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Perception of volatiles associated with sex and food by different adult forms of the black bean aphid, *Aphis fabae*

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Abstract. Electroantennograms (EAGs) were recorded from adult male and asexual forms (winged and wingless virginoparae and gynoparae) of the black bean aphid, *Aphis fabae*, during stimulation with two sex pheromone components, (+)-(4a*S*,7*S*,7a*R*)-nepetalactone and (−)-(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol, as well as six plant volatiles, i.e. (*E*)-2-hexenal, (*E*)-2-hexenol-1, (*Z*)-3-hexenol-1, (*Z*)-3-hexenyl acetate, hexanal and allyl isothiocyanate. The male antennae are 1000–10,000 times more sensitive to nepetalactol and nepetalactone than to the plant compound (*E*)-2-hexenal. Besides this marked difference of EAG peak responses in males, the EAG rise and decay are slower for the sex pheromone components. Males are also much more sensitive to the sex pheromone components than asexual females. This high sensitivity correlates with a predominance of antennal secondary rhinaria, the major sites of pheromone perception in the male. However, it is the primary rhinaria on the antennae of the wingless asexual females that are responsible for pheromone perception. Male antennae are as responsive as the asexual female antennae to the plant volatiles. The specialization of the male for mate location is discussed.

Key words. *Aphis fabae*, electroantennogram, male aphids, odours, olfaction, plant volatiles, rhinaria, semiochemicals, sex pheromones.

Introduction

In temperate climates many aphid species have an annual life cycle that alternates between asexual and sexual reproduction (Hille Ris Lambers, 1966). The asexual period of development begins with the hatching of the overwintering egg in spring and continues through a number of female generations throughout spring and summer until the sexual males and females are induced in autumn, frequently cued by the short day-lengths (Lees, 1966). Although the males and sexual females (oviparae) are monomorphic, the asexual generations are represented by a number of different adult forms. During summer (or under artificial long-day conditions in the laboratory) black bean aphid, *Aphis fabae* Scopoli, adults may be winged, developing under crowded conditions, or wingless, developing under more isolated conditions (Shaw, 1970). Short days induce both the winged males and later the wingless oviparae. In this host-alternating species the oviparae are preceded by a short-day-promoted winged female, the gynopara, which migrates from the summer host (e.g. broad bean, *Vicia faba* L.) to the winter host (spindle, *Euonymus europaeus* L.) where the sexual part of the life cycle occurs.

Host-plant location by asexual female aphids, predominantly undertaken by the winged adults, has an olfactory component (Pickett *et al.*, 1992). The antennae bear three types of olfactory organs, the proximal and distal primary rhinaria and the secondary rhinaria which are often reduced or lacking in the wingless adults (Anderson & Bromley, 1987). A number of studies have shown that electrophysiological recordings of the whole antenna produces electroantennogram reponses to a number of volatiles. Wohlers & Tjallingii (1983) showed that antennae responded to (*E*)-β-farnesene, a plant volatile and an aphid alarm pheromone; Yan & Visser (1982) and Van Giessen *et al.* (1992) used a variety of plant volatiles. It

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is also possible to record from individual rhinaria in the aphid antenna and to locate specific neurones responding to a number of volatiles (Bromley & Anderson, 1982; Wadhams, 1990). This single-cell recording technique has been successfully used, in conjunction with gas chromatography, to locate active volatiles in complex mixtures derived from plant or insect sources (Wadhams, 1990). Indeed, this combined technique was utilized in the identification of the aphid sex pheromone components (+)-(4a*S*,7*S*,7a*R*)-nepetalactone and (−)-(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (Dawson *et al.*, 1987).

