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Olfactory antennal responses to plant volatiles in apterous virginoparae of the vetch aphid *Megoura viciae*

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Abstract

Electroantennogram (EAG) responses were recorded from apterous virginoparae of the vetch aphid *Megoura viciae* Buckton (Homoptera, Aphididae) to more than eighty volatile compounds in order to investigate its sensory ability to perceive plant odours. The response profile of *M. viciae* reveals a differential sensitivity for the array of plant volatiles tested. The whole group of general green leaf volatiles is very stimulatory. In addition to (*E*)-2-hexenal, the following compounds of this group elicit large EAG responses: (*E*)-2-heptenal, 1-octenol-3, hexyl acetate, (*Z*)-3-hexenyl acetate, hexanol-1, hexanal, 2-heptanone and 3-octanone. Relatively large EAGs are also produced by 4-methoxybenzaldehyde (*p*-anisaldehyde), hexanonitrile, heptanonitrile, 1,6-hexanedithiol, butyl isothiocyanate, 4-pentenyl isothiocyanate, (−)-(1*S*)-β-pinene, (+)-(*S*)-carvone, (−)-(*R*)-carvone, α-terpineol, linalool and citronellal. The nitriles are the most effective of all plant volatiles tested. Structure-activity relationships occur in various groups of chemicals and members of the green leaf volatiles, benzaldehydes, isothiocyanates and monoterpenes are ranked accordingly. In the group of green leaf volatiles, aliphatic aldehydes are more stimulating than the corresponding alcohols. EAG responses to series of saturated aliphatic alcohols and aldehydes reveal that C_6 and C_7 compounds are the most stimulatory. Dose-response curves show that the rank order of EAG response amplitudes hardly changes at lower dosages. It is concluded that *M. viciae* perceives general plant volatiles as well as more-specific components, such as nitriles and isothiocyanates, associated with the odour blends of non-host plant species.

Introduction

In their search for host plants, the insect's perception of host-plant odour triggers positive anemotaxis. Such behaviour increases, compared to random movements, the probability of encountering host plants (Visser, 1986, 1988). In aphids, evidence for olfactory orientation is accumulating (Pickett *et al.*, 1992b) and the existence of an odour-conditioned anemotaxis has been shown in walking *Cryptomyzus korschelti* (Visser & Taanman, 1987). It is noteworthy that the walking tracks of wingless *C. korschelti* adults in response to host-plant odour look similar to those of Colorado potato beetles *Leptinotarsa decemlineata* (Thiery & Visser, 1986) and differ only through the speed of locomotion (Visser, 1988). Although for aphids it is expected that the winged forms (alatae) are devoted to migration and host-plant selection, also the wingless adults (apterae) often move from one plant to another and confront the difficulties in finding new resources (Hodgson, 1991).

The aphid antennae bear specific sets of olfactory neurones, placoid sensilla in the so-called distal and proximal primary rhinaria on segments six and five, respectively, and, in alate aphids, the secondary rhinaria on segments three and four (Shambaugh *et al.*, 1978; Bromley *et al.*, 1979). The neural responses of these sensilla to plant odours can be recorded in two ways: (1) by penetration of an individual placoid sensillum with an electrode, i.e., single-unit recording, or (2) by recording the overall response of an antennal preparation with the electroantennogram (EAG) technique

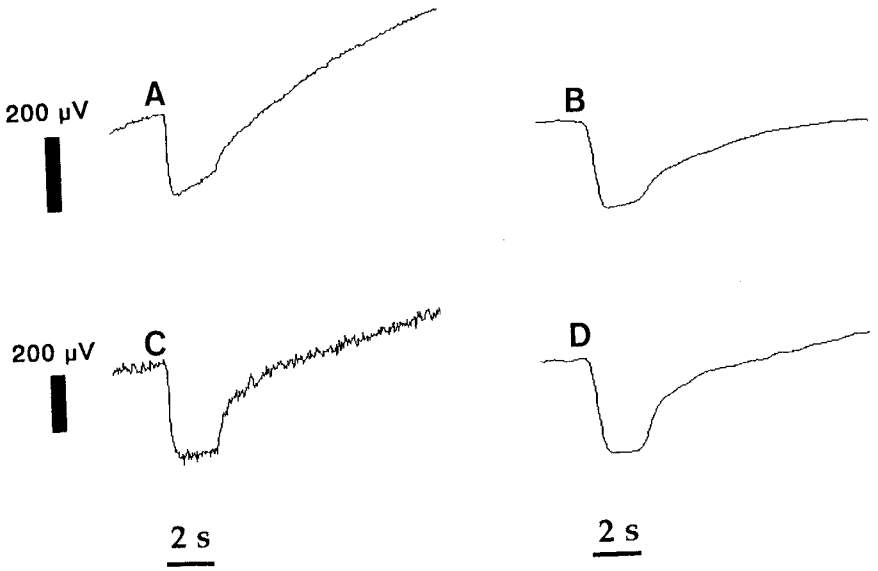


Fig. 1. EAGs from apterous virginoparae of *Megoura viciae* to (*E*)-2-hexenal (at the source in log 2 dilution). B: same recording as A, but corrected for DC drift. D: same recording as C, but smoothed.

(Boeckh *et al.*, 1965). The EAG response reflects the sum of receptor potentials in the antennal population of olfactory neurones, whereas single-unit recordings reveal the action potentials of individual sensory neurones, which in aphids are frequently mixed as multi-spike trains (Bromley & Anderson, 1982).

