007/s10340-014-0640-0>
- R Core Team (2019) R: A language and environment for statistical computing. Version 3.6.0. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org>



- Rim H, Uefune M, Ozawa R, Takabayashi J (2018) An omnivorous arthropod, *Nesidiocoris tenuis*, induces gender-specific plant volatiles to which conspecific males and females respond differently. *Arthropod-Plant Interact*. <https://doi.org/10.1007/s11829-018-9612-2>
- Ripley B (2016) tree: classification and regression trees. <https://CRAN.R-project.org/package=tree>
- Riudavets J, Castañe C (1998) Identification and evaluation of native predators of *Frankliniella occidentalis* (Thysanoptera: Thripidae) in the Mediterranean. *Environ Entomol* 27:86–93. <https://doi.org/10.1093/ee/27.1.86>
- Roda A, Nyrop J, Dicke M, English-Loeb G (2000) Trichomes and spider-mite webbing protect predatory mite eggs from intraguild predation. *Oecologia* 125:428–435. <https://doi.org/10.1007/s004420000462>
- Röse USR, Manukian A, Heath RR, Tumlinson JH (1996) Volatile semiochemicals released from undamaged cotton leaves: a systemic response of living plants to caterpillar damage. *Plant Physiol* 111:487–495. <https://doi.org/10.1104/pp.111.2.487>
- Sabelis MW, Afman BP, Slim PJ (1984) Location of distant spider mite colonies by *Phytoseiulus persimilis*: localization and extraction of a kairomone. In: Griffiths DA, Bowman CE (eds) *Acarology* 6. Ellis Horwood, Chichester
- Sabelis MW, Bakker FM (1992) How predatory mites cope with the web of their Tetranychid prey - a functional view on dorsal chaetotaxy in the Phytoseiidae. *Exp Appl Acarol* 16:203–225. <https://doi.org/10.1007/BF01193804>
- Sabelis MW, Janssen A, Bruin J et al (1999) Interactions between arthropod predators and plants: a conspiracy against herbivorous arthropods? In: Bruin J, van der Geest LPS, Sabelis MW (eds) *Ecology and evolution of the acari*. Kluwer Academic Publishers, The Netherlands, pp 207–229
- Sabelis MW, Takabayashi J, Janssen A et al (2007) Ecology meets plant physiology: herbivore-induced plant responses and their indirect effects on arthropod communities. In: Ohgushi T, Craig TP, Price PW (eds) *Ecological communities: plant mediation in indirect interaction webs*. Cambridge University Press, Cambridge, pp 188–218. <https://doi.org/10.1017/CBO9780511542701.010>
- Sabelis MW, van de Baan HE (1983) Location of distant spider mite colonies by phytoseiid predators: demonstration of specific kairomones emitted by *Tetranychus urticae* and *Panonychus ulmi*. *Entomol Exp Appl* 33:303–314. <https://doi.org/10.1111/j.1570-7458.1983.tb03273.x>
- Shimoda T, Dicke M (2000) Attraction of a predator to chemical information related to nonprey: when can it be adaptive? *Behav Ecol* 11:606–613. <https://doi.org/10.1093/beheco/11.6.606>
- Takabayashi J, Dicke M, Posthumus MA (1994a) Volatile herbivore-induced terpenoids in plant mite interactions - Variation caused by biotic and abiotic factors. *J Chem Ecol* 20:1329–1354. <https://doi.org/10.1007/BF02059811>
- Takabayashi J, Dicke M, Takahashi S et al (1994b) Leaf age affects composition of herbivore-induced synomones and attraction of predatory mites. *J Chem Ecol* 20:373–386. <https://doi.org/10.1007/BF02064444>
- Takabayashi J, Sabelis MW, Janssen A et al (2006) Can plants betray the presence of multiple herbivore species to predators and parasitoids? The role of learning in phytochemical information networks. *Ecol Res* 21:3–8. <https://doi.org/10.1007/s11284-005-0129-7>
