

## Behavioral responses of a herbivorous mite and its potential predatory mirid to synthetic plant volatiles

N. Giagoudis<sup>1</sup>, E. Anastasaki<sup>2</sup>, P. Milonas<sup>2</sup>, G. Broufas<sup>1</sup> and M. Pappas<sup>1\*</sup>

**Summary** Herbivore-induced plant volatiles (HIPVs) mediate tritrophic interactions among plants, herbivores and their natural enemies. Using Y-tube olfactometer bioassays, we evaluated the behavioral responses of the zoophytophagous predator *Macrolophus pygmaeus* (Hemiptera: Miridae) and the herbivorous mite *Tetranychus urticae* (Acari: Tetranychidae) to ten synthetic volatile compounds associated with HIPV blends emitted by pepper (*Capsicum annuum*) plants under herbivore attack. Each compound (methyl salicylate [MeSA], farnesene, [mix of isomers] decanal, (+)-3-carene, (–)-β-pinene, (1S)-(–)-α-pinene, nonanal, hexanal, (Z)-3-hexenyl acetate, and 1H-indole) was tested at three concentrations (1, 10 and 100 ppm) against a hexane solvent control. At 100 ppm, *M. pygmaeus* was significantly attracted to MeSA and farnesene and significantly repelled by (+)-3-carene; neither attractant elicited a response at 10 ppm. *Tetranychus urticae* showed no attraction to any compound but was strongly repelled by (+)-3-carene at all three concentrations tested. Results demonstrate that HIPVs can differentially influence predator and herbivore behaviour in a dose-dependent manner. The identification of (+)-3-carene as a potent repellent for *T. urticae* and of MeSA and farnesene as attractants for *M. pygmaeus* provides a basis for volatile-based IPM strategies that integrate pest deterrence with natural enemy attraction within the crop, which could represent a practical tool supporting conservation biological control in pepper.

**Additional Keywords:** herbivore-induced volatiles, integrated pest management, predator recruitment, tritrophic interactions

### Introduction

Plants have developed a wide range of defense strategies against herbivores, generally categorised as direct or indirect. Direct defenses include physical barriers, such as trichomes and thickened cuticles, as well as chemical traits, including secondary metabolites such as alkaloids and phenolic compounds, and defense proteins such as protease inhibitors, all of which decrease plant palatability or impair herbivore fitness. Indirect defenses operate by producing and emitting chemical signals that recruit natural enemies of herbivores, primarily volatile organic compounds (VOCs) released into

the surrounding environment (Dicke and Sabelis, 1988; Vet and Dicke, 1992). These defenses can be constitutive (always present at baseline levels) or induced in response to damage (Karban and Baldwin, 1997; Heil and Baldwin, 2002).

Plants emit a complex mixture of VOCs, including terpenoids, green leaf volatiles (GLVs), benzenoids, and amino acid derivatives, whose composition is influenced by plant species, developmental stage, and environmental factors (Dudareva *et al.*, 2006; Holopainen and Gershenzon, 2010). During herbivore attack, plants produce a specific subset of these compounds, designated herbivore-induced plant volatiles (HIPVs), generated via active biosynthetic pathways triggered by elicitors in herbivore secretions and saliva (Hare, 2011).

HIPVs serve as informational signals within tritrophic interactions, transmitting cues regarding plant condition and the presence and nature of attacking herbivores to natural enemies such as parasit-

<sup>1</sup> Democritus University of Thrace, Department of Agricultural Development, Laboratory of Agricultural Entomology and Zoology, Orestiada, Greece

<sup>2</sup> Benaki Phytopathological Institute, Scientific Directorate of Entomology and Agricultural Zoology, Laboratory of Biological Control, Kifissia, Athens, Greece.

\* Corresponding author: mpappa@agro.duth.gr

oids, predatory mites, and insects (Dicke *et al.*, 1990; Turlings *et al.*, 1990). Through these tritrophic interactions, plants can indirectly reduce herbivore pressure by facilitating the location and recruitment of natural enemies (Turlings and Erb, 2018; Otto *et al.*, 2025). Prior exposure to HIPVs can prime plant defenses, enabling faster and stronger responses to subsequent attack (Riahi *et al.*, 2022). Nevertheless, the composition of HIPV blends exhibits substantial variability: both the plant species and the identity of the attacking herbivore influence the qualitative and quantitative composition of the emitted blend (Geervliet *et al.*, 1997; Van den Boom *et al.*, 2004), and infestation intensity can further modify the chemical profile (Ayelo *et al.*, 2021). This specificity means that responses observed in a particular plant-herbivore pairing cannot be broadly generalised, and that HIPVs may not always confer an indirect defensive advantage, at times attracting generalist herbivores or failing to elicit the expected responses in natural enemies (Peñaflor and Bento, 2013).

GLVs are key categories within HIPV blends, which are rapidly emitted upon cell membrane disruption via the lipoxygenase pathway and form part of the general wound response, as well as monoterpenes and sesquiterpenes whose biosynthesis is more specifically linked to herbivore recognition (Matsui, 2006). The production of GLVs is primarily regulated through the jasmonic acid (JA) and salicylic acid (SA) signaling pathways, JA predominantly activated by tissue-damaging herbivores such as chewing insects and cell-content feeders, while SA more strongly associated with responses to phloem-feeding insects and biotrophic pathogens, though neither pathway is exclusive to a single attacker type and substantial overlap occurs (Dicke and Baldwin, 2010; Thaler *et al.*, 2012). Crosstalk between these pathways enables plants to fine-tune their volatile emission profile in response to the identity of specific attackers, producing qualitatively distinct HIPV blends that reflect the nature of the biotic challenge (Zhang *et al.*, 2020).

Pepper (*Capsicum annuum*) is an economically important vegetable crop cultivated worldwide and is susceptible to a broad range of herbivorous pests, making it a relevant system for studying arthropod-induced plant defense responses (Florenco-Ortiz *et al.*, 2018). Among the key pests affecting pepper, the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a polyphagous herbivore of major economic significance. In tomato, *T. urticae* feeding has been shown to activate both the JA and SA pathways and to trigger the emission of volatile terpenoids and methyl salicylate, components of indirect defense (Kant *et al.*, 2004; Alba *et al.*, 2015). Analogous responses have been directly demonstrated in pepper: *T. urticae* infestation of *C. annuum* induced both JA and SA signaling, substantially altered the volatile emission profile, including a pronounced increase in methyl salicylate emission, and enhanced the attractiveness of infested plants to predatory arthropods (Zhang *et al.*, 2020).