The vetch aphid *Megoura viciae* Buckton is a non host-alternating species which feeds exclusively on Leguminosae such as *Vicia* and *Lathyrus* species (Hille Ris Lambers, 1949). Under long-day conditions these aphids reproduce parthenogenetically, i.e., the summer forms (virginoparae), while short days induce the formation of the sexual forms. Since these aphids are relatively robust and easily maintained as a clonal culture, which excludes genotypic variation, they have often been used for physiological experiments (see e.g. Lees, 1967). Apterous virginoparae of *M. viciae* bear, in addition to the two primary rhinaria, 10–18 secondary rhinaria on their third antennal segment (Hille Ris Lambers, 1949). The present study was undertaken in order to investigate the aphid's sensory ability to perceive plant odours. For that purpose we recorded EAG peak responses from apterous virginoparae of *M. viciae* to more than eighty volatile compounds. The analysis of shape characteristics of EAG waveforms, which relate to the events underlying sensory transduction, has been summarized elsewhere (Visser & Piron, 1994).

Materials and methods

Insects. A colony of *M. viciae* was started with one virginopara from the Lees clone (Lees, 1967). Aphids were reared continuously on broad bean plants *Vicia faba* L. cv. Minica under long-day conditions (L17:D7) at 19 °C, 60% r.h. during the day and 16 °C, 60% r.h. during the night, and reproduced parthenogenetically. From this colony apterous virginoparae were used in the experiments.

Plant volatiles. The plant volatiles were obtained from commercial sources, i.e., Aldrich, Fluka, ICN/K&K, Merck and Roth, or from the Biological & Ecological Chemistry Department, Rothamsted UK (3-butenyl and 4-pentenyl isothiocyanates), and were $\geq$ 96% pure, except for α-terpinene (90%), myrcene (91%), α-ionone and heptanonitrile (92%), 1,4-cineole (93%), γ-terpinene (94%), (*Z*)-2-hexenol-1, hexanol-3, β-ionone and allyl isothiocyanate (95%). Table 1 shows the complete list of plant volatiles used. Code numbers were given to volatiles in order to shorten the legend of Fig. 3.

Antennal preparation for EAG. For the preparation of EAG recordings the head of an aphid was removed just behind the eyes. One antenna was amputated and the tip of the processus terminalis of the remaining antenna was cut leaving the distal primary rhinarium intact.

Table 1. List of plant volatiles used for recording EAGs from *Megoura viciae* apterous virginoparae, together with code numbers. Those without code numbers are used in Fig. 4

Code	Chemical	Code	Chemical
0	Paraffin oil	45	(−)-(1*S*)-α-Pinene
2	(*E*)-2-Hexenal	46	(−)-(1*S*)-β-Pinene
3	(*Z*)-2-Hexenol-1	47	δ-3-Carene
4	(*E*)-2-Hexenol-1	48	Myrcene
5	(*Z*)-3-Hexenol-1	49	(+)-(*S*)-Carvone
6	(*E*)-3-Hexenol-1	50	(−)-(*R*)-Carvone
7	1-Octenol-3	54	α-Terpinene
8	Hexyl acetate	55	γ-Terpinene
9	(*E*)-2-Hexenyl acetate	56	α-Terpineol
10	(*E*)-2-Hexenyl propionate	57	α-Ionone
11	(*Z*)-3-Hexenyl acetate	58	β-Ionone
12	(*Z*)-3-Hexenyl propionate	59	Linalool
	Pentanol-1	60	Geraniol
15	Hexanol-1	61	Nerol
	Heptanol-1	62	Citral
	Octanol-1	63	1,4-Cineole
	Nonanol-1	64	1,8-Cineole
	Decanol-1	65	Citronellal
20	Hexanol-2	66	(+)-Citronellol
21	Hexanol-3	67	(+)-Limonene
	Butanal	68	Terpinolene
	Pentanal	69	Sabinene
24	Hexanal	70	(−)-(*E*)-Caryophyllene
	Heptanal	71	α-Humulene
	Octanal	72	Chamazulene
	Nonanal	74	(−)-(*R*)-α-Phellandrene
	Decanal	75	(−)-α-Bisabolol
29	3-Pentanone	77	*m*-Cresol
30	2-Hexanone	79	2-Phenylethyl acetate
31	3-Hexanone	80	(*E*, *E*)-Farnesol
32	2-Heptanone	81	(*E*, *E*)-Farnesyl acetate
33	3-Heptanone	84	Hexylamine
34	3-Octanone	85	Hexanonitrile
35	Benzylalcohol	86	Heptanonitrile
36	Benzaldehyde	87	1-Hexanethiol
37	2-Methoxybenzaldehyde	88	1,6-Hexanedithiol
38	3-Methoxybenzaldehyde	89	Butyl isothiocyanate
39	4-Methoxybenzaldehyde	90	*tert*-Butyl isothiocyanate
40	2-Hydroxybenzaldehyde	91	Allyl isothiocyanate
41	4-Isopropylbenzaldehyde	92	Pyridine
42	1,2-Dimethoxybenzene	102	(*E*)-2-Heptenal
43	Hexylbenzene	126	3-Butenyl isothiocyanate
44	(+)-(1*R*)-α-Pinene	127	4-Pentenyl isothiocyanate

