

## The aphids' peripheral perception of plant volatiles

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### Introduction

In aphids the olfactory receptor neurones are housed in placoid sensilla on the antennae, the so-called distal and proximal primary rhinaria on segments six and five, respectively, and, in winged aphids, the secondary rhinaria on segments three and four (Shambaugh *et al.*, 1978; Bromley *et al.*, 1979). The overall responses of these receptors to plant odours can be studied by the electroantennogram (EAG) technique. In this way, it can be shown which plant volatiles are detected and, thus, are likely to play a rôle in the discrimination between the odour blends of host and non-host plant species during orientation and landing. EAGs in response to plant volatiles have been recorded in a number of aphid species, i.e., the English grain aphid *Sitobion avenae* (F.) and the rose-grain aphid *Metopolophium dirhodum* (Walk.) (Yan & Visser, 1982; Visser & Yan, 1995), the black bean aphid *Aphis fabae* Scop. (Hardie *et al.*, 1995), and the vetch aphid *Megoura viciae* Buckton (Visser & Piron, 1994, 1995). In addition, EAG responses to sex pheromone components have been recorded in *A. fabae* (Hardie *et al.*, 1994). Furthermore, details of EAG waveforms have been reported which are related to the elements underlying sensory transduction such as transport to receptor sites, receptor interaction and the subsequent deactivation of odour molecules (Visser & Piron, 1994; Hardie *et al.*, 1995).

In the present study a comparison is made between the EAG response profiles of four aphid species, namely *M. viciae*, *A. fabae*, the peach-potato aphid *Myzus persicae* (Sulz.) and the cabbage aphid *Brevicoryne*

*brassicae* (L.). These aphids were selected as they represent variation in aphid biology and consist of species (a) with an overlapping host-plant range, *M. viciae* and *A. fabae* on beans, *M. persicae* and *B. brassicae* on Chinese cabbage, (b) with different degrees of host-plant specialization, *M. viciae*, *A. fabae* and *B. brassicae* are oligophagous, *M. persicae* is polyphagous, and (c) with an alternation between summer and winter hosts, *A. fabae* and *M. persicae*, or (d) with a persistent presence on the same host plants throughout the year, *M. viciae* and *B. brassicae*.

### Materials and methods

**Aphids.** All aphid colonies were maintained as clones. *M. viciae* (Lees clone) was reared on broad bean plants *Vicia faba* L. (Visser & Piron, 1995), *A. fabae* (Kennedy clone) on tick bean seedlings *Vicia faba* (Hardie *et al.*, 1995), and *B. brassicae* (Tjallingii clone from Spain) and *M. persicae* (Dieleman clone M3) were reared on Chinese cabbage *Brassica chinensis* L. cv. Granaat under long-day conditions (L16:D8) at 22 °C. For EAG recordings apterous virginoparae (wingless summer forms) of *M. viciae* and alate virginoparae (winged summer forms) of *A. fabae*, *B. brassicae* and *M. persicae* were used.

**EAG recordings.** EAGs were recorded to 35 plant volatiles (see Table 1) as described by Visser & Piron (1995). All volatiles were ≥95% pure except for heptanonitrile (92%), (*E*, *E*)- $\alpha$ -farnesene (92%) and (*E*)- $\beta$ -farnesene (58%) which also contained 35%  $\alpha$ -

Table 1. Plant volatiles used for recording aphid EAGs, together with code numbers

Nr	Chemical	Nr	Chemical	Nr	Chemical
0	Paraffin oil (control)	12	3-Methoxybenzaldehyde	24	(+)-Citronellol
1	( <i>E</i> )-2-Hexenal	13	4-Methoxybenzaldehyde	25	Sabinene
2	( <i>E</i> )-2-Hexenol-1	14	2-Hydroxybenzaldehyde	26	(-)-( <i>E</i> )-Caryophyllene
3	( <i>Z</i> )-3-Hexenol-1	15	(-)-(1 <i>S</i> )- $\alpha$ -Pinene	27	( <i>E, E</i> )- $\alpha$ -Farnesene
4	( <i>Z</i> )-3-Hexenyl acetate	16	(-)-(1 <i>S</i> )- $\beta$ -Pinene	28	( <i>E</i> )- $\beta$ -Farnesene
5	Hexanol-1	17	(+)-( <i>S</i> )-Carvone	29	Hexanonitrile
6	Hexanal	18	(-)-( <i>R</i> )-Carvone	30	Heptanonitrile
7	( <i>E</i> )-2-Heptenal	19	$\alpha$ -Terpineol	31	Butyl isothiocyanate
8	2-Hexanone	20	Linalool	32	<i>tert</i> -Butyl isothiocyanate
9	2-Heptanone	21	Geraniol	33	Allyl isothiocyanate
10	Benzaldehyde	22	Nerol	34	3-Butenyl isothiocyanate
11	2-Methoxybenzaldehyde	23	Citronellal	35	4-Pentenyl isothiocyanate