The zoophytophagous predatory mirid *Macrolophus pygmaeus* (Rambour) (Hemiptera: Miridae) is a commercially deployed biological control agent widely used in greenhouse cropping systems, known to exploit volatile cues to locate herbivore-infested plants and orient toward suitable prey (Lins *et al.*, 2014; Pérez-Hedo *et al.*, 2018a). Beyond direct predation, the phytophagy of *M. pygmaeus* also triggers the release of plant volatiles that can recruit additional natural enemies, generating cascading tritrophic effects that amplify its biocontrol value (Zhang *et al.*, 2022). Moreover, the volatile blend induced by mirid phytophagy has been shown to influence the colonization behavior of subsequent herbivores on the same plant (Silva *et al.*, 2021a), suggesting that these predators contribute to crop protection through multiple, interconnected chemical mechanisms. However, whereas the responses of *M. pygmaeus* to complex plant volatile blends have been characterised in tomato-based systems (Pérez-Hedo *et al.*, 2018b), its behavioral responses to individually tested synthetic HIPV com-

pounds from pepper remain poorly understood. The volatile blend emitted by *Capsicum annuum* plants under arthropod attack has been characterised in response to multiple herbivore guilds, including zoophytophagous mirids (Bouagga *et al.*, 2018), the spider mite *T. urticae* (Zhang *et al.*, 2020; Zhang *et al.*, 2023), thrips (Esmaily *et al.*, 2025), aphids (Ali *et al.*, 2022), and lepidopteran larvae (Cardoza and Tumlinson, 2006, Yuan *et al.*, 2022), revealing a chemically diverse HIPV repertoire spanning green leaf volatiles, monoterpenes, sesquiterpenes, aliphatic aldehydes, and aromatic compounds. Whether individual constituents of this blend differ in their capacity to attract or repel *M. pygmaeus* and *T. urticae* remains to be established.

Interest in using HIPVs as tools for integrated pest management (IPM) has grown significantly. Based on push-pull principles, where stimuli are employed to make the protected resource less attractive to pests while guiding them toward alternative, appealing sources (Cook *et al.*, 2007), the strategic use of synthetic HIPVs to both repel herbivorous pests and attract natural enemies is seen as a promising volatile-based IPM strategy (Peñaflor and Bento, 2013). Methyl salicylate (MeSA) has been shown in field tests to attract beneficial arthropods, including predatory insects and parasitoids (James, 2003), whereas (E)- $\beta$ -farnesene, an aphid alarm pheromone, acts as a kairomone, drawing predators and parasitoids to aphid-infested plants (Francis *et al.*, 2004). However, behavioral responses to individual VOCs depend heavily on dose and context and can differ markedly between single compounds and complex blends (Kaplan, 2012, Holopainen and Blande, 2013). Therefore, systematic testing of these compounds under controlled conditions is crucial for their effective and informed use in volatile-based IPM approaches.

This study aimed to analyse how *M. pygmaeus* and *T. urticae* respond to ten plant-derived volatile compounds chosen from the pepper HIPV blend, tested with Y-tube olfactometer bioassays at three different

concentrations. We hypothesised that specific compounds in the pepper HIPV blend would affect the two species differently, acting as attractants for the predator *M. pygmaeus* and as repellents for the herbivore *T. urticae*, and that these effects would depend on concentration. This could inform targeted use of individual volatiles in biological control and volatile-based IPM strategies based on push-pull principles.

## Materials and Methods

### Insects

The *M. pygmaeus* colony was established from approximately 250 individuals obtained from the commercially available product MACROLOPHUS (Bio-insecta, Greece). The colony was maintained on young tomato plants (*Solanum lycopersicum* L., cv. Ace 55, 2-3 weeks old) in 200 mL plastic pots with a peat substrate (Klasmann-TS2), housed in insect-proof cages (47.5 × 47.5 × 47.5 cm, BugDorm, MegaView Science Co., Ltd.) under controlled conditions: 25 ± 2°C, 16:8 h photoperiod (L:D), and 65 ± 5% relative humidity (RH). A constant supply of *Ephestia kuehniella* (Lepidoptera: Pyralidae) eggs on posted strips was provided as a food supplement, with bee pollen added weekly.

In all experiments, young adult females, aged 7 to 10 days, were used. Before the bioassays, females were individually starved for 24 hours in 4 cm-diameter plastic containers with mesh lids, each containing a small piece of moistened cotton to maintain humidity. During starvation, the insects were maintained at 25 ± 2°C, with a 16:8-hour light: dark cycle and a relative humidity of 60 ± 5%.

### Spider mites

The *T. urticae* colony was established from approximately 700 mites collected from field-infested tomato plants and maintained in the laboratory for several years. A sub colony of the spider mite population was established and maintained on pep-

per plants (*C. annuum* L., cv. П13) for several generations before the bioassays. The colony was maintained on intact pepper plants potted in 12 cm diameter plastic pots with peat substrate (Klasmann-TS2), in insect-proof cages (47.5 × 47.5 × 47.5 cm, BugDorm, MegaView Science Co., Ltd.) under: 25 ± 2°C, 16:8 h L:D, 60–70% RH.

Adult inseminated females approximately 5 days old were used in the bioassay. A starvation period of 24 hours before bioassays was used, during which the adult females were individually placed in 1.5 mL Eppendorf tubes. The opening of the Eppendorf was covered with thin mesh to facilitate proper ventilation, whereas at the tip of the tube, a small hole was drilled to allow contact with a water-soaked cotton wool layer used to maintain proper humidity.

### Synthetic compounds

Ten compounds were selected to represent the major chemical classes documented in the volatile blend of *Capsicum annuum* plants under arthropod attack (aliphatic aldehydes, green leaf volatile esters, aromatic compounds, monoterpenes, and sesquiterpenes) on the basis of their occurrence in pepper HIPV profiles characterised in response to mirid phytophagy (Bouagga *et al.*, 2018), *T. urticae* infestation (Zhang *et al.*, 2020; Zhang *et al.*, 2023), *Spodoptera litura* (Lepidoptera: Noctuidae) herbivory (Yuan *et al.*, 2022), *Frankliniella occidentalis* (Thy-

sanoptera: Thripidae) infestation (Esmaily *et al.*, 2025), and *M. persicae* infestation (Ali *et al.*, 2022), with the exception of nonanal, for which a direct pepper-specific herbivore-induced reference was not identified in the available literature; its inclusion reflects its chemical class representativeness as a C9 aliphatic aldehyde produced via the lipoxigenase pathway in herbivore-damaged Solanaceous plants (Matsui, 2006, Hare, (2011). The compounds belong to five chemical groups: (1) aliphatic aldehydes: hexanal (≥98%; TCI, Japan), nonanal (≥97%; Sigma-Aldrich, Germany), and decanal (≥97%; TCI, Japan); (2) green leaf volatiles: (Z)-3-hexenyl acetate (≥98%; Sigma-Aldrich, Germany); (3) aromatic compounds: methyl salicylate (MeSA; ≥99%; Sigma-Aldrich, Germany) and 1H-indole (≥98%; Sigma-Aldrich, Germany); (4) monoterpenes: (+)-3-carene (≥90%; TCI, Japan), (–)-β-pinene (≥94%; TCI, Japan), and (1S)-(–)-α-pinene (≥97%; TCI, Japan); and (5) sesquiterpenes: farnesene, mixture of isomers (≥80%; Sigma-Aldrich, Germany). Details of all compounds are summarised in Table 1.

