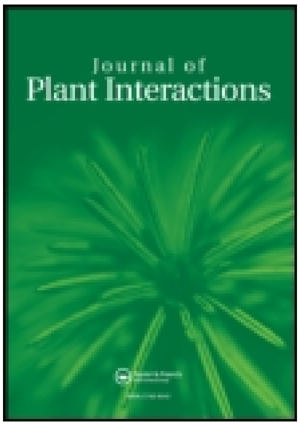

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SHORT COMMUNICATION

Interactions between tomato volatile organic compounds and aphid behaviour

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In the tritrophic system consisting of tomato, *Solanum lycopersicum* (L.), the aphid *Macrosiphum euphorbiae* (Thomas) and its natural enemy, the parasitoid *Aphidius ervi* (Haliday), it has been shown that the release of volatile organic compounds following aphid attack is responsible for attracting aphid parasitoids in wind tunnel experiments. The main compounds involved in these multitrophic interactions have been characterized and quantified. In this work, the possible activity of such compounds on plant direct defences against the aphid *M. euphorbiae* was assessed in laboratory tests. The selected compounds were applied to uninfested tomato plants, either by evaporation or contact, and performance of aphids, in terms of plant acceptance, fixing behaviour and aphid development, calculated in standard conditions. The results showed that two compounds, namely methyl salicylate and *cis*-hex-3-en-1-ol, alter aphid performance. These two compounds have been reported to be those eliciting the best response by *A. ervi* in terms of flight behavior (wind tunnel bioassay) and antennal stimulation (EAG bioassay).

Keywords: *Aphidius ervi*; *Macrosiphum euphorbiae*; direct defences; indirect defences; multitrophic interactions

Introduction

In most cases, the release of attractive compounds, quantitatively and/or qualitatively, is elicited by the feeding activity of the phytophagous insect, (induced indirect defence). However, it has been rarely assessed whether these same VOCs can directly affect the behaviour or the development of the insect pest. In the system consisting of tomato, *Solanum lycopersicum* (L.), the aphid *Macrosiphum euphorbiae* (Thomas) and its natural enemy, the endophagous parasitoid *Aphidius ervi* (Haliday), the profile of the VOCs released by tomato plants following aphid infestation has been quantified and characterized (Sasso et al. 2007). Such VOCs were subsequently tested for olfactory recognition (EAG) and parasitoid attractiveness (wind tunnel bioassay), and a final 'chart' was compiled on the basis of the combined results (Sasso et al. 2009). All these observations were restricted to the highest level of the trophic chain, i.e. the parasitoid *A. ervi*, leaving unexplored the possible direct effects of the same VOC on the performance of the aphid *M. euphorbiae*. This paper tries to answer the question: do the VOCs involved in parasitoid response (indirect defences) have any direct effect on aphid performance (direct defences)? Should the answer be positive, the possibility of applying such compounds in a push–pull strategy (Cook et al. 2007) would be feasible in tomato crops.

Materials and methods

Plants and insects

Plants for the bioassays were tomatoes cv San Marzano, grown in sterilized soil in a greenhouse at the following conditions: 24 ± 2°C and 70 ± 10% UR, and a 16:8 light/dark photoperiod. Plants were watered every other day with approximately 250 ml of tap water.

The tomato and potato aphid *Macrosiphum euphorbiae* is permanently reared in an environmental cabinet at the Department of Entomology on tomato plants, cv San Marzano at the same conditions as described above. The original strain was field-collected in 2003 in Scafati (Campania, Italy) on tomato cv S. Marzano.

After completing the assays, the plants were kept in a greenhouse at the conditions described above for one week to check their status.

Compounds

The compounds selected for the tests were chosen on the basis of the results reported in Sasso et al. (2009). They were: *cis*-hex-3-en-1-ol (purity >98%, CAS no. 928-96-1), β-caryophyllene (purity ≥80%, CAS no. 87-44-5), (−)-α-pinene (purity = 97.0%, CAS no. 77785-26-4), methyl salicylate (purity = 98%, CAS no. 119-36-8) and *cis*-jasmone (purity >85%, CAS no. 488-10-8), all purchased from Sigma-Aldrich.