farnesene isomers. Chemicals were dissolved in paraffin oil at 1% v/v. Stimulation cartridges were prepared by applying 25  $\mu$ l of each paraffin oil solution onto a piece of filter paper which was subsequently placed in a Pasteur pipette. The aphid antenna was stimulated for 2 s by pushing air through the Pasteur pipette into an air flow directed over the preparation. EAG waveforms were recorded and the largest deflection of the baseline was measured as the absolute EAG response. Absolute EAG responses were normalised and expressed as percentage responses relative to the responses of adjacent standards consisting of 1% (*E*)-2-hexenal at the source (Visser & Piron, 1995). All plant volatiles were tested on at least 10 different antennal preparations of each aphid species. Means and 95% confidence intervals were calculated, and conclusions were restricted to significant differences.

## Results and discussion

The absolute EAG response to the standard, 1% (*E*)-2-hexenal, is  $420 \pm 120 \mu$ V for *M. viciae*,  $220 \pm 60 \mu$ V for *A. fabae*,  $440 \pm 120 \mu$ V for *M. persicae*, and  $250 \pm 75 \mu$ V for *B. brassicae* (mean  $\pm$  95% c.i.).

The mean relative EAG responses of the four aphid species to 35 plant volatiles are shown in Figure 1. For comparison, the mean aphid EAG response profile is also presented and consists of the means of the EAG responses in the four aphid species to each of the plant volatiles tested. From this profile it is obvious that aphids, in general, show distinct sensitivities for (a) the general green leaf volatiles, i.e., (*E*)-2-hexenal (Figure 1: nr 1), (*E*)-2-hexenol-1 (2),

(*Z*)-3-hexenyl acetate (4), hexanol-1 (5), hexanal (6), (*E*)-2-heptenal (7), and 2-heptanone (9), (b) the benzaldehydes, e.g., 4-methoxybenzaldehyde (13), (c) the carvones, (-)-(*R*)-carvone (18) being the most effective, (d) the monoterpene aldehyde citronellal (23), (e) the nitriles, i.e., hexanonitrile (29) and heptanonitrile (30), and (f) some isothiocyanates, e.g., butyl (31) and 4-pentenyl isothiocyanate (35). It is noteworthy that single-unit responses to 4-pentenyl and 3-butenyl isothiocyanates have been recorded in *A. fabae* as well as in *B. brassicae* (Nottingham *et al.*, 1991).

Moreover, structure-activity relationships occur that are preserved in the individual response profiles of the different aphid species (Figure 1). In the isothiocyanates, the rank order of sensitivity remains the same, with butyl (31) and 4-pentenyl isothiocyanate (35) eliciting large responses. Citronellal (23) always evokes larger responses than (+)-citronellol (24). The same is true for (-)-(*R*)-carvone (18) compared to (+)-(*S*)-carvone (17), and 2-heptanone (9) compared to 2-hexanone (8).

Each of the aphid species studied differs in varying degrees from the general response profile. The present discussion of the species-specific traits is restricted to the major deviations from the mean aphid response profile. In the non host-alternating *M. viciae* the antennal sensitivity is reduced for some of the general green leaf volatiles, i.e., (*E*)-2-hexenol-1 (2), (*Z*)-3-hexenol-1 (3), (*Z*)-3-hexenyl acetate (4), and hexanol-1 (5). For this species, however, alate forms still remain to be tested. In the host-alternating *A. fabae*, which in the summer also feeds on beans and utilizes the spindle tree *Euonymus europaeus* L. as winter host, antennae are more responsive to (*E*)-2-hexenol-1 (2) and less so