The existence of a sex pheromone produced by sexual female aphids, which affected male behaviour, was first demonstrated 20 years ago (Pettersson, 1971, 1973; Marsh, 1972). These authors concluded that the pheromone was volatile and detected by the secondary rhinaria on the male antennae but was only active over a short distance from the female. This latter observation led to the idea that males of host-alternating species would firstly locate their winter host which is, to a great extent, aphid species specific and then locate the oviparae (see Hardie, 1991). The chemical identification of sex pheromone components and their synthesis has allowed more extensive investigations and it is now known that males can locate pheromone sources in the field without recourse to host-plant cues although there may be an interaction between plant volatiles and pheromone (Campbell *et al.*, 1990; Hardie *et al.*, 1992, and unpublished). The sex pheromone released by *A.fabae* oviparae has been identified as a mixture of the nepetalactone and nepetalactol isomers, reported above, in a 29:1 blend, respectively (Dawson *et al.*, 1990). The present paper examines the perception of volatile compounds, both sex pheromone components and plant-derived volatiles, by recording electroantennograms from the antennae of different asexual females of the black bean aphid, *Aphis fabae*, winged and wingless virginoparae (summer forms), the winged gynoparae (autumn females) and the male. Comparison is drawn between the olfactory responses and the morphology of the antennae.

Materials and Methods

Insects. A clone of *Aphis fabae*, originally isolated by Kennedy in 1946 (Kennedy & Booth, 1951), was cultured on tick bean (*V.faba*) seedlings at 15°C in Gallenkamp incubators. Wingless and winged virginoparae were reared under long-day conditions (LD 16:8 h); apterous adults developed in sparse populations on seedlings while alates were induced by crowding young larvae on bean stipules (growing tip removed). Transfer of long-day insects to short days (15°C, LD 12:12 h) induced the development of gynoparae and males (for details see Hardie, 1980). Winged virginoparae and gynoparae were used shortly after first take-off (<24 h old), apterae and males were <72 h old.

Electroantennograms (EAGs). Antennal preparation involved amputation of one antenna at the base and removing the anterior part of the head capsule close to the eyes. A glass micro-pipette, filled with 0.1 M KCl, formed the indifferent electrode and was inserted into the head such that the tip lodged close to the base of the remaining antenna. The extreme tip of the last, sixth antennal segment was then severed and the cut end sleeved with a 0.1 M KCl-filled recording electrode. Chlorided silver wires connected the electrodes to a Grass P16 amplifier via an HIP16A input probe. Amplified signals were observed on a Philips PM3302 storage oscilloscope and recorded using a Krenz TRC 4010 transient recorder attached to an Estate AT386 computer (Visser & Piron, 1994, and unpublished).

The storage of electrical recordings was triggered on odour injection into the air stream directed over the antennal preparation and lasted for 20.5 s in total. The odour stimulus reached the antennal preparation 0.5 s after injection, but the start of this 2 s odour injection was taken as time 0.0. The data were analysed by software developed in Asyst 3.1 (Visser & Piron, 1994, and unpublished) with signal correction for DC drift and noise. The EAG parameters examined were: (1) Peak response, the largest negative potential (μV) recorded in 0.0–2.5 s from the onset of stimulus injection. (2) Mean response, the mean potential (μV) recorded in 1.5–2.0 s. (3) Rise, the mean potential reached in 0.0–1.0 s relative to the mean response in per cent. (4) Decay, the mean decrease in potential reached in 2.5–3.5 s relative to the mean response in per cent.

The peak responses were normalized and expressed as a percentage in relation to adjacent EAG peaks produced in response to the standard odour (*E*)-2-hexenal (see below).

Odour stimuli, presentation and protocol. The volatile chemicals comprised natural plant volatiles together with sex pheromone components. (*E*)-2-hexenal, (*E*)-2-hexenol-1, (*Z*)-3-hexenol-1 and (*Z*)-3-hexenyl acetate were purchased from Roth, hexanal from Fluka and allyl isothiocyanate from Aldrich. The first five compounds represent general green leaf volatiles and the latter is a volatile associated with cruciferous non-host plants. The sex pheromone components (+)-(4a*S*,7*S*,7a*R*)-nepetalactone and (−)-(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol were prepared from cat mint plants, *Nepeta cataria* (after Dawson *et al.*, 1989). Purities of the chemicals were 95–99%. The compounds were dissolved in paraffin oil (Uvasol, Merck), normally 10 μl in 1 ml paraffin oil to give a log 2 dilution (v/v).