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Induction assay

Tomato plants cv. San Marzano at the age of three weeks were transferred singly in a perspex cage (4.7 dm^3), together with 10 μl of a volatile pure compound spotted on filter paper (Glinwood et al. 2007). Assuming the complete evaporation of VOC in the box, we obtained the following concentrations: *cis*-hex-3-en-1-ol 18.01 μM, β-caryophyllene 9.38 μM, (−)-α-pinene 13.38 μM, methyl salicylate 16.42 μM, *cis*-jasmone 12.18 μM. The box was sealed, and the plant was exposed to volatiles for 24 hrs. Then, in the open air, the cages were left open and plants were exposed to clean air for 20 min. A 2 ml Eppendorf tube, containing ten aphids (apterous fourth instars or newly moulted adults), weighed as a pool, was placed with its tip in the soil, and the opening next to the tomato stem, so that aphids were free to climb on the plant and choose their feeding site. Each treatment was repeated six times. The box was sealed again, and aphid acceptance of plants receiving different treatments was assessed at 3, 24 and 48 hrs. Aphids, dead or alive, were sorted as: (1) Remained in Eppendorf, (2) Wandering on cage walls, pot or tube, (3) Fixed on plant. Only at the observation programmed at 48 hrs (endpoint) were the cages opened, and the remaining living adult aphids were collected and weighed as a pool. The pooled weights measured before aphid release (T0) and at the end of the assay (T48) were compared by paired *t*-test. Furthermore, the nymphs produced during the assay were counted. Control cages were equally set up, containing clean filter paper.

Contact assay

Tomato plants cv. San Marzano at the age of three weeks were sprayed with 10 μl of compound diluted in 2 ml of water with 1% non-ionic wetting agent (Bagnante Adesivo S, SIVAM®). This resulted in the following concentrations of VOC: *cis*-hex-3-en-1-ol 38.48 mM, β-caryophyllene 20.04 mM, (−)-α-pinene 28.59 mM, methyl salicylate 35.07 mM, *cis*-jasmone 26.02 mM. After partial drying (15 min in the open air), the plants were collocated singly into the Perspex cages and exposed to ten aphids in an Eppendorf tube, as described above. Control plants were treated with a solution of water and wetting agent. Each treatment was repeated four times. The time course of the assessment of plant acceptance was the same as described above. Weight modifications and fertility were measured as described above.

Results

Induction assay

At 3 hrs from the introduction of aphids in the cage, no difference was observed in the fixing behaviour of aphids exposed to the different VOCs and to control treatments (Table 1). At 24 hrs from the onset of the assay, only plants treated with methyl salicylate induced an alteration of aphid acceptance behaviour, resulting in a lower number of aphids on the plant and with their stylets inserted ($G = 17.98$; $n = 60$; DF = 5). This result was confirmed at 48 hrs ($G = 26.612$; $n = 60$; DF = 5), when also *cis*-exenol showed similar activity ($G = 9.48$; $n = 60$; DF = 4) The resulting values of G were compared with the critical values of χ^2 (9.47 and 11.07 for DF = 4 and DF = 5)

Table 1. Fixing behaviour on plants following induction bioassay.

	3 hrs	24 hrs	48 hrs
Methyl salicylate	60	47*	25*
β-Caryophyllene	62	73	53
cis-Jasmone	68	75	58
cis-Hex-3-en-1-ol	72	65	37*
(−)-α-Pinene	75	73	60
Control	72	78	60

Note: The number of aphids fixed on plants at different time intervals after aphid release is expressed in the table as a percentage. Each replicate was made up of 10 individuals. *G*-test for the goodness of fit. Asterisks denote significantly different groups within the same column. $P < 0.05$, $n = 60$.

Of all the compounds, only treatment with methyl salicylate resulted in reduced fertility (see Table 2). Adult aphid biomass at T48 was generally and slightly lower with respect to the pool of ten original aphids at T0, because of mortality during the assay, but only the aphids feeding on methyl salicylate treated plants weighed significantly less ($t = -5.071$, DF = 10, $P \leq 0.001075$) with respect to the weights of the ten original aphids. Seven days after the completion of the assay, plants did not present any visible problem in terms of growth or pathogen infection.

Contact assay

At 3 and 24 hrs (Table 3) from the introduction of aphids in the cage, no difference was observed in the fixing behaviour of aphids exposed to the different VOCs and to control. At 48 hrs, plants treated with *cis*-hex-3-en-1-ol ($G = 11.739$; $n = 40$; DF = 5) hosted a lower number of aphids apparently feeding (stylet

Table 2. Reproductive behaviour on plants following induction and contact bioassays (number of nymphs produced in 48 hrs).