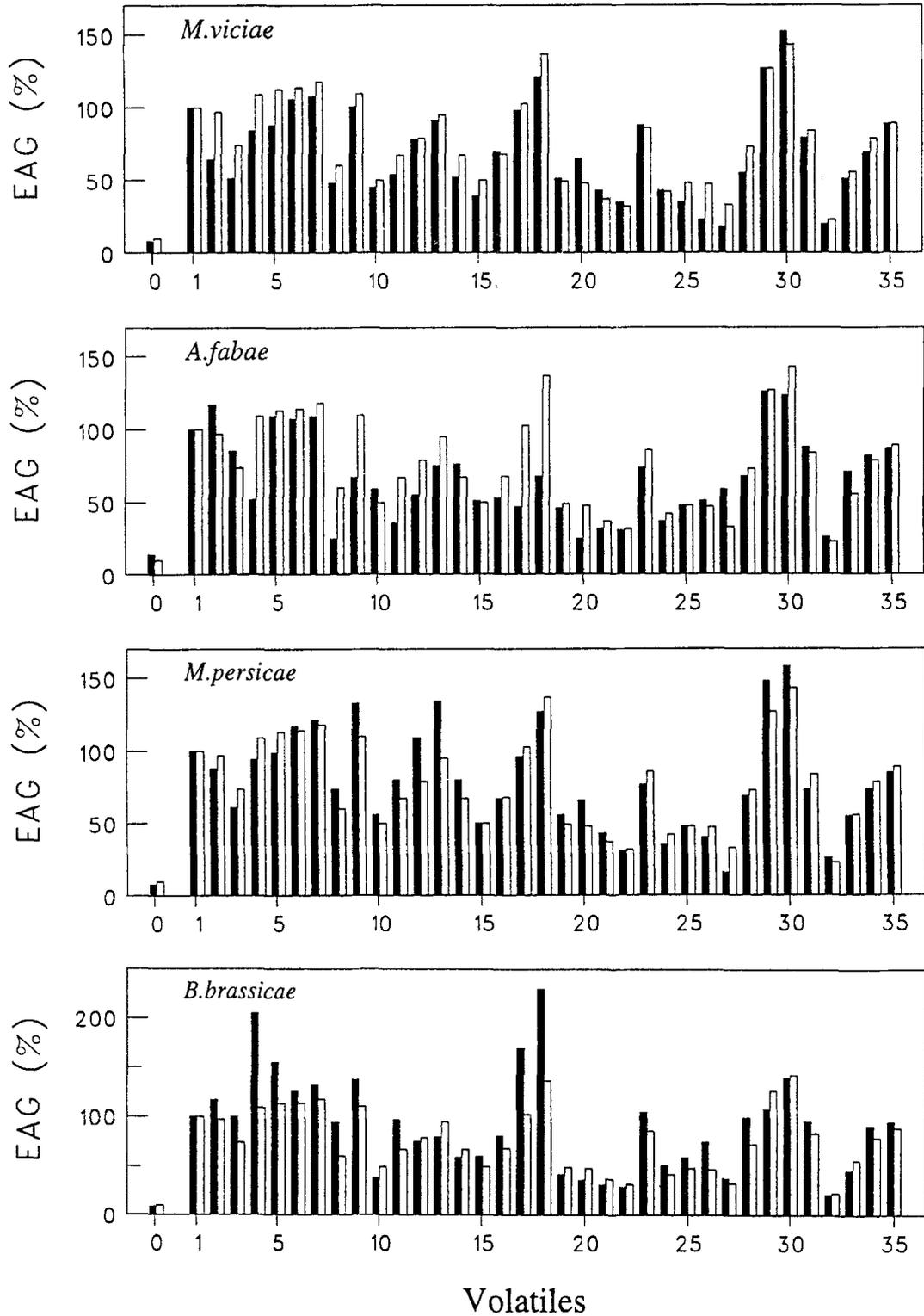


Figure 1. EAG response profiles of four aphid species (black bars), i.e., *Megoura viciae*, *Aphis fabae*, *Myzus persicae* and *Brevicoryne brassicae*, together with the mean aphid response profile to 35 plant volatiles (open bars). See Table 1 for code to volatiles.

to (*Z*)-3-hexenyl acetate (4), 2-heptanone (9), (+)-(*S*)-carvone (17) and (–)-(*R*)-carvone (18). The response profile of the polyphagous *M. persicae* appears rather similar to the mean aphid profile but shows slightly increased sensitivity for 2-heptanone (9), 3-methoxy- (12) and 4-methoxybenzaldehyde (13), and hexano- (29) and heptanonitrile (30). The oligophagous *B. brassicae*, as *M. persicae* reared on Chinese cabbage, is quite different from the mean aphid: larger responses to (*E*)-2-hexenol-1 (2), (*Z*)-3-hexenol-1 (3), (*Z*)-3-hexenyl acetate (4), hexanol-1 (5), 2-hexanone (8), 2-heptanone (9), 2-methoxybenzaldehyde (11), both (+)-(*S*)- (17) and (–)-(*R*)-carvone (18), and (*E*)- $\beta$ -farnesene (28). Here the response profile is dominated by (*Z*)-3-hexenyl acetate (4) and (–)-(*R*)-carvone (18).

From the present data set it is concluded that the four aphid species studied show some general features in their response profiles. It is also striking that aphid species which do not feed on cruciferous plants, i.e., *M. viciae* and *A. fabae*, respond to the components typical for these plants, i.e., the isothiocyanates and nitriles (Tollsten & Bergström, 1988). On the other hand, aphid species do differ in their response profiles, e.g., the sensitivity of *B. brassicae* for (*Z*)-3-hexenyl acetate, a component present in the headspace of unbruised cabbage (Tollsten & Bergström, 1988). The differential sensory perception in these aphid species of general as well as plant-specific volatile components should assist in the olfactory discrimination between host and non-host plants.

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