The paraffin oil solutions were pipetted, in 25 μl aliquots, onto 0.8 × 6 cm pieces of filter paper (Schleicher and Schuell 589², Germany) which were inserted into glass Pasteur pipettes. The stimulus was defined as the log dilution at this point, i.e. at the odour source. The pipette was then attached to an air line and the volatile delivered, over a 2 s interval, into the main air flow over the antennal preparation. The air was purified by passage through Chrompack moisture and charcoal filters and then re-humidified by bubbling through de-ionized water before use. Air flow was controlled at 1800 ml/min for the main air flow which was directed over the preparation by a 1 cm diameter glass tube (i.e. 40 cm/s). The Pasteur pipette containing the volatile was inserted through a small hole

in the wall of the glass tube and air forced through at 60 ml/min for 2 s. Air flows were controlled by Brooks mass flow controllers and the stimulus via a three-way solenoid valve.

The antennal preparations proved rather delicate, EAG responses tended to decrease with time and the preparations were short-lived (<20 min). There were also differences in EAG amplitude between antennae. To overcome this variability, all test stimuli were preceded and followed by a standard stimulus with (*E*)-2-hexenal (log 2 dilution at source). Test responses could then be normalized against the adjacent standards. A period of 30 s was allowed between stimuli. Dose–response data were obtained using serial dilutions at the source.

Scanning electron and light microscopy. Male *A.fabae* were immersed in acetone and left for at least 7 days before critical point drying, sputter coating with gold–palladium and examination in a Philips 535 M scanning electron microscope. The numbers of secondary rhinaria were counted using a light microscope. For this purpose, antennal pairs, connected by head capsule cuticle, were severed from adult males and winged virginoparae, dehydrated in ethanol, cleared in xylene and mounted in canada balsam prior to viewing.

Results

Examples of EAGs recorded from the antenna of a male *A.fabae* are presented in Fig. 1 where stimulations with log 2 dilution nepetalactone and log 2 dilution nepetalactol are bracketed by log 2 dilution standard, (*E*)-2-hexenal. The average response of the male antennae to the standard was $220 \pm 60\,\mu V$ (mean ±95% C.I.). The dose–response curves of these three compounds are shown in Fig. 2. Although there are significant differences between the sex pheromone components at log 6, 5 and 4 dilutions, with the antenna appearing more sensitive to nepetalactone, the sensitivities are statistically identical at other stimulus dilutions. Male antennae are much more sensitive to both sex pheromone components than they are to the plant volatile standard. Comparison of the EAG responses to nepetalactone at log 5 dilution and the response to nepetalactol at log 4 dilution with the response to (*E*)-2-hexenal at log 1 dilution reveals a similar potency. Thus nepetalactone and nepetalactol are some 10,000 and 1000 times more potent, respectively, than the plant volatile (*E*)-2-hexenal.

Comparisons were also drawn between the rise and decay times for EAGs recorded after stimulation with sex pheromone components and with the green-leaf volatile (*E*)-2-hexenal (Fig. 3). The same EAG recordings were used as in Fig. 2, although the lowest dilutions were excluded because the EAG responses did not differ from the blank control. For the main part, rise and decay speeds are slower for the sex pheromone components than for the plant volatile.