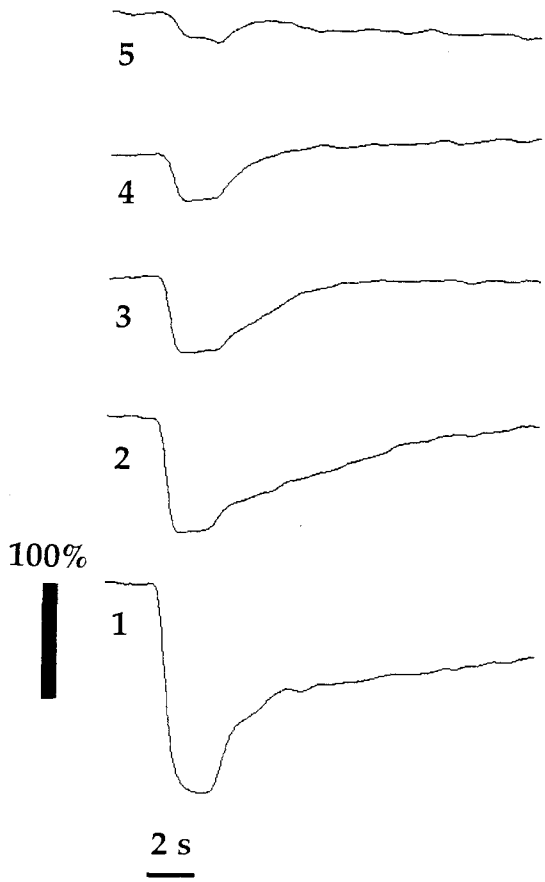
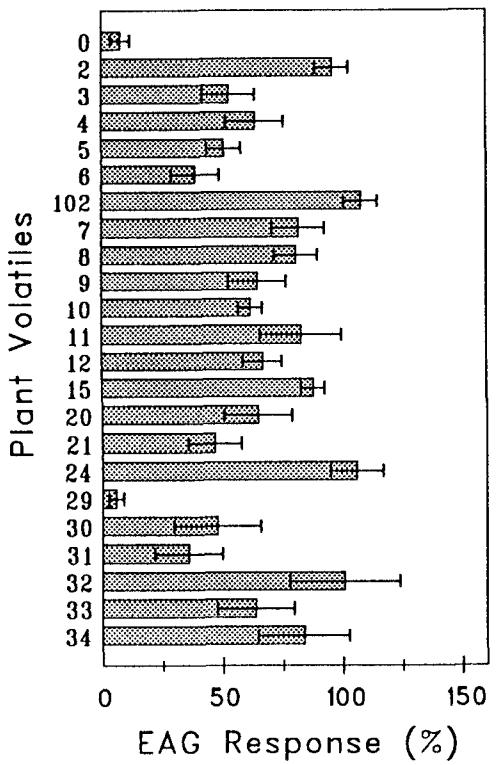
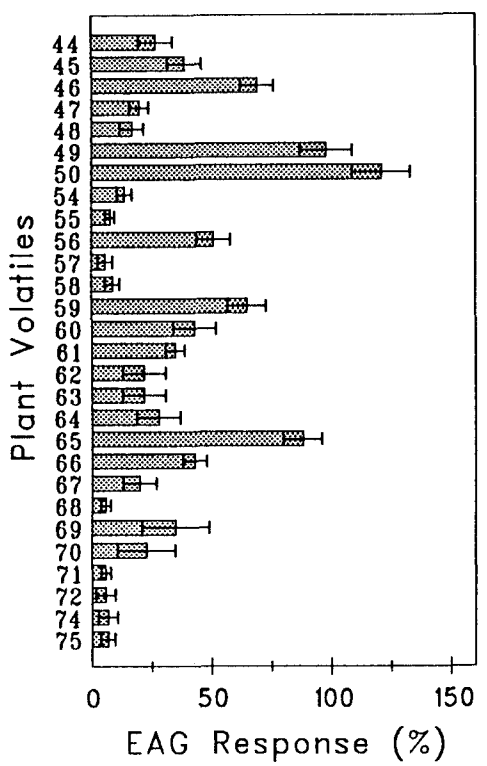


Fig. 2. EAGs from an apterous virginopara of *Megoura viciae* to (*E*)-2-hexenal (at the source in log 1 to log 5 dilutions). Bar 100% indicates relative response amplitude of the standard (*E*)-2-hexenal (log 2 dilution).

The electrodes consisted of glass capillaries filled with 0.1 M KCl. The ground electrode was inserted into the open side of the head while the recording electrode was sleeved over the antennal tip. Ag-AgCl wires connected the preparation to the amplification and recording devices consisting of an input probe and DC amplifier (Grass HIP16A & P16D, rise time set at 30 ms), an oscilloscope (Philips PM3302), and a transient-recorder (Krenz TRC 4010, 12 bits ADC) connected to a personal computer (Estate 80386 & 80387).