For each liquid compound, a primary stock solution was prepared by dissolving 10 µL of the neat compound (measured using a calibrated 100 µL glass pipette) in hexane to a final volume of 10 mL. Given its higher density and to achieve a tractable working range, farnesene was prepared using 100 µL of neat compound in 10 mL hex-

**Table 1.** Synthetic volatile compounds tested in Y-tube olfactometer bioassays with their chemical group, purity, and supplier.

| Compound                       | Chemical group            | Purity | Supplier               |
|--------------------------------|---------------------------|--------|------------------------|
| Hexanal                        | Aliphatic aldehyde        | ≥98%   | TCI, Japan             |
| Nonanal                        | Aliphatic aldehyde        | ≥97%   | Sigma-Aldrich, Germany |
| Decanal                        | Aliphatic aldehyde        | ≥97%   | TCI, Japan             |
| (Z)-3-Hexenyl acetate          | Green leaf volatile ester | ≥98%   | Sigma-Aldrich, Germany |
| Methyl salicylate (MeSA)       | Aromatic compound         | ≥99%   | Sigma-Aldrich, Germany |
| 1H-Indole                      | Aromatic compound         | ≥98%   | Sigma-Aldrich, Germany |
| (+)-3-Carene                   | Monoterpene               | ≥90%   | TCI, Japan             |
| (–)-β-Pinene                   | Monoterpene               | ≥94%   | TCI, Japan             |
| (1S)-(–)-α-Pinene              | Monoterpene               | ≥97%   | TCI, Japan             |
| Farnesene (mixture of isomers) | Sesquiterpene             | ≥80%   | Sigma-Aldrich, Germany |

ane. Resulting stock concentrations, calculated from compound density, ranged from approximately 800 to 1174 mg/L for most compounds and 8790 mg/L for farnesene. 1H-indole, being a solid at room temperature, was prepared by dissolving 1 mg in 10 mL of hexane to give a stock concentration of 100 mg/L. Working solutions at 1, 10, and 100 ppm (mg/L) were prepared by serial dilution of the respective stock solutions in hexane to a final volume of 10 mL. These concentrations were selected to span a biologically relevant range enabling characterization of dose-dependent behavioral responses, consistent with concentration ranges used in comparable olfactometer studies (Silva *et al.*, 2021b; Ayelo *et al.*, 2021), rather than to replicate specific field residue levels, which vary considerably with plant species, herbivore load, and environmental conditions (Holopainen and Blande, 2013). All solutions were stored in sealed glass vials at 4°C in the dark until use and freshly prepared on the day of each experiment. The quantity of active ingredient deposited per replicate corresponded to 1000, 100, and 10 ng for the 100, 10, and 1 ppm working solutions, respectively, calculated from 10 µL of the applied working solution. The hexane control consisted of 10 µL of pure hexane applied under identical conditions.

### Olfactometer assays

Behavioral responses of *M. pygmaeus* and *T. urticae* to the ten synthetic volatile compounds were assessed in a glass Y-tube olfactometer (4 cm internal diameter; main arm 25 cm long, lateral arms 17 cm long, 60° angle between lateral arms). Each lateral arm was connected via Teflon tubing to a 4 L glass container serving as an odor-source chamber: one containing the test compound and the other containing the hexane control. Compressed air was supplied by a low-pressure compressor, first passing through an activated carbon filter and a humidification flask to remove background contaminants, before entering the glass chambers and the olfactometer. Air flow was adjusted to 1.2 L/min per arm us-

ing a flowmeter (KYTOLA, Finland). For each odor source, 10 µL of the test compound or hexane control was applied by pipette onto a filter paper strip (1 × 2 cm) placed in a glass Petri dish on a sheet of aluminum foil. The loaded strip was left to stand for 2 min in the open air to allow solvent evaporation before being transferred to the bottom of the respective glass chamber. All Y-tube experiments were conducted between 10:00 and 17:00 h, at  $25 \pm 2$  °C and  $70 \pm 10\%$  RH.

A single female individual was introduced at the base of the main arm downwind using an aspirator (*M. pygmaeus*) or a fine brush (*T. urticae*) and observed for up to 10 min, following the general procedure described by Lins *et al.* (2014). Individuals were considered to have made a choice when they moved at least 3 cm into one of the lateral arms. Individuals who did not choose a lateral arm within 10 min were classified as unresponsive and excluded from analysis. Each individual was tested only once. After every 10 replicates, the lateral arm positions were switched to minimize positional bias. After every 20 replicates, the Y-tube and glass chambers were washed with unscented soap and acetone and left to dry completely, and the filter paper sources were replaced. For each treatment (compound and concentration), at least 40 individuals were tested.

Bioassays began at the highest concentration (100 ppm). Bioassays at lower concentrations (10 and 1 ppm) were conducted only when a statistically significant response had been observed at the preceding higher concentration. For *M. pygmaeus*, sequential concentration testing was applied exclusively to compounds eliciting statistically significant attraction at the preceding concentration; repellent responses were not evaluated at lower concentrations, as the sequential protocol was designed specifically to characterise the concentration threshold of predator attraction.

### Data analysis

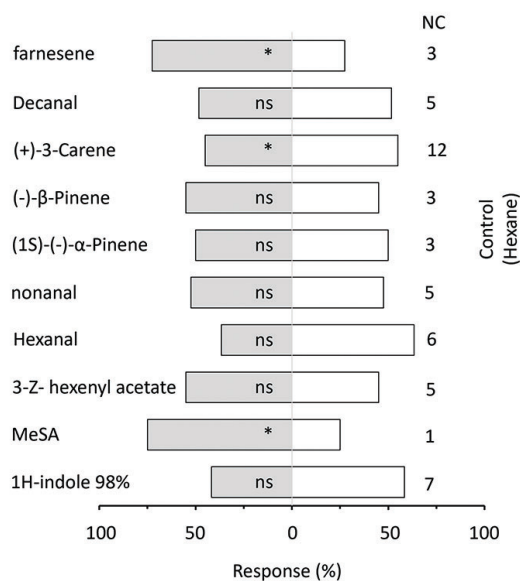
Data were analysed using the chi-square goodness-of-fit test ( $\chi^2$ ) to assess whether

the proportion of individuals choosing each arm differed significantly from a 50:50 random distribution ( $\alpha = 0.05$ ). Analyses were performed independently for each compound, concentration, and species. Statistics were performed using IBM Corp. (2020).

## Results

### Responses of *M. pygmaeus*

At 100 ppm, female *M. pygmaeus* showed a significant preference for two volatile compounds, farnesene and MeSA, over the hexane control, and a significant avoidance of (+)-3-carene (farnesene vs. hexane:  $\chi^2 = 4.26$ ,  $P = 0.039$ ; MeSA vs. hexane:  $\chi^2 = 5.33$ ,  $P = 0.021$ ; (+)-3-carene vs. hexane:  $\chi^2 = 17.36$ ,  $P < 0.001$ ) (Fig. 1). In accordance with the sequential testing protocol, which applied to attractant responses in *M. pygmaeus* only,



**Figure 1.** Behavioral responses of *Macrolophus pygmaeus* adult females in a Y-tube olfactometer to ten synthetic volatile compounds associated with the herbivore-induced plant volatile (HIPV) blend of *Capsicum annuum*, each tested at 100 ppm against a hexane solvent control. Grey bars represent the percentage of responding females choosing the test compound arm; white bars represent the percentage choosing the hexane control arm. An asterisk indicates significant preference ( $\chi^2$  test:  $P < 0.05$ ). NC= indicates the number of mirids that did not make a choice; ns= nonsignificant; MeSA= methyl salicylate.

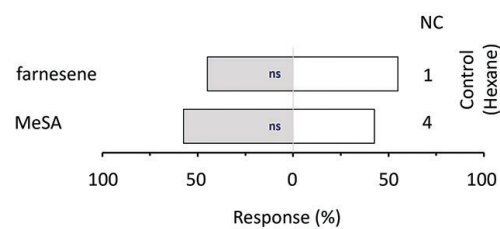
the repellent response to (+)-3-carene was not evaluated at lower concentrations. No significant positive or negative responses were observed for the remaining seven volatile compounds.