Antennal preparations from other adult forms of *A.fabae* also reveal olfactory receptors for the sex pheromone components (Fig. 4). Comparison of the EAGs from wingless and winged virginoparae, as well as gynoparae, indicate similar sensitivities with nepetalactone producing responses slightly greater than the standard (100%) whereas nepetalactol and the standard produce identical responses. The increased sensitivity of the male antennae can be correlated, at least to some extent, by the increased num-

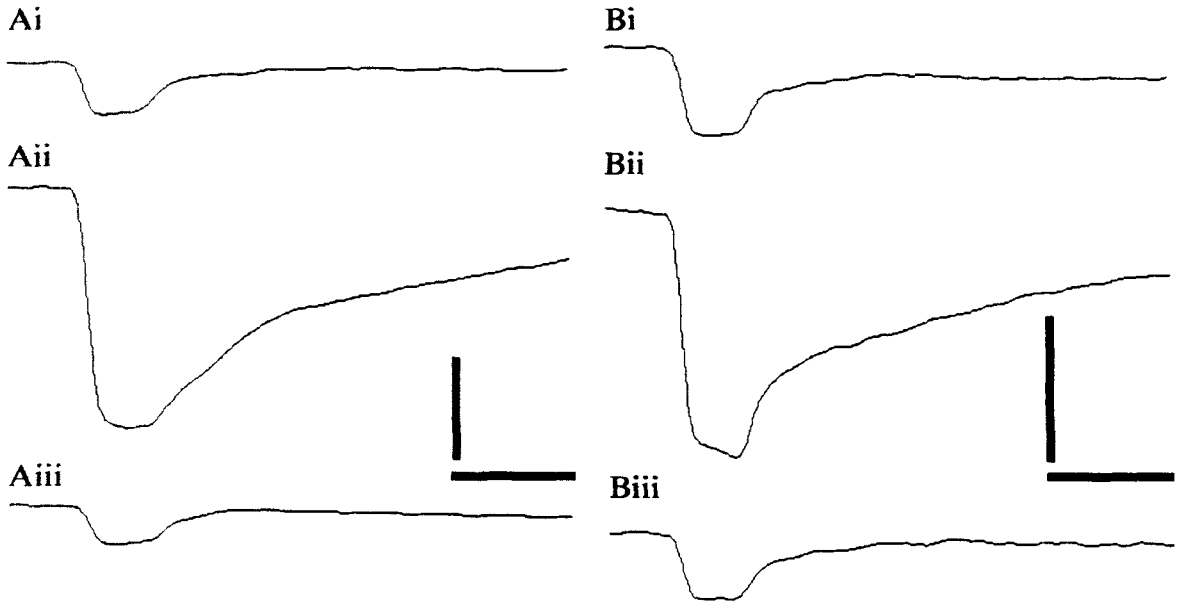

Fig. 1. Electroantennogram recordings from male antennae. (A) Top and bottom responses (Ai and Aiii) to standard, (*E*)-2-hexenal, preceding and following the response to nepetalactone (Aii). (B) as (A) but centre response (Bii) to nepetalactol. Odour dilution at the source was log 2 in all cases. scale bars are 500 μV, 4 s.

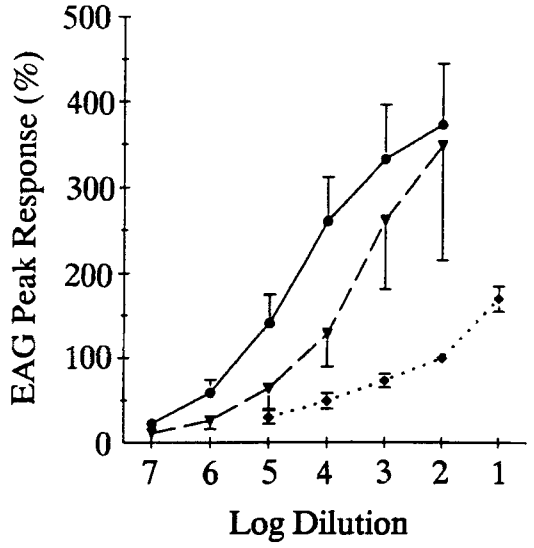

Fig. 2. Dose–response curves of male antennae for the green-leaf volatile, (*E*)-2-hexenal (diamonds, dotted line; $n = 11-16$) and sex pheromone components, nepetalactone (circles, solid line; $n = 15$) and nepetalactol (triangles, dashed line; $n = 12-15$). Bars indicate 95% C.I.