Stimulus delivery and protocol. In order to control release rates, plant volatiles were dissolved in paraffin oil (Merck, Uvasol), in most cases to 1% v/v (log 2 dilution). As reported in the results (Figs. 2 & 5–7), serial dilutions from a limited number of plant volatiles were also used. Fresh stimulation cartridges were prepared each day by applying 25 μl of each paraffin oil





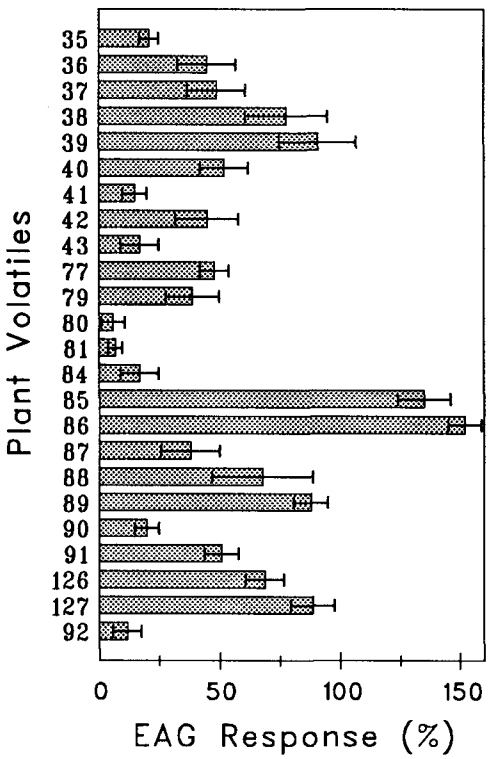


Fig. 3. EAG response profile from apterous virginoparae of *Megoura viciae* to an array of plant volatiles (at the source in log 2 dilution). EAG peak responses are expressed relative to the standard (*E*)-2-hexenal (log 2 dilution). Means ±95% c.i. (n=10–15). See Table 1 for key to plant volatiles.

solution onto a piece of filter paper (6 × 0.8 cm, Schleicher & Schuell 589[2]) which was subsequently placed in a Pasteur pipette.

Air was purified by passage through moisture and charcoal filters (Chrompack 7971 & 7972) and then re-humidified by bubbling through water. The main air flow was directed over the preparation by a glass tube (10 mm i.d.) and passed continuously over the antenna at a rate of 40 cm/s (30 ml/s). The tip of the stimulation cartridge was inserted into a small hole, 20 cm upstream of the preparation, in the side of the glass tube. By operation of a 3-way solenoid valve (Lee LFAA1200618H) purified air was blown for 2 s through the stimulation cartridge at a rate of 1 ml/s. In this way, the odour was injected into the main air stream and reached the antenna 0.5 s later. Air flow speeds were set by a mass flow control unit (Brooks 5878 with two 5850TRs).

The dilution of plant volatiles in paraffin oil has proven to be convenient (Visser, 1979; Ma & Visser, 1978). The paraffin oil solution in the stimulation car-

tridge acted as slow-release formulation that allowed the same cartridge to be used for repeated stimulations, up to 15 times, without a detectable change in stimulus intensity. For some of the plant volatiles, calibration curves have been measured using a flame ionisation detector (Ma & Visser, 1978). After correction for the present method of odour delivery, these curves revealed that cartridges containing log 2 dilutions of (*E*)-2-hexenol-1, (*Z*)-3-hexenol-1, hexanol-1 and (*E*)-2-hexenal stimulated the antennal preparation with 3.3×10^{13} to 9.8×10^{13} molecules/ml of air.

The antennal preparation lasted for about 25 min. In order to compensate for its decrease in responsiveness in the course of time, the antenna was stimulated by a standard, i.e., a Pasteur pipette containing 25 μl of (*E*)-2-hexenal in log 2 dilution, immediately prior to and after each stimulation with test compounds. Individual antennae were tested with a blank control (paraffin oil) and 10 different plant volatiles or 2 dilution series. Intervals between all stimulations were 30 s.

Data collection and analysis. The recordings were digitized and stored for 20.5 s, starting 4 s prior to the injection of odour into the main air stream (Krenz TRS software 4.03a). Software was developed in ASYST 3.1 to analyse the digitized EAG waveforms (ADC rate 25 Hz). Owing to their small amplitudes, EAG recordings from aphid antennae often showed DC drift and noise. The 4-second recording prior to odour injection was used to calculate the actual DC drift which was subsequently subtracted from the whole array of data representing the EAG waveform. Furthermore, the array of data was subjected to the Smooth function of ASYST (cutoff frequency at 0.1). The effects of these corrections on digitized EAG waveforms are shown in Fig. 1. Because of the distinct improvement all EAGs were first subjected to these corrections before further analysis.

From each corrected EAG waveform 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 (EAG peak responses in %). All plant volatiles and serial dilutions were tested on at least 9 different antennal preparations. Means and 95% confidence intervals (2-tailed 95% c.i., Student-*t* distribution; Sokal & Rohlf, 1981) were calculated from these data sets.

Results

The absolute EAG response of apterous virginoparae to the standard, i.e., (*E*)-2-hexenal in log 2 dilution at the source, is 420 ± 120 μV (mean ± 95% c.i.). Typical EAG waveforms recorded from *M. viciae* antennae in response to a dilution series of (*E*)-2-hexenal are shown in Fig. 2. Antennae of *M. viciae* are very receptive to the leaf aldehyde (*E*)-2-hexenal, which elicits EAG responses in a dose-related fashion (Fig. 2).