	Induction	Contact
Methyl salicylate	25*	23
β-Caryophyllene	47	31
cis-Jasmone	53	28
cis-Hex-3-en-1-ol	56	34
(−)-α-Pinene	52	40
Control	72	39

Note: Kolmogorov-Smirnoff test for the goodness of fit. Asterisks denotes significantly different groups within the same column. $P < 0.05$. Each replicate was made of 10 individuals, six replicates were treated for induction and four for contact assay.

Table 3. Fixing behaviour on plants following contact bioassay.

	3 hrs	24 hrs	48 hrs
Methyl salicylate	93	70	60
β-Caryophyllene	85	77	77
cis-Jasmone	97	75	72
cis-Hex-3-en-1-ol	87	77	48*
(−)-α-Pinene	90	72	72
Control	87	77	75

Note: The number of aphids fixed on plants at different time intervals after aphid release is expressed as in the table as a percentage. Each replicate was made of 10 individuals. *G*-test for the goodness of fit. Asterisks denote significantly different groups within the same column. $P < 0.05$, $n = 40$.

inserted). Fertility and biomass were not affected by VOC treatments.

The resulting values of *G* were compared with the critical values of χ^2 (11.07 for DF = 5). Seven days after completion of the assay, plants did not present any visible problem in terms of growth or pathogen infection.

Discussion

Among the volatile compounds released at the highest level by tomato plants following *M. euphorbiae* infestation, only two seem to alter the behaviour and the performances of the aphid: methyl salicylate and *cis*-hexen-1-ol.

Methyl salicylate has been reported as an aphid-related compound in a number of different crop plants (Zhu & Park 2005; Sasso et al. 2007) and, not surprisingly, it is recognized by the aphid parasitoid *A. ervi* even at a concentration as low as 0.01 mg/ml (Sasso et al. 2009). When applied as vapor, this compound showed a significant negative effect on aphid fixing behaviour and fertility in accordance with previous studies in the olfactometer (Hardie et al. 1994; Glinwood et al. 2007). Unexpectedly, on the tomato, this compound seemed to have no direct effect on aphid behavior and performance when sprayed on the plant, even though a lower number (but not significant) of fixed aphids were observed at the end of the assay. With regard to reproduction, the outcome of methyl salicylate fumigation has a negative impact on aphid fertility, in accordance with the application of BTH, a synthetic salicylate mimic and an inducer of the salicylate pathway in tomato (Cooper et al. 2004; Thaler et al. 2010).

Conversely, *cis*-hexen-1-ol seemed to affect plant acceptance of *M. euphorbiae* both through induction and contact. However, the contact effect of this compound appeared only after 48 hrs from treatment, when it is not possible to exclude an 'induction' effect at all. In a different system (*Vicia faba* and the black bean aphid, *Aphis fabae*), *cis*-hexen-1-ol proved to be attractive towards the aphids (Webster et al. 2010) and this is surprising, considering that *A. fabae* and *M. euphorbiae* share many host plants, even though they show a respective preference for Leguminosae and Solanaceae (Blackman & Eastop 2000). The effect of *cis*-hexen-1-ol on aphid reproduction seems to be variable. In our system there was no effect (Table 2) whilst on tobacco and potato this compound negatively affected the fertility of two *Myzus* species (Hildebrand et al. 1993; Vancanneyt et al. 2001). The mobility, in terms of plant uptake, and the specificity of the induction of defence genes, have been reported for *cis*-hexen-1-ol both in model (lima bean) and crop (maize) plants (Arimura et al. 2001; Farag et al. 2005) and this makes it an ideal candidate for alternative methods of pest control.

The behavior of *M. euphorbiae* was not affected by *cis*-jasmone application. This compound has been reported to be repellent for the aphids *Sitobion avenae* (Bruce et al. 2003) and *Aphis gossypii* (Moraes et al. 2009) but highly attractive towards the aphid parasitoid *A. ervi* (Birkett et al. 2000; Sasso et al. 2009). These contrasting results can only be explained by a specific plant-mediation.

The negative effects of methyl salicylate and *cis*-hexen-1-ol on aphid behaviour and reproduction coupled to a strong attractiveness towards aphid parasitoids make these compounds ideal candidates for alternative and sustainable control of aphid pests.

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