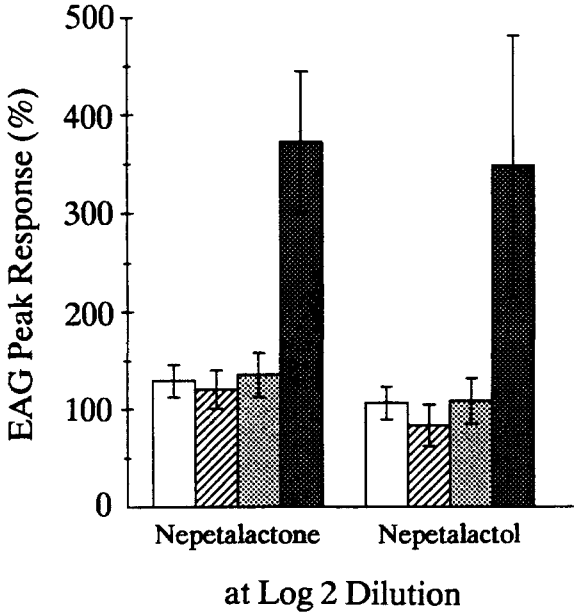

Fig. 4. Comparison of the normalized EAG peak responses of different *Aphis fabae* morphs, wingless virginoparae (outline; $n = 11$ and $n = 11$), winged virginoparae (hatched; $n = 11$ and $n = 11$), gynoparae (grey; $n = 12$ and $n = 12$) and males (dark grey; $n = 15$ and $n = 12$) to the sex pheromone components, nepetalactone and nepetalactol, respectively. Means ±95% C.I.

bers of secondary rhinaria present (Fig. 5). The scanning electron micrograph reveals secondary rhinaria on the third, fourth and fifth antennal segments. Light microscope counts for males indicated 69 ± 1 (mean ±SEM; $n = 21$) secondary rhinaria on the third segments of antennal pairs, 36 ± 1 on the fourth segments and 20 ± 1 on the fifth segments, a total of 126 ± 2 secondary rhinaria on each antennal pair. The antennae of winged virginoparae were counted as 31 ± 1 secondary rhinaria ($n = 27$) on the third segments of antennal pairs, 0.8 ± 0.2 on the fourth segments but none on the fifth segments, a total of 32 ± 1 secondary rhinaria per antennal pair. Wingless virginoparae possessed no secondary rhinaria.

The peak EAG responses of winged virginoparae to five other plant volatiles were compared with the responses of