Response profile. In order to study the sensory specificity of the olfactory receptor system, we screened 74 volatile compounds, in log 2 dilution at the source, for their relative EAG peak responses (Fig. 3). The resulting EAG response profile reveals a differential sensitivity for the array of plant volatiles tested. The whole group of general green leaf volatiles (Visser *et al.*, 1979) is very stimulatory. Besides (*E*)-2-hexenal (code 2), the following compounds of this group elicit large EAG responses: (*E*)-2-heptenal (102), 1-octenol-3 (7), hexyl acetate (8), (*Z*)-3-hexenyl acetate (11), hexanol-1 (15), hexanal (24), 2-heptanone (32) and 3-octanone (34). Various volatile compounds belonging to other chemical classes, also release relatively large EAG responses, e.g., 4-methoxybenzaldehyde (39, *p*-anisaldehyde), hexanonitrile (85), heptanonitrile (86), 1,6-hexanedithiol (88), butyl isothiocyanate (89), 4-pentenyl isothiocyanate (127), (−)-(1*S*)-β-pinene (46), (+)-(*S*)-carvone (49), (−)-(*R*)-carvone (50), α-terpineol (56), linalool (59) and citronellal (65). It is striking that hexanonitrile (85) and heptanonitrile (86) elicit the largest EAGs of all plant volatiles tested. In contrast to the foregoing list, several volatile compounds elicit EAGs which do not differ in amplitudes from the EAGs obtained with pure paraffin oil (0), their 95% confidence intervals overlap with these blank controls. The non-stimulatory compounds include 3-pentanone (29), 4-isopropylbenzaldehyde (41, cuminaldehyde), hexylbenzene (43), (*E*, *E*)-farnesol (80), (*E*, *E*)-farnesyl acetate (81), hexylamine (84), pyridine (92), α-terpinene (54), γ-terpinene (55), α-ionone (57), β-ionone (58), terpinolene (68), α-humulene (71), chamazulene (72), (−)-(*R*)-α-phellandrene (74) and (−)-α-bisabolol (75).

Structure-activity relationships. Besides the response profile shown in Fig. 3, we recorded the EAG responses of *M. viciae* to series of saturated aliphatic alcohols and aldehydes (Fig. 4). The array of plant volatiles tested, thus, contains series of homologous molecules, e.g.,

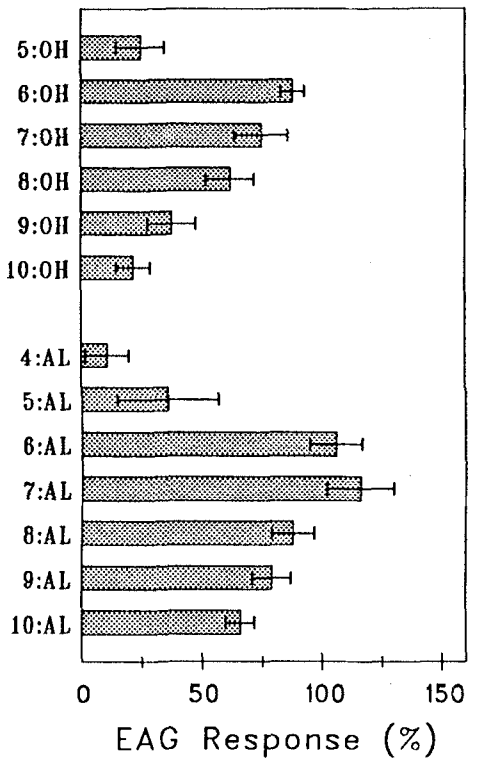


Fig. 4. EAG response profile from apterous virginoparae of *Megoura viciae* to series of saturated aliphatic alcohols (:OH) and aldehydes (:AL) with varying numbers of C atoms (at the source in log 2 dilution). EAG peak responses are expressed relative to the standard (*E*)-2-hexenal (log 2 dilution). Means ±95% c.i. (n=10–14).

the group of general green leaf volatiles, with modifications in chain lengths, functional groups, double-bond positions and geometry of atoms. The difference in EAG responses to members of such series exposes the stimulatory capacity of particular molecular structures. The EAG responses to the green leaf volatiles show that the aldehydes are more stimulatory than the alcohols: (*E*)-2-hexenal (2) > (*E*)-2-hexenol-1 (4), and hexanal (24) > hexanol-1 (15). The saturated C_6 alcohol, hexanol-1 (15), elicits larger EAGs than the corresponding mono-unsaturated alcohols, i.e., (*Z*)-2-hexenol-1 (3), (*E*)-2-hexenol-1 (4), (*Z*)-3-hexenol-1 (5) and (*E*)-3-hexenol-1 (6). In the EAGs for mono-unsaturated alcohols (*E*)-2-hexenol-1 (4) stimulates more than (*E*)-3-hexenol-1 (6), indicating that for the *E*-isomers the double-bond position at C_2 is more effective than at C_3. However, this does not occur for the *Z*-isomers (3 *vs* 5). When (*E*)-2-hexenol-1 (4), (*Z*)-3-hexenol-1 (5) and hexanol-1 (15) are compared with the corresponding acetate (9, 11, 8) and propionate esters (10, 12), only (*Z*)-3-hexenyl acetate (11) and (*Z*)-3-hexenyl propionate (12) elicit larger EAGs than the original alcohol. For hexanols the position of the alcohol group at C_1 is essential as EAGs show that hexanol-1 (15) > hexanol-2 (20) = hexanol-3 (21). 2-Heptanone (32) elicits larger EAGs than the C_5 (29) and C_6 equivalents (30, 31), while 3-heptanone (33) differs only from 3-pentanone (29). EAG responses to the series of alcohols and aldehydes reveal that C_6 and C_7 compounds are the most stimulatory (Fig. 4). In addition, the conclusion that aldehydes elicit larger EAGs than the corresponding alcohols is further substantiated as heptanal > heptanol-1, octanal > octanol-1, nonanal > nonanol-1 and decanal > decanol-1.