- Tamó C, Roelfstra L-L, Guillaume S, Turlings TCJ (2006) Odour-mediated long-range avoidance of interspecific competition by a solitary endoparasitoid: a time-saving foraging strategy. *J Anim Ecol* 75:1091–1099. <https://doi.org/10.1111/j.1365-2656.2006.01128.x>
- Turlings T, Lengwiler UB, Bernasconi ML, Wechsler D (1998) Timing of induced volatile emissions in maize seedlings. *Planta* 207:146–152. <https://doi.org/10.1007/s004250050466>
- Turlings T, Tumlinson J, Lewis W (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250:1251–1253. <https://doi.org/10.1126/science.250.4985.1251>
- Turlings T, Tumlinson JH (1992) Systemic release of chemical signals by herbivore-injured corn. *Proc Natl Acad Sci U S A* 89:8399–8402. <https://doi.org/10.1073/pnas.89.17.8399>
- Turlings T, Wackers F, Vet LEM et al (1993) Learning of host-finding cues by hymenopterous parasitoids. In: Papaj DR, Lewis AC (eds) *Insect learning: ecology and evolutionary perspectives*. Chapman & Hall, New York, pp 51–78. [https://doi.org/10.1007/978-1-4615-2814-2\\_3](https://doi.org/10.1007/978-1-4615-2814-2_3)
- Urbaneja A, Montón H, Mollá O (2009) Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis*. *J Appl Entomol* 133:292–296. <https://doi.org/10.1111/j.1439-0418.2008.01319.x>
- van den Boom CEM, van Beek TA, Posthumus MA et al (2004) Qualitative and quantitative variation among volatile profiles induced by *Tetranychus urticae* feeding on plants from various families. *J Chem Ecol* 30:69–89. <https://doi.org/10.1023/B:JOEC.0000013183.72915.99>
- van Lenteren JC (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *Biocontrol* 57:1–20. <https://doi.org/10.1007/s10526-011-9395-1>
- van Wijk M, de Bruijn PJA, Sabelis MW (2008) Predatory mite attraction to herbivore-induced plant odors is not a consequence of attraction to individual herbivore-induced plant volatiles. *J Chem Ecol* 34:791–803. <https://doi.org/10.1007/s10886-008-9492-5>
- van Wijk M, de Bruijn PJA, Sabelis MW (2010) The predatory mite *Phytoseiulus persimilis* does not perceive odor mixtures as strictly elemental objects. *J Chem Ecol* 36:1211–1225. <https://doi.org/10.1007/s10886-010-9858-3>
- Venables WN, Ripley BD (2002) *Modern applied statistics with S*. Springer, New York
- Wäckers FL (2001) A comparison of nectar-and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *J Insect Physiol* 47:1077–1084. [https://doi.org/10.1016/S0022-1910\(01\)00088-9](https://doi.org/10.1016/S0022-1910(01)00088-9)
- Walter DE (1996) Living on leaves: mites, tomenta, and leaf domatia. *Annu Rev Entomol* 41:101–114. <https://doi.org/10.1146/annurev.en.41.010196.000533>
- Zhang NX, Messelink GJ, Alba JM et al (2018) Phytophagy of omnivorous predator *Macrolophus pygmaeus* affects performance of herbivores through induced plant defences. *Oecologia* 186:101–113. <https://doi.org/10.1007/s00442-017-4000-7>
- Zhang NX, Messelink GJ, Verdonkschot S, Janssen A (2019a) Plant feeding by an omnivorous predator affects plant phenology and omnivore performance. *Biol Control* 135:66–72. <https://doi.org/10.1016/j.biocontrol.2019.05.006>
- Zhang NX, Stephan JG, Björkman C, Puentes A (2021) Global change calls for novel plant protection: reviewing the potential of omnivorous plant-inhabiting arthropods as predators and plant defence inducers. *Curr Opin Insect Sci* 47:103–110. <https://doi.org/10.1016/j.cois.2021.06.001>
- Zhang NX, van Wieringen D, Messelink GJ, Janssen A (2019b) Herbivores avoid host plants previously exposed to their omnivorous predator *Macrolophus pygmaeus*. *J Pest Sci* 92:737–745. <https://doi.org/10.1007/s10340-018-1036-3>