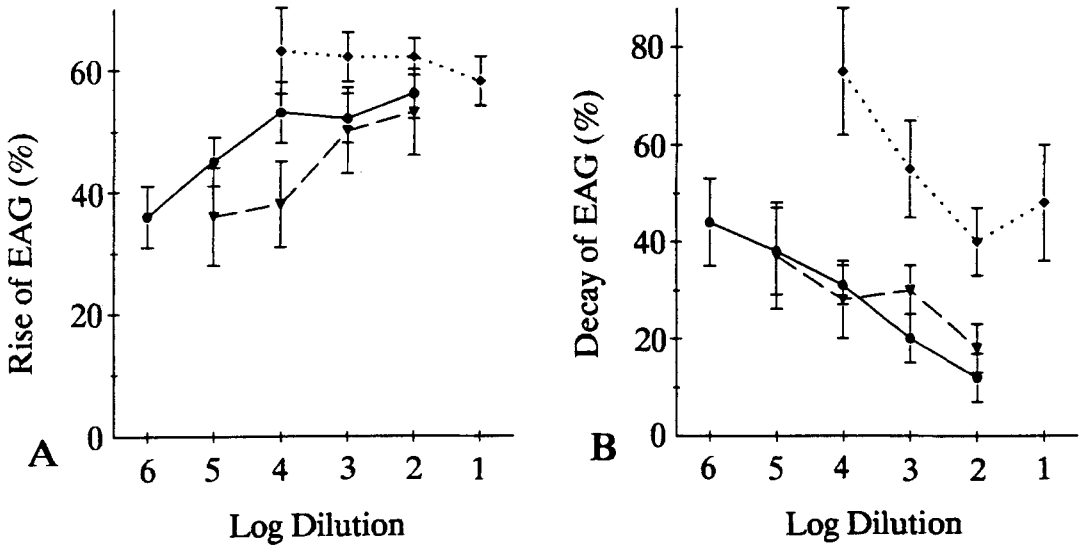

Fig. 3. Dose–response relationships of rise (A) and decay (B) for the green-leaf volatile, (*E*)-2-hexenal (diamonds, dotted line; $n = 11-14$) and sex pheromone components, nepetalactone (circles, solid line; $n = 15$) and nepetalactol (triangles, dashed line; $n = 12-15$). Bars indicate 95% C.I.

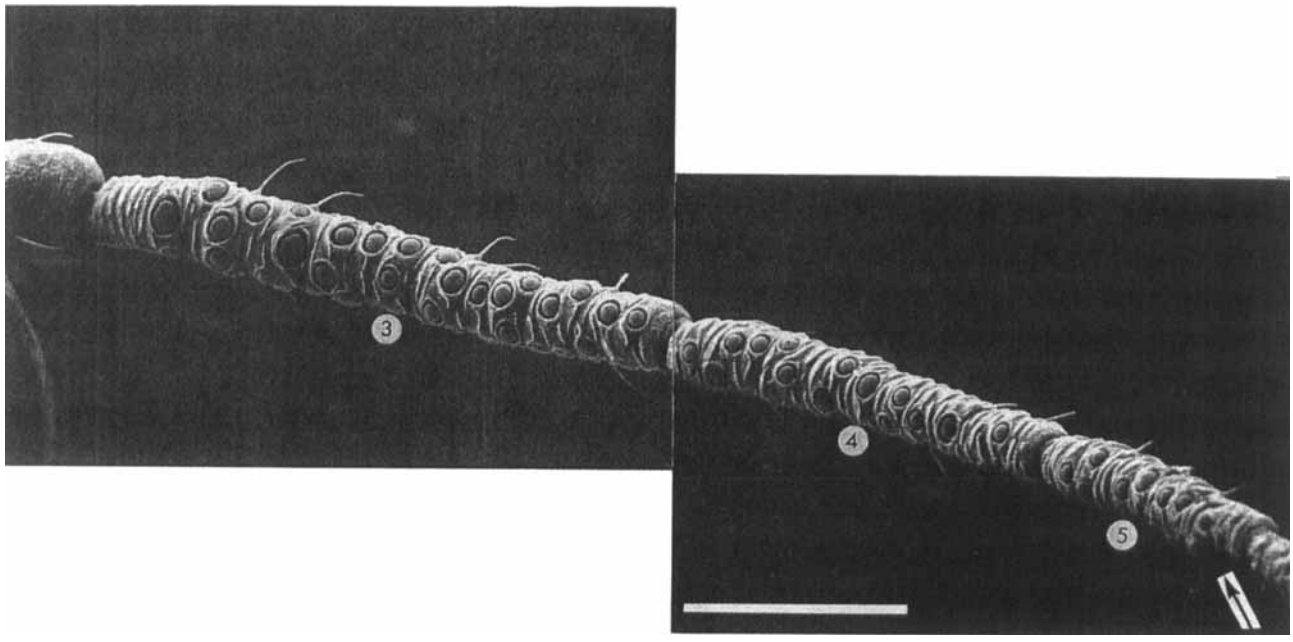

Fig. 5. Scanning electron micrograph of the antenna of a male *Aphis fabae* showing the secondary rhinaria present on the third, fourth and fifth segments. Arrow indicates the position of the proximal primary rhinarium. Scale bar = 0.1 mm.

males (Fig. 6). No differences were detected between the two aphid forms for the EAG peak responses to the plant volatiles.