Although the number of homologous compounds is limited in the other chemical classes, structure-activity relationships are obviously present. In the group of benzene derivatives, EAG amplitudes are ordered into 4-methoxybenzaldehyde (39, *p*-anisaldehyde) = 3-methoxybenzaldehyde (38, *m*-anisaldehyde) > 2-methoxybenzaldehyde (37, *o*-anisaldehyde) = 2-hydroxybenzaldehyde (40, salicylaldehyde) = benzaldehyde (36) > benzylalcohol (35). Isothiocyanates stimulate EAGs with amplitudes for 4-pentenyl (127) = butyl (89) > 3-butenyl (126) > allyl (91) > *tert*-butyl (90). For EAGs to monoterpenes, (−)-(1*S*)-β-pinene (46) is larger than (+)-(1*R*)-α-pinene (44) and (−)-(1*S*)-α-pinene (45), and (−)-(*R*)-carvone (50) > (+)-(*S*)-carvone (49). The rank order for EAGs to monoterpene alcohols is linalool (59) > geraniol (60) = nerol (61). Citronellal (65) elicits much larger EAGs than (+)-citronellol (66).

Dose-response relationships. The EAG response profile of *M. viciae* was recorded by application of the chemicals in stimulation cartridges at log 2 dilution. The question remains whether the rank order of EAG response amplitudes changes at other dosages. For that reason we tested dilution series of a limited number of plant volatiles. Chemicals were selected from groups which elicit distinct EAG responses at log 2 dilution, namely, (*E*)-2-hexenal, hexanal and (*E*)-2-heptenal (Fig. 5), (*Z*)-3-hexenol-1 and (*Z*)-3-hexenyl acetate (Fig. 6), and hexanonitrile and butyl isothiocyanate (Fig. 7).

The dose-response curves of the leaf aldehydes are rather similar, although slight deviations occur at the lower and highest concentrations (Fig. 5). At log 5 dilution the EAG responses to the leaf aldehydes do not differ from pure paraffin oil. At log 4 dilution the EAG response to (*E*)-2-heptenal is significantly

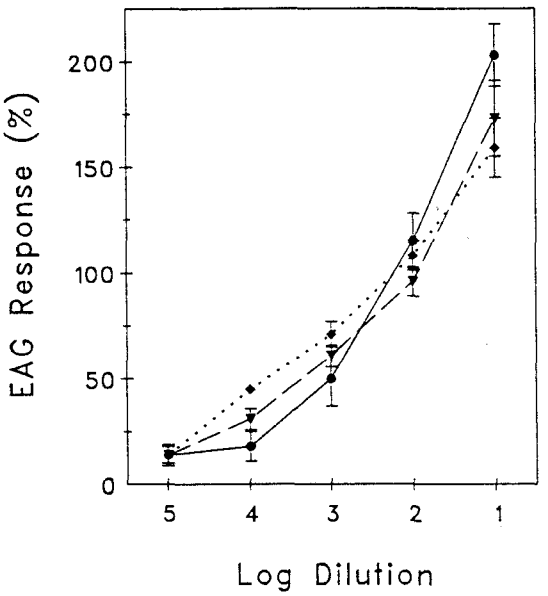


Fig. 5. Dose-response relationships for relative EAG peak responses from apterous virginoparae of *Megoura viciae* to hexanal (circles, solid line), (*E*)-2-hexenal (triangles, broken line) and (*E*)-2-heptenal (diamonds, dotted line). Means ±95% c.i. (n=12–13).

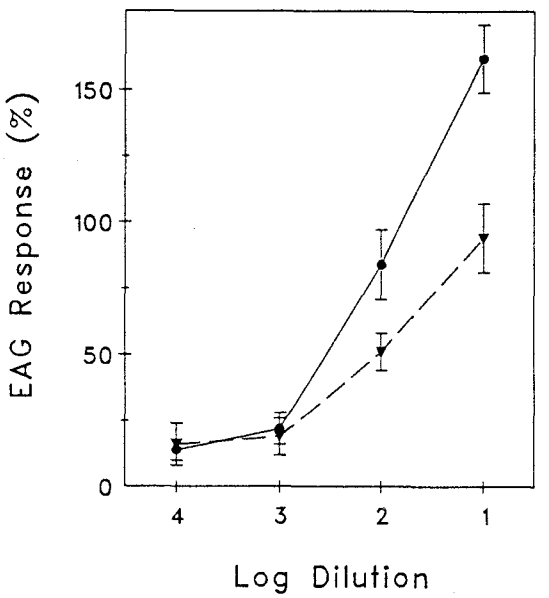


Fig. 6. Dose-response relationships for relative EAG peak responses from apterous virginoparae of *Megoura viciae* to (*Z*)-3-hexenyl acetate (circles, solid line) and (*Z*)-3-hexenol-1 (triangles, broken line). Means ±95% c.i. (n=12–14).

larger than those to (*E*)-2-hexenal and hexanal, while, at log 1 dilution hexanal shows the largest response