Consequently, only the two compounds that elicited a positive response at 100 ppm (MeSA and farnesene) were further evaluated at a lower concentration (10 ppm). However, at this concentration, female *M. pygmaeus* did not show a significant preference for either compound (MeSA vs. hexane:  $\chi^2 = 0.45$ ,  $P = 0.501$ ; farnesene vs. hexane:  $\chi^2 = 0.20$ ,  $P = 0.654$ ) (Fig. 2). Therefore, testing was not extended to 1 ppm for these compounds.

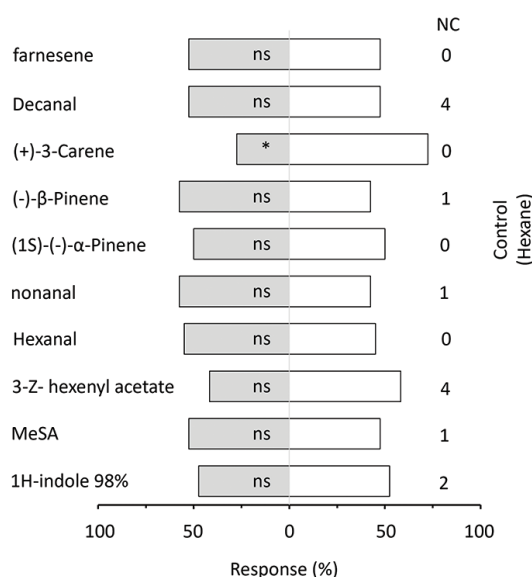
### Responses of *T. urticae*

At 100 ppm, female *T. urticae* showed no attraction to any of the ten compounds tested. A significant avoidance response was observed for (+)-3-carene relative to the hexane control ((+)-3-carene vs. hexane:  $\chi^2 = 28.06$ ,  $P < 0.001$ ) (Fig. 3). No other compound elicited a significant positive or negative response.

Given this result, (+)-3-carene was further evaluated at lower concentrations to characterise the dose-response relationship. The avoidance effect persisted at 10



**Figure 2.** Behavioral responses of *Macrolophus pygmaeus* adult females in a Y-tube olfactometer to two synthetic volatile compounds associated with the herbivore-induced plant volatile (HIPV) blend of *Capsicum annuum*, each tested at 10 ppm against a hexane solvent control. Grey bars represent the percentage of responding females choosing the test compound arm; white bars represent the percentage choosing the hexane control arm. An asterisk indicates significant preference ( $\chi^2$  test:  $P < 0.05$ ). NC= indicates the number of mirids that did not make a choice; ns= nonsignificant; MeSA= methyl salicylate.

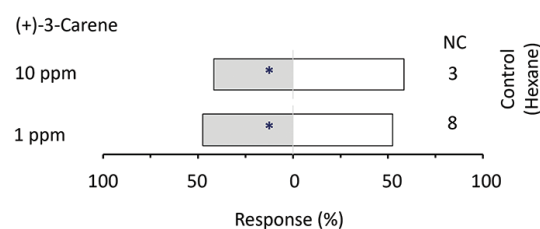


**Figure 3.** Behavioral responses of *Tetranychus urticae* adult females in a Y-tube olfactometer to ten synthetic volatile compounds associated with the herbivore-induced plant volatile (HIPV) blend of *Capsicum annuum*, each tested at 100 ppm against a hexane solvent control. Grey bars represent the percentage of responding females choosing the test compound arm; white bars represent the percentage choosing the hexane control arm. An asterisk indicates significant preference ( $\chi^2$  test:  $P < 0.05$ ). NC= indicates the number of spider mites that did not make a choice; ns= nonsignificant; MeSA= methyl salicylate.

ppm ( $\chi^2 = 28.63$ ,  $P < 0.001$ ) and remained significant at 1 ppm ( $\chi^2 = 16.15$ ,  $P < 0.001$ ) (Fig. 4), demonstrating consistent repellent activity across the entire concentration range tested.

## Discussion

The present study demonstrated that *M. pygmaeus* and *T. urticae* respond differently to individual volatile compounds. *Macropothus pygmaeus* was significantly attracted to MeSA and farnesene and repelled by (+)-3-carene at 100 ppm, whereas *T. urticae* showed no attraction to any compound tested but was consistently repelled by (+)-3-carene across all three concentrations evaluated. These results are consistent with the established role of HIPVs as mediators of tritrophic plant-herbivore-natural enemy



**Figure 4.** Behavioral responses of *Tetranychus urticae* adult females in a Y-tube olfactometer to (+)-3-Carene, tested at 10 and 1 ppm against a hexane solvent control. Grey bars represent the percentage of responding females choosing the tested compound arm; white bars represent the percentage choosing the hexane control arm. An asterisk indicates significant preference ( $\chi^2$  test:  $P < 0.05$ ). NC= indicates the number of spider mites that did not make a choice; ns= nonsignificant; MeSA= methyl salicylate.

interactions, first demonstrated for parasitic wasps by Turlings *et al.* (1990) and subsequently extended to a broad range of arthropod natural enemies (Turlings and Erb, 2018). They also add to existing knowledge by characterizing the behavioral responses of the zoophytophagous mirid predator and its mite prey to individually tested synthetic volatiles related to the pepper HIPV blend, underscoring the importance of evaluating individual compounds in isolation rather than inferring behavioral activity from blend composition alone.

The attraction of *M. pygmaeus* to MeSA is consistent with its well-established role as a semiochemical for natural enemy recruitment across a broad range of arthropod communities (James, 2003; James and Price, 2004; Mallinger *et al.*, 2011). MeSA emission is typically triggered by activation of the shikimate pathway following phytophagy by zoophytophagous predators, herbivore feeding, or pathogen attack (Pérez-Hedo *et al.*, 2018a; Silva *et al.*, 2021b). Characterization of HIPVs induced by *M. pygmaeus* and *Nesidiocoris tenuis* (Hemiptera: Miridae) phytophagy in tomato identified MeSA as one of the major differential volatile peaks alongside several GLVs, confirming its relevance as a chemical signal associated with mirid activity on Solanaceous crops (Pérez-Hedo *et al.*, 2018a). Silva *et al.* (2021b) further

demonstrated through Y-tube olfactometer trials with synthetic compounds that MeSA as well as (Z)-3-hexenyl propanoate, and (Z)-3-hexenyl acetate attracted *M. pygmaeus*, suggesting that both aromatic and fatty acid-derived volatile esters function as attractants for predatory mirids. The present results support MeSA's attractiveness to *M. pygmaeus* in the pepper system. The absence of a significant response to (Z)-3-hexenyl acetate in pepper may reflect plant species effect/differentiation and/or concentration-dependent threshold effects, given that the two studies used different dilution protocols, or intrinsic differences between the predator populations tested, including possible effects of colony origin and rearing conditions on olfactory sensitivity. Both studies used naïve, previously unexposed adult females, so differential prior experience cannot account for the discrepancy.