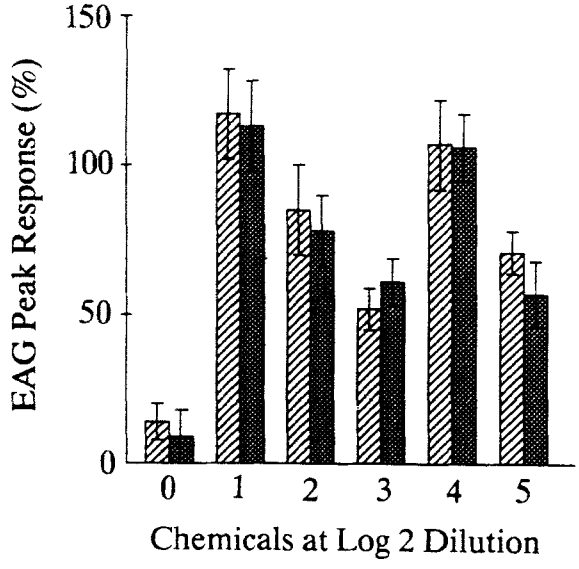

Fig. 6. Comparison between normalized EAG peak responses of winged virginoparae (hatched; $n = 12-19$) and males (dark grey; $n = 10-13$) of *Aphis fabae* to plant volatiles. Key: 0 ± paraffin oil blank; 1 = (*E*)-2-hexenol-1; 2 = (*Z*)-3-hexenol-1; 3 = (*Z*)-3-hexenyl acetate; 4 = hexanal; 5 = allyl isothiocyanate. Means ±95% C.I.; response to the standard (*E*)-2-hexenal is 100% in all cases.

Discussion

Under the present experimental protocol, EAG responses are described in relation to a bracketing standard response. A comparison between EAGs from different adult forms is valid as antennae from males and the asexuals produce EAGs of similar absolute values to (*E*)-2-hexenal at log 2 dilution. The peak value of 220 ± 60 μV (mean ±95% C.I.) recorded from male antennae is not significantly different from those recorded from asexual forms (270 ± 90 μV from wingless virginoparae; 220 ± 60 μV from winged virginoparae; 190 ± 60 μV from gynoparae) (Hardie *et al.*, 1994).

Experiments involving removal of antennal segments indicated that the secondary rhinaria of male aphids were responsible for perception of sex pheromone (Pettersson, 1971; Marsh, 1975) and, more recently, single-cell recording techniques have confirmed that, in males, they contain olfactory neurones responsive to the sex pheromone components, nepetalactone and nepetalactol (Dawson *et al.*, 1987, 1990). The present recordings from whole antennae of asexual female forms of *A.fabae* show that they, also, can perceive sex pheromone although they are much less sensitive than the male antennae. Indeed, individual cells responding to the two components have been found in the primary rhinaria of alate virginoparae of several species including *A.fabae*, as well as in secondary rhinaria of other asexual forms (C. M. Woodcock, unpublished). However, the similarity in response by winged and wingless virginoparae and gynoparae indicates that, in these forms, the primary rhinaria may provide the major component to the EAGs as the wingless virginoparae have no secondary rhinaria. Although there are no records of virginoparae

responding behaviourally to sex pheromone, there is evidence that gynoparae of some species may use the sex pheromone as an aggregation pheromone (Campbell *et al.*, 1990; Hardie *et al.*, unpublished). The composition of the sex pheromone released by oviparae of *A.fabae* comprises a blend of the nepetalactone and nepetalactol isomers used in the present experiments in a ratio of 29:1 (Pickett *et al.*, 1992) and males show maximal behavioural responsiveness to this blend (Hardie *et al.*, 1990). The high sensitivity of the antennae of male *A.fabae* to these sex pheromone components undoubtedly reflects a specialization for mate location.