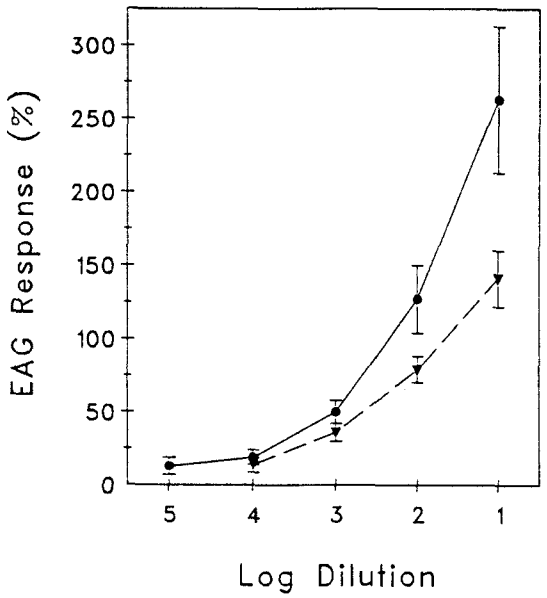


Fig. 7. Dose-response relationships for relative EAG peak responses from apterous virginoparae of *Megoura viciae* to hexanonitrile (circles, solid line) and butyl isothiocyanate (triangles, broken line). Means ±95% c.i. (n=9–15).

which differs significantly from the response to (*E*)-2-heptenal.

The dose-response curves for (*Z*)-3-hexenol-1 and (*Z*)-3-hexenyl acetate show that the distinction in EAG response amplitudes, i.e., larger EAGs for the acetate than the alcohol, occurs at both log 2 and log 1 dilution (Fig. 6). At log 4 and log 3 dilution the EAG responses to these volatiles do not differ from the blank controls.

Hexanonitrile elicits larger EAG responses than butyl isothiocyanate at log dilutions ranging from 3 to 1 (Fig. 7). The EAG responses to both hexanonitrile at log 5 dilution and butyl isothiocyanate at log 4 dilution, do not differ from the pure paraffin oil response.

Discussion

Plant volatiles. The plant volatiles used in the present study were selected for three reasons, namely, (1) their reported occurrence in all kind of plant odour blends (see e.g., Metcalf, 1987), (2) various compounds are known to act as kairomones or to elicit sensory responses in insects (Visser, 1986; Metcalf, 1987), and (3) to complete series of homologous compounds in order to study structure-activity relationships.

Most compounds listed in Table 1 represent plant volatiles which are not considered to be specific for one particular plant species. Members of the general green leaf volatiles are always present in the odour of green leaves. These compounds, like (*E*)-2-hexenal, (*Z*)-3-hexenol-1, hexanal and hexanol-1, are formed from linolenic and linoleic acids by an enzyme system which is bound to the thylakoid membrane of chloroplasts (Hatanaka, 1993). The significance of the green odour complex as kairomones for insects has been recognized previously (Visser *et al.*, 1979; Visser, 1983). Furthermore, other compounds, such as the group of benzaldehydes, the mono- and sesquiterpenes with derivative alcohols and aldehydes, are common plant constituents (Visser, 1986; Metcalf, 1987).

On the other hand, the isothiocyanates are specific for cruciferous species. Allyl, 3-butenyl and 4-pentenyl isothiocyanate are produced from glucosinolates (Cole, 1976) and are present in headspace samples of *Brassica* and *Sinapis* species (Tollsten & Bergström, 1988). In addition, macerated bud and leaf samples of *Brassica* species contain 5-hexenonitrile (Tollsten & Bergström, 1988). The odour of onion is dominated by sulfur-containing compounds, and methane-, propane- and allylthiol are present in the steam-distilled oil (Boelens *et al.*, 1971). Sulfides, however, also occur in disrupted tissues of *Brassica* species (Tollsten & Bergstöm, 1988; Chin & Lindsay, 1993). The bouquet of a given plant species is, thus, determined by the particular blend of non-specific constituents, although for some plant species, specific compounds are involved as well (Visser, 1986).

Perception of plant volatiles. Since all aphids used in the present study belong to the same genotype (see Materials and methods), the variation in EAG responses is considered to arise from phenotypic and methodological sources.

The antennal olfactory receptors of *M. viciae* respond clearly to general green leaf volatiles with (*E*)-2-hexenal, (*E*)-2-heptenal, hexanal and 2-heptanone dominating this part of the response profile. Receptivity for the green leaf volatiles is a common feature in phytophagous insects, although species-specific traits occur (Visser, 1983, 1986). In aphids, the mono-unsaturated and saturated C_6 aldehydes always elicit large EAG responses as has been reported for the English grain aphid *Sitobion avenae* and the rose-grain aphid *Metopdophium dirhodum* (Yan & Visser, 1982; Visser & Yan, 1995), the pea aphid *Acyrthosiphon pisum* (van Giessen *et al.*, 1994), and different forms of the black bean aphid *Aphis fabae* (Hardie *et al.*, 1994, 1995). Nevertheless, response profiles for the other green leaf volatiles differ between aphid species. For example, in *M. dirhodum* the receptivity for (*Z*)-3-hexenol-1 is increased as compared to *S. avenae* (Visser & Yan, 1995), while in *A. fabae* the mono-unsaturated and saturated C_6 alcohols elicit EAGs of similar size as the corresponding aldehydes (Hardie *et al.*, 1995). The position of the recording electrode can be manipulated such that EAGs are recorded from both the distal and proximal primary rhinarium, or from the proximal rhinarium alone. In *A. pisum* such recordings revealed that the distal rhinarium was more sensitive to aliphatic alcohols than to aldehydes, while the reverse occurred in the proximal rhinarium (van Giessen *et al.*, 1994). The series of saturated aldehydes and alcohols show a characteristic peak for C_6 and C_7 compounds in *M. viciae*, but also, for the alcohols, in *S. avenae* and *M. dirhodum* (Yan & Visser, 1982; Visser & Yan, 1995). However, this should not be considered specific for aphid species as EAGs of *L. decemlineata* beetles express the same phenomenon (Visser, 1979).