To the best of the authors' knowledge, no prior study has demonstrated that a mirid predator is attracted to farnesene as an individually tested synthetic compound. Maselou *et al.* (2019) reported that *M. pygmaeus* nymphs were significantly attracted to *Myzus persicae*-(Hemiptera: Aphididae) infested aubergine plants whose headspace contained (E)- $\beta$ -farnesene among other induced volatiles; however, the individual behavioral contribution of farnesene was not evaluated in isolation. This is noteworthy given previous findings showing that the monoterpenes ocimene and limonene, and the sesquiterpene caryophyllene, did not attract *M. pygmaeus*, *N. tenuis*, or *Dicyphus bolivari* (Hemiptera: Miridae) in olfactometer assays with tomato-derived HIPVs (Silva *et al.*, 2021b), suggesting that terpene attractiveness in mirid predators is highly compound-specific rather than a chemical class-level property. The commercial farnesene preparation used in the present study consists of a mixture of sesquiterpene isomers rather than a single geometric form (Wang *et al.*, 2024). Identification of the specific isomers responsible for the observed attractant activity will be necessary before practical conclusions can be drawn, and comparison with

the kairomonal activity of (E)- $\beta$ -farnesene, which functions as the aphid alarm pheromone and has been reported to attract the coccinellid *Adalia bipunctata* (Coleoptera: Coccinellidae) (Francis *et al.*, 2004), may help clarify the molecular basis of this response.

Attraction to both MeSA and farnesene was not sustained at 10 ppm, indicating that the behavioral responses of *M. pygmaeus* to these compounds are dose-dependent and may occur only above certain threshold concentrations. Dose-dependent responses to mirid volatiles have been reported by Ayelo *et al.* (2021), who found that individual monoterpenes attracted *N. tenuis* only at doses 100-fold above the natural emission rate of a single *T. absoluta*-infested tomato plant, equivalent to the simultaneous release from 100 plants, whereas lower doses were ineffective. This threshold effect aligns with the broader literature, which shows that arthropod behavioral responses to plant volatiles are strongly concentration-dependent, with both the magnitude and direction of responses varying with dose (Ayelo *et al.*, 2021). The absence of a response at 10 ppm does not preclude the ecological relevance of MeSA and farnesene, as natural HIPV concentrations in the field vary considerably with plant species and herbivore load (Geervliet *et al.*, 1997; Van den Boom *et al.*, 2004), as well as with environmental conditions, including wind speed, and temperature that govern volatile plume dispersion and persistence (Holopainen and Blande, 2013). Additionally, the inexperienced adult females used in this study may represent a conservative estimate of responsiveness. Lins *et al.* (2014) demonstrated that *N. tenuis* individuals were attracted to HIPVs only after prior experience with the odor source, and similar learning effects have been documented in anthocorid predators (Drukker *et al.*, 2000) and predatory mites (de Boer *et al.*, 2005).

The most consistent finding of this study was the strong, persistent avoidance of (+)-3-carene by both *M. pygmaeus* and *T. urticae*, with the repellent effect in *T. urticae* maintained across all three concentra-

tions evaluated (100, 10, and 1 ppm). This response is of particular ecological interest given that 3-carene has been reported to attract other mirid predators under different experimental conditions. Ayelo *et al.* (2021) found that 3-carene attracted *N. tenuis* only at a dose approximately 100-fold above the natural emission rate of a single *T. absoluta*-infested tomato plant, with lower doses ineffective. The contrasting responses, i.e. repellency in *M. pygmaeus* and *T. urticae* in the present study versus attraction in *N. tenuis* in Ayelo *et al.* (2021), may reflect species-specific differences in olfactory receptor sensitivity and behavioral ecology among mirid predators or differences in the plant-herbivore system. It should also be noted that both the present study and Ayelo *et al.* (2021) evaluated compounds individually, and responses to single compounds do not necessarily predict behavioral outcomes when the same compounds are presented as components of complex volatile mixtures (Mumm and Dicke, 2010). Nevertheless, Ayelo *et al.* (2021) found a repellence effect from (E)- $\beta$ -caryophyllene to *N. tenuis* at the highest dose tested, demonstrating that terpenoids can elicit avoidance responses in mirid predators and that the deterrent potential of this chemical class is compound- and species-specific.

The consistent avoidance of (+)-3-carene by *T. urticae* across a two-orders-of-magnitude range of concentrations suggests a robust, dose-persistent deterrent signal, potentially indicating a plant chemical cue associated with unsuitable or overexploited host tissue. Considering that (+)-3-carene was detected in the headspace volatile profile of *C. annuum* leaves infested by *T. urticae* (Zhang *et al.*, 2023), its repellent effect in the present study supports the hypothesis of an induced volatile direct defense function against the attacking herbivore in pepper, although this interpretation requires direct experimental validation under plant-based conditions. Additionally, the repellent effect of 3-carene on *T. urticae* has been demonstrated through contact-based leaf-disc assays with essential oil components (Da Ca-

mara *et al.*, 2015, Tak and Isman, 2017). These studies used enantiomerically unresolved material under contact conditions rather than in a volatile phase. Although not directly comparable, they still support the current result that this monoterpene acts as a repellent against *T. urticae*.

*Tetranychus urticae* showed no attraction to any of the ten compounds tested individually, including (1S)-(-)- $\alpha$ -pinene. This contrasts with Mérida-Torres *et al.* (2023), who reported that  $\alpha$ -pinene at 10 ng (equivalent to the amount deposited on the filter paper at the lowest concentration tested in the present study) attracted *T. urticae* in olfactometer bioassays using a colony collected from strawberry plants. Several methodological and biological differences may account for this discrepancy. First, Mérida-Torres *et al.* (2023) did not specify the enantiomeric form of  $\alpha$ -pinene used, suggesting a racemic or commercially unspecified preparation, whereas the present study employed the specific (1S)-(-) enantiomer, which differs substantially in biological activity. Second, the colony used in the present study was reared on pepper, whereas the Mérida-Torres *et al.* (2023) colony originated from strawberry. *Tetranychus urticae* populations adapted to different host plants are known to differ in their volatile-mediated responses (Maeda *et al.*, 2001). More broadly, the absence of attraction to any individually tested compound is consistent with the view that *T. urticae* host orientation is driven by the integrated perception of complex volatile blends rather than responses to single constituents (Mumm and Dicke, 2010).

Overall, the results demonstrate that individual HIPVs associated with pepper differ substantially in their capacity to influence the behaviour of *T. urticae* and *M. pygmaeus*, and that these effects are concentration-dependent and species-specific. The identification of MeSA and farnesene as attractants for *M. pygmaeus* at higher concentrations, combined with the consistent, dose-persistent repellency of (+)-3-carene toward *T. urticae*, provides a preliminary basis for ex-

ploring volatile-based strategies within integrated pest management. In the context of greenhouse pepper production, where *M. pygmaeus* is routinely introduced as a biological control agent, MeSA and farnesene could function as within-crop attractants to recruit and retain the released predator, analogous to the attract-and-reward concept in conservation biological control, in which synthetic HIPVs are used to draw natural enemies into crop environments and complementary resources sustain their populations (Simpson *et al.*, 2011; Kaplan, 2012). Simultaneously, (+)-3-carene may serve as a deterrent volatile that reduces *T. urticae* colonisation pressure, creating a chemical push component that complements the predator-attracting pull. This combined push-attract approach, drawing on both push-pull principles (Cook *et al.*, 2007; Peñaflor and Bento, 2013) and conservation biological control strategies, represents a conceptually coherent framework for the targeted deployment of individual pepper HIPVs in sustainable pest management.