It is noteworthy that the rise and decay parameters of the EAG responses in male *A.fabae* are smaller for the sex pheromone components than for the green-leaf volatile (*E*)-2-hexenal. These parameters reflect the time course of the EAG. Rise is proportional to transport of the odour molecules to the receptors in the dendritic membranes of the olfactory neurones, whereas decay is related to the inactivation via release/removal from the receptors and metabolism (see Dickens *et al.*, 1993). In other words, the antennae of male aphids have a slower response to the sex pheromone components than to the plant volatile. The opposite, however, occurs in the EAGs of male *Spodoptera exigua* moths which repond much faster to sex pheromone components released by *S.exigua* females than to green-leaf volatiles (Dickens *et al.*, 1993). It appears that the process of sensory transduction underlying pheromone perception differs between aphids and moths.

Although the males appear specialized for locating the oviparae, within the limits of the compounds tested in the present work, their antennae are as responsive to plant volatiles as are the antennae of asexual forms. That is, the EAG responses were similar for the general green leaf volatiles which are found in many plant species (*E*)-2-hexenal, (*E*)-2-hexenol-1, (*Z*)-3-hexenol-1, (*Z*)-3-hexenyl acetate and hexanal; Visser *et al.*, 1979) as well as the Crucifera-specific allyl isothiocyanate which represents a non-host volatile (Tollsten & Bergström, 1988). For the main part, it appears that the primary rhinaria are the major organs responding to plant volatiles in males (Pettersson, 1970) and asexual forms (Pickett *et al.*, 1992; Van Giessen *et al.*, 1992), but the secondary rhinaria of asexuals also possess receptors for (*E*)-β-farnesene (an aphid alarm pheromone and plant volatile) (Nault *et al.*, 1973) and other plant compounds (C. M. Woodcock, unpublished). The idea that male aphids initially located the host plant and then the oviparae (Steffan, 1987; Guldemond, 1990) has recently been superseded by the finding that male aphids can locate a synthetic sex pheromone source in the field in the absence of host-plant cues (Campbell *et al.*, 1990; Hardie *et al.*, 1992). Nevertheless, there is some evidence that there may be an olfactory interaction between host plant and sex pheromone that improves the efficiency of mate location (Campbell *et al.*, 1990; Hardie *et al.*, unpublished). Indeed, Pettersson (1970) showed that male bird cherry-oat aphid, *Rhopalosiphum padi*, responded in an olfactometer to volatiles from the primary host, *Prunus padus*. This may not be the case for all aphid species because male *Cryptomyzus* spp. showed no response to host-plant volatiles in a similar experiment (Guldemond *et al.*, 1993).

The secondary rhinaria of male *A.fabae* are some 4 times more prevalent than those of the winged virginopara and extend to the fifth antennal segment. These findings confirm earlier reports of Jones (1944) for *A.fabae* and Pettersson (1970) for *R.padi*. There is therefore a correlation between the specialization of the male antenna to respond to the volatile sex pheromones and the number of secondary rhinaria. However, it has to be appreciated that the analogous organs in winged females are seemingly less specialized for the perception of sex pheromone components. There are certainly olfactory cells in the secondary rhinaria that respond to nepetalactone and nepetalactol (see above) but the increased sensitivity in the male is not fully accounted for by the increase in number of secondary rhinaria. The presence of secondary rhinaria in winged females, and the lack or reduced numbers in wingless females, seems more likely to be connected with host plant location (Pickett *et al.*, 1992).

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