For the remaining part of volatiles tested the most striking feature of the *M. viciae* response profile is the distinct sensitivity for non-host plant compounds, like the nitriles, the isothiocyanates, and, to a lesser extent, the thiols. It is tempting to postulate that the antennae of *M. viciae* contain repellent receptors that allows the olfactory discrimination of cruciferous species, non-hosts for *M. viciae*, during its search for host plants. This is indeed true for *A. fabae* which is repelled by 4-pentenyl isothiocyanate (Nottingham *et al.*, 1991) and heptanonitrile (R. Isaacs, pers. comm.) in olfactometer bioassays. In the latter case, heptanonitrile was tested because the EAG study exposed its potential (Hardie *et al.*, 1995).

Nowadays the perception of isothiocyanates by the proximal primary rhinarium is documented for a number of aphid species. Single-unit recordings revealed a graded response in *A. fabae* as well as in the cabbage aphid *Brevicoryne brassicae*, 4-pentenyl being more stimulatory than 3-butenyl isothiocyanate (Nottingham *et al.*, 1991). Also in the damson-hop aphid *Phorodon humuli*, responses were obtained to 4-pentenyl isothiocyanate (Pickett *et al.*, 1992a). In the turnip aphid *Lipaphis erysimi*, the rank order of stimulatory intensity is 3-butenyl > allyl ≫ 4-pentenyl > 2-butyl isothiocyanate (Dawson *et al.*, 1987). The function of the perception of isothiocyanates, however, differ considerably between aphid species. For some species, like *B. brassicae* and *L. erysimi*, they act as attractants, for

others, like *A. fabae* and *P. humuli*, they are repellent (Nottingham *et al.*, 1991; Pickett *et al.*, 1992a), but, in both ways, they assist the olfactory discrimination between host and non-host plant species. It is noteworthy that the action of isothiocyanates is not restricted to host-plant selection but that they also substantially increase the alarm response to (*E*)-β-farnesene in *L. erysimi* (Dawson *et al.*, 1987).

Bromley & Anderson (1982) suggested in their early study on the responses of primary rhinaria in the lettuce aphid *Nasonovia ribisnigri* that these sensilla may be more important in detecting terpenes. The interpretation of their data, however, is difficult as multi-spike recordings were classified only in terms of excitation, inhibition and no-effect. Moreover, most of the recordings were obtained at relatively high concentrations. The suggestion that the rhinaria are tuned to terpenes is not supported by their data at low doses, since, in the proximal primary rhinarium, (*E*)-2-hexenal still elicits responses while terpenes are below the response threshold. The proximal primary rhinarium of *P. humuli* contains separate receptors for (*E*)-2-hexenal and β-caryophyllene. In an olfactometer bioassay, the mixture of these two components in the natural ratio is more attractive than (*E*)-2-hexenal alone (Campbell *et al.*, 1993). In *M. viciae*, several terpenes and derivatives produce distinct EAG peaks, namely, (−)-(1*S*)-β-pinene, α-terpineol, linalool, citronellal, (+)-(*S*)-carvone and (−)-(*R*)-carvone. The last compound even elicits larger EAGs than (*E*)-2-hexenal. Large numbers of the willow-carrot aphid *Cavariella aegopodii* were collected in yellow water traps baited with carvone (Chapman *et al.*, 1981). Again, the interaction with other plant volatiles turned out to be important as linalool reduced trap catches. The bird-cherry-oat aphid *Rhopalosiphum padi* is attracted to benzaldehyde, a compound expected to be present in the odour of its winter host *Prunus padus* (Pettersson, 1970. In *M. viciae*, benzaldehyde derivatives elicit EAG responses which can be ranked for structure-activity relationship, 4-methoxybenzaldehyde being as effective as the standard (*E*)-2-hexenal.

Considering the response profile of *M. viciae* to the large array of plant volatiles tested and the foregoing literature, it is concluded that the population of antennal olfactory receptors in this species is tuned for the perception of general as well as more-specific plant odour components. This perceptual ability is expected to assist *M. viciae* to discriminate the positive signals associated with its host plant species as well as the negative signals present in the odour blends of non-host plants. One should realize, however, that such olfactory discrimination may not be the mere evaluation of just a few positive and negative signals but probably involves the interaction of volatiles making up complicated odour blends. The further study of odour perception, both at the sensory and behavioural level, may expose the intriguing possibilities of plant odour manipulation to be applied in the control of insect pest populations, as suggested by several authors (Chapman *et al.*, 1981; Thiery & Visser, 1986; Metcalf, 1987; Nottingham *et al.*, 1991; Pickett *et al.*, 1992a, 1992b).

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