Finally, it is important to consider a number of limitations of the current study. First, Y-tube olfactometer bioassays present stimuli under simplified, controlled conditions that do not capture the complexity of field exposure, including the heterogeneity of volatile plumes, the presence of competing background odors, and the influence of wind and temperature on volatile dispersion and persistence (Holopainen and Blande, 2013). Second, behavioral responses were assessed to individually tested compounds, whereas natural HIPV exposure in the field involves complex multicomponent blends; responses to single compounds do not necessarily predict outcomes in complex mixtures (Mumm and Dicke, 2010). Third, naive, previously unexposed females were used, and prior olfactory experience can substantially alter responsiveness in both mirid predators and predatory mites (de Boer *et al.*, 2005; Lins *et al.*, 2014). These considerations underscore the importance of validating the present findings under semi-field and field conditions. Specifical-

ly, future studies should evaluate whether MeSA and farnesene can recruit and retain *M. pygmaeus* within greenhouse pepper crops, and whether (+)-3-carene can reduce *T. urticae* colonisation under realistic agronomic conditions, accounting for volatile plume dynamics, competing stimuli, natural enemy dispersal, and the influence of repeated or prolonged exposure on behavioral responses.

*The project ECOBOOST was funded by the General Secretariat for Research and Technology of the Ministry of Development and Investments under the PRIMA Programme. PRIMA is an Art.185 initiative supported and co-funded under Horizon 2020, the European Union's Programme for Research and Innovation (PRIMA2021-09).*

## Literature cited

- Alba, J.M., Schimmel, B.C.J., Glas, J.J., Ataide, L.M.S., Pappas, M.L., Villarroel, C.A., Schuurink, R.C., Sabelis, M.W. and Kant, M.R. 2015. Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytologist*, 205(3): 828–840. <https://doi.org/10.1111/nph.13075>
- Ali, M.Y., Naseem, T., Zhang, J., Pan, M., Zhang, F. and Liu, T.X. 2022. Plant volatiles and herbivore induced plant volatiles from chili pepper act as attractant of the aphid parasitoid *Aphelinus varipes* (Hymenoptera: Aphelinidae). *Plants*, 11(10): 1350. <https://doi.org/10.3390/plants11101350>
- Ayelo, P.M., Yusuf, A.A., Pirk, C.W.W., Chailleux, A., Mohamed, S.A. and Deletre, E. 2021. Terpenes from herbivore-induced tomato plant volatiles attract *Nesidiocoris tenuis* (Hemiptera: Miridae), a predator of major tomato pests. *Pest Management Science*, 77(11): 5255–5267. <https://doi.org/10.1002/ps.6568>
- Bouagga, S., Urbaneja, A., Rambla, J.L., Flors, V., Granell, A., Jaques, J.A. and Pérez-Hedo, M. 2018. Zoophytophagous mirids provide pest control by inducing direct defences, antixenosis and attraction to parasitoids in sweet pepper plants. *Pest Management Science*, 74(6): 1286–1296. <https://doi.org/10.1002/ps.4838>
- Cardoza, Y.J. and Tumlinson, J.H. 2006. Compatible and incompatible *Xanthomonas* infections differentially affect herbivore-induced volatile emission by pepper plants. *Journal of Chemical Ecology*, 32(8): 1755–1768. <https://doi.org/10.1007/s10886-006-9107-y>

- Cook, S.M., Khan, Z.R. and Pickett, J.A. 2007. The use of push-pull strategies in integrated pest management. *Annual Review of Entomology*, 52: 375–400. <https://doi.org/10.1146/annurev.ento.52.110405.091407>
- Da Camara, C.A., Akhtar, Y., Isman, M.B., Seffrin, R.C. and Born, F.S. 2015. Repellent activity of essential oils from two species of Citrus against *Tetranychus urticae* in the laboratory and greenhouse. *Crop Protection*, 74: 110–115. <https://doi.org/10.1016/j.cropro.2015.04.014>
- de Boer, J.G., Posthumus, M.A. and Dicke, M. 2005. Identification of volatiles that are used in discrimination between plants infested with prey or nonprey herbivores by a predatory mite. *Journal of Chemical Ecology*, 31(11): 2473–2493. <https://doi.org/10.1007/s10886-005-7813-8>
- Dicke, M. and Sabelis, M.W. 1988. How plants obtain predatory mites as bodyguards. *Netherlands Journal of Zoology*, 38(2–4): 148–165.
- Dicke, M., van Beek, T.A., Posthumus, M.A., Ben Dom, N., van Bokhoven, H. and de Groot, A.E. 1990. Isolation and identification of volatile kairomones that affect acarine predator-prey interactions. *Journal of Chemical Ecology*, 16(2): 381–396. <https://doi.org/10.1007/BF01021771>
- Dicke, M. and Baldwin, I.T. 2010. The evolutionary context for herbivore-induced plant volatiles: Beyond the 'cry for help.' *Trends in Plant Science*, 15(3): 167–175. <https://doi.org/10.1016/j.tplants.2009.12.002>
- Drukker, B., Bruin, J. and Sabelis, M.W. 2000. Anthorcid predators learn to associate herbivore-induced plant volatiles with presence or absence of prey. *Physiological Entomology*, 25(3): 260–265. <https://doi.org/10.1046/j.1365-3032.2000.00190.x>
- Dudareva, N., Negre, F., Nagegowda, D.A. and Orlova, I. 2006. Plant volatiles: Recent advances and future perspectives. *Critical Reviews in Plant Sciences*, 25(5): 417–440. <https://doi.org/10.1080/07352680600899973>
- Esmaily, M., Ayoobi, A., Jin, G., Mandal, E., Shahmohammadi, N., Kim, C., Kim, K., Park, H., Khan, F., Lee, D., Kim, H.-J., Ham, E., Choi, H.W., Jeon, Y., Sung, J. and Kim, Y. 2025. Methyl hexenoate and linalool emitted by hot peppers repel western flower thrips (*Frankliniella occidentalis*) and attract their predator (*Orius laevigatus*). *Scientific Reports*, 15: 42959. <https://doi.org/10.1038/s41598-025-26633-6>
- Florencio-Ortiz, V., Novák, O. and Casas, J.L. 2018. Local and systemic hormonal responses in pepper (*Capsicum annuum* L.) leaves under green peach aphid (*Myzus persicae* Sulzer) infestation. *Journal of Plant Physiology*, 231: 356–363. <https://doi.org/10.1016/j.jplph.2018.10.015>
- Francis, F., Lognay, G. and Haubruge, E. 2004. Olfactory responses to aphid and host plant volatile releases: (E)-beta-farnesene an effective kairomone for the predator *Adalia bipunctata*. *Journal of Chemical Ecology*, 30(4): 741–755. <https://doi.org/10.1023/B:JOEC.0000028429.13413.a2>
- Geervliet, J.B.F., Posthumus, M.A., Vet, L.E.M. and Dicke, M. 1997. Comparative analysis of headspace volatiles from different caterpillar-infested or uninfested food plants of *Pieris* species. *Journal of Chemical Ecology*, 23(11): 2935–2954. <https://doi.org/10.1023/A:1022575619612>
- Hare, J.D. 2011. Ecological role of volatiles produced by plants in response to damage by herbivorous insects. *Annual Review of Entomology*, 56: 161–180. <https://doi.org/10.1146/annurev-ento-120709-144753>
- Heil, M. and Baldwin, I.T. 2002. Fitness costs of induced resistance: Emerging experimental support for a slippery concept. *Trends in Plant Science*, 7(2): 61–67. [https://doi.org/10.1016/S1360-1385\(01\)02186-0](https://doi.org/10.1016/S1360-1385(01)02186-0)
- Holopainen, J.K. and Blande, J.D. 2013. Where do herbivore-induced plant volatiles go? *Frontiers in Plant Science*, 4: 185. <https://doi.org/10.3389/fpls.2013.00185>
- Holopainen, J.K. and Gershenzon, J. 2010. Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science*, 15(3): 176–184. <https://doi.org/10.1016/j.tplants.2010.01.006>
- IBM Corp. 2020. IBM SPSS Statistics for Windows (Version 27.0) [Computer software]. IBM Corp.
- James, D.G. 2003. Synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. *Environmental Entomology*, 32(5): 977–982. <https://doi.org/10.1603/0046-225X-32.5.977>
- James, D.G. and Price, T.S. 2004. Field-testing of methyl salicylate for recruitment and retention of beneficial insects in grapes and hops. *Journal of Chemical Ecology*, 30(8): 1613–1628. <https://doi.org/10.1023/B:JOEC.0000045587.98461.79>
- Kant, M.R., Ament, K., Sabelis, M.W., Haring, M.A. and Schuurink, R.C. 2004. Differential timing of spider mite-induced direct and indirect defenses in tomato plants. *Plant Physiology*, 135(1): 483–495. <https://doi.org/10.1104/pp.103.038315>
- Kaplan, I. 2012. Attracting carnivorous arthropods with plant volatiles: The future of biocontrol or playing with fire? *Biological Control*, 60(2): 77–89. <https://doi.org/10.1016/j.biocontrol.2011.10.017>
- Karban, R. and Baldwin, I.T. 1997. Induced responses to herbivory. University of Chicago Press.
- Lins, J.C., Van Loon, J.J.A., Bueno, V.H.P., Lucas-Barbosa, D., Dicke, M. and Van Lenteren, J.C. 2014. 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(6): 707–718. <https://doi.org/10.1007/s10526-014-9605-x>

- Maeda, T., Takabayashi, J., Yano, S. and Takafuji, A. 2001. Variation in the olfactory response of 13 populations of the predatory mite *Amblyseius womersleyi* (Acari: Phytoseiidae, Tetranychidae) to *Tetranychus urticae*-infested plant volatiles (Acari: Phytoseiidae, Tetranychidae). *Experimental and Applied Acarology*, 25: 55–64. <https://doi.org/10.1023/A:1010667812788>
- Mallinger, R.E., Hogg, D.B. and Gratton, C. 2011. Methyl salicylate attracts natural enemies and reduces populations of soybean aphids (Hemiptera: Aphididae) in soybean agroecosystems. *Journal of Economic Entomology*, 104(1): 115–124. <https://doi.org/10.1603/EC10181>
- Maselou, D.A., Anastasaki, E. and Milonas, P.G. (2019). The role of host plants, alternative food resources and herbivore induced volatiles in choice behavior of an omnivorous predator. *Frontiers in Ecology and Evolution*, 6: 241. <https://doi.org/10.3389/fevo.2018.00241>
- Matsui, K. 2006. Green leaf volatiles: Hydroperoxide lyase pathway of oxylipin metabolism. *Current Opinion in Plant Biology*, 9(3): 274–280. <https://doi.org/10.1016/j.pbi.2006.03.002>
- Mérida-Torres, N.M., Cruz-López, L., Malo, E.A. and Cruz-Esteban, S. 2023. Attraction of the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), to healthy and damaged strawberry plants mediated by volatile cues. *Experimental and Applied Acarology*, 91(3): 413–427 DOI:10.1007/s10493-023-00852-w
- Mumm, R. and Dicke, M. 2010. Variation in natural plant products and the attraction of bodyguards involved in indirect plant defense. *Canadian Journal of Zoology*, 88(7): 628–667. <https://doi.org/10.1139/Z10-032>
- Otto, P., Ninkovic, V., Meiners, T., Pashalidou, F.G., Fortuna, T.M., Louis, J., Milonas, P. and Cusumano, A. 2025. Multifunctionality of plant VOCs in agroecological systems: perspectives for biological pest control. *Entomologia Generalis*, 45: 931–949. <https://doi.org/10.1127/entomologia/3506>
- Peñaflor, M.F.G.V. and Bento, J.M.S. 2013. Herbivore-induced plant volatiles to enhance biological control in agriculture. *Neotropical Entomology*, 42(4): 331–343. <https://doi.org/10.1007/s13744-013-0147-z>
- Pérez-Hedo, M., Arias-Sanguino, Á. M. and Urbaneja, A. 2018b. Induced tomato plant resistance against *Tetranychus urticae* triggered by the phytophagy of *Nesidiocoris tenuis*. *Frontiers in Plant Science*, 9: 1419. <https://doi.org/10.3389/fpls.2018.01419>
- Pérez-Hedo, M., Rambla, J.L., Granell, A. and Urbaneja, A. 2018a. Biological activity and specificity of Miridae-induced plant volatiles. *BioControl*, 63(2): 203–213. <https://doi.org/10.1007/s10526-017-9854-4>
- Riahi, C., González-Rodríguez, J., Alonso-Valiente, M., Urbaneja, A. and Pérez-Hedo, M. 2022. Eliciting plant defenses through herbivore-induced plant volatiles' exposure in sweet peppers. *Frontiers in Ecology and Evolution*, 9: 776827. <https://doi.org/10.3389/fevo.2021.776827>
- Silva, D.B., Weldegergis, B.T., Van Loon, J.J.A. and Bueno, V.H.P. 2017. Qualitative and quantitative differences in herbivore-induced plant volatile blends from tomato plants infested by either *Tuta absoluta* or *Bemisia tabaci*. *Journal of Chemical Ecology*, 43(1): 53–65. <https://doi.org/10.1007/s10886-016-0807-7>
- Silva, D.B., Jiménez, A., Urbaneja, A., Pérez-Hedo, M. and Bento, J.M. 2021a. Changes in plant responses induced by an arthropod influence the colonization behavior of a subsequent herbivore. *Pest Management Science*, 77(9): 4168–4180. <https://doi.org/10.1002/ps.6454>
- Silva, D.B., Urbaneja, A. and Pérez-Hedo, M. 2021b. Response of mirid predators to synthetic herbivore-induced plant volatiles. *Entomologia Experimentalis et Applicata*, 169(1): 125–132. <https://doi.org/10.1111/eea.12970>
- Simpson, M., Gurr, G.M., Simmons, A.T., Wratten, S.D., James, D.G., Leeson, G. and Nicol, H.I. 2011. Attract and reward: combining chemical ecology and habitat manipulation to enhance biological control in field crops. *Journal of Applied Ecology*, 48(3): 580–590. <https://doi.org/10.1111/j.1365-2664.2010.01946.x>
- Steenbergen, M., Abd-el-Halim, A., Bleeker, P., Dicke, M., Escobar-Bravo, R., Cheng, G., Haring, M.A., Kant, M.R., Kappers, I., Klinkhamer, P.G.L., Leiss, K.A., Legarra, S., Macel, M., Mouden, S., Pieterse, C.M.J., Sarde, S.J., Schuurink, R.C., De Vos, M., Van Wees, S.C.M. and Broekgaarden, C. 2018. Exploiting thrips-induced defences to combat pests on crops. *Journal of Experimental Botany*, 69(8): 1837–1848. doi: 10.1093/jxb/ery060.
- Tak, J.-H. and Isman, M. B. 2017. Acaricidal and repellent activity of plant essential oil-derived terpenes and the effect of binary mixtures against *Tetranychus urticae* Koch (Acari: Tetranychidae). *Industrial Crops and Products*, 108: 786–792. <https://doi.org/10.1016/j.indcrop.2017.07.040>
- Thaler, J.S., Humphrey, P.T. and Whiteman, N.K. 2012. Evolution of jasmonate and salicylate signal crosstalk. *Trends in Plant Science*, 17(5): 260–270. <https://doi.org/10.1016/j.tplants.2012.02.010>
- Turlings, T.C.J. and Erb, M. 2018. Tritrophic interactions mediated by herbivore-induced plant volatiles: Mechanisms, ecological relevance, and application potential. *Annual Review of Entomology*, 63: 433–452. <https://doi.org/10.1146/annurev-ento-020117-043507>
- Turlings, T.C.J., Tumlinson, J.H. and Lewis, W.J. 1990. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science*, 250(4985): 1251–1253. <https://doi.org/10.1126/science.250.4985.1251>
- Van Den Boom, C.E., van Beek, T.A., Posthumus, M.A., de Groot, A. and Dicke, M. 2004. Qualita-

- tive and quantitative variation among volatile profiles induced by *Tetranychus urticae* feeding on plants from various families. *Journal of Chemical Ecology*, 30: 69–89. <https://doi.org/10.1023/B:JOEC.0000013183.72915.99>
- Vet, L.E.M. and Dicke, M. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, 37: 141–172. <https://doi.org/10.1146/annurev.en.37.010192.001041>
- Wang, S., Zhou, J., Zhan, C., Qiao, J., Caiyin, Q. and Huang, M. 2024. Fine-tuning the function of farnesene synthases for selective synthesis of farnesene stereoisomers. *Journal of Agricultural and Food Chemistry*, 72(49): 27355–27364. <https://doi.org/10.1021/acs.jafc.4c07338>
- Yuan, X., Liu, W., Zhang, X., He, Y. and Yin, K. 2022. Repellence or attraction: secondary metabolites in pepper mediate attraction and defense against *Spodoptera litura*. *Pest Management Science*, 79(1): 303–313. <https://doi.org/10.1002/ps.7107>
- Zhang, Y., Bouwmeester, H.J. and Kappers, I. F. 2020. Combined transcriptome and metabolome analysis identifies defence responses in spider mite-infested pepper (*Capsicum annuum*). *Journal of Experimental Botany*, 71(1): 330–343, <https://doi.org/10.1093/jxb/erz422>
- Zhang, N.X., van Loon, J.J.A., de Bruijn, P.J.A. and Dicke, M. 2022. The omnivorous predator *Macrolophus pygmaeus* induces production of plant volatiles that attract a specialist predator. *Journal of Chemical Ecology*, 48(2): 166–177. <https://doi.org/10.1007/s10340-021-01463-3>
- Zhang, Y., Kashkooli, A.B., Blom, S., Zhao, T., Bouwmeester, H.J. and Kappers, I.F. 2023. The Capsicum terpenoid biosynthetic module is affected by spider-mite herbivory. *Plant Molecular Biology*, 113: 303–321. <https://doi.org/10.1007/s11103-023-01390-0>

Received: 19 April 2026; Accepted: 7 June 2026

## Συμπεριφορικές αποκρίσεις ενός φυτοφάγου ακάρεως και του δυνητικού θηρευτή του Miridae σε συνθετικές πτητικές φυτικές ενώσεις

N. Γιαγκούδης, E. Αναστασάκη, Π. Μυλωνάς, Γ. Μπρούφας και Μ. Παππά

**Περίληψη** Οι πτητικές ενώσεις που επάγονται από τα φυτά ως απόκριση στην τροφική δραστηριότητα φυτοφάγων εχθρών (HIPVs) επηρεάζουν τις τριτροφικές αλληλεπιδράσεις μεταξύ φυτών, φυτοφάγων και των φυσικών εχθρών τους. Στην παρούσα εργασία αξιολογήσαμε σε βιοδοκιμές με ολφακτομέτρο τύπου Υ, τις συμπεριφορικές αποκρίσεις του ζωοφυτοφάγου αρπακτικού *Macrolophus pygmaeus* (Hemiptera: Miridae) και του φυτοφάγου ακάρεως *Tetranychus urticae* (Acari: Tetranychidae) σε δέκα συνθετικές πτητικές ενώσεις που σχετίζονται με μίγματα HIPVs που εκλύονται από φυτά πιπεριάς (*Capsicum annuum*) μετά την προσβολή τους από φυτοφάγους εχθρούς. Κάθε ένωση (Μεθυλικός εστέρας του σαλικυλικού οξέος [MeSA], φαρνεσένιο [μίγμα ισομερών], δεκανάλη, (+)-3-καρένιο, (–)-β-πινένιο, (1S)-(–)-α-πινένιο, νονανάλη, εξανάλη, οξικό (Z)-3-εξενυλ εστέρας του οξικού οξέος και 1H-ινδόλιο) αξιολογήθηκε σε τρεις συγκεντρώσεις (1, 10 και 100 ppm) έναντι του μάρτυρα (εξάνιο, διαλύτης των πτητικών ενώσεων). Στα 100 ppm, το *M. pygmaeus* έδειξε σημαντική προσέλκυση προς το MeSA και το φαρνεσένιο και σημαντική αποφυγή του (+)-3-καρενίου. Κανένα από τα δύο δεν προκάλεσε απόκριση στα 10 ppm. Ο κοινός τετράνυχος *T. urticae* δεν έδειξε προσέλκυση προς καμία ένωση, αλλά έντονη αποφυγή του (+)-3-καρενίου και στις τρεις συγκεντρώσεις που εξετάστηκαν. Τα αποτελέσματα δείχνουν ότι οι HIPVs μπορούν να επηρεάσουν διαφορετικά τη συμπεριφορά των αρπακτικών και των φυτοφάγων εχθρών με τρόπο που εξαρτάται από τη δόση εφαρμογής. Ο εντοπισμός του (+)-3-καρενίου ως ισχυρού απωθητικού για τον τετράνυχον *T. urticae*, και του MeSA και του φαρνεσενίου ως ελκυστικών για το *M. pygmaeus* διαμορφώνει πλαίσιο για στρατηγικές Ολοκληρωμένης Φυτοπροστασίας μέσω της εφαρμογής πτητικών ενώσεων, συνδυάζοντας την απώθηση φυτοφάγων με την προσέλκυση του φυσικού εχθρού τους εντός της καλλιέργειας, κάτι που θα μπορούσε να αποτελέσει ένα πρακτικό εργαλείο που υποστηρίζει τη βιολογική αντιμετώπιση μέσω της διατήρησης φυσικών εχθρών στην πιπεριά.