PERCEPTION OF PLANT ODOUR COMPONENTS BY THE VETCH APHID *MEGOURA VICIAE*: SHAPE CHARACTERISTICS OF ELECTROANTENNOGRAM RESPONSES

J.H. Visser & P.G.M. Piron

Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands.

Key words: olfaction, semiochemicals, kairomones, transduction.

Summary

Electroantennogram responses to plant volatiles were recorded from *Megoura viciae* apterous virginoparae. The response profile shows a differential sensitivity to general green leaf volatiles, *i.e.*, hexanal, (*E*)-2-hexenal, hexanol-1, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenol-1, (*Z*)-3-hexenol-1, 2-hexanone, and more specific plant volatiles, *i.e.*, *p*-anisaldehyde, ß-pinene, linalool, citronellal, hexanonitrile, 1,6-hexanedithiol. Hexanonitrile, an expected repellent, elicits the strongest response. Shape characteristics of the electroantennogram recordings reveal component-specific sensory transduction.

INTRODUCTION

Host-plant selection by phytophagous insects involves a concatenation of insect behaviours such as orientation, landing, acceptance, nutrition and reproduction. The ultimate selection of a host plant is, therefore, regarded as the outcome of a whole series of insect responses to the complex set of physical and chemical plant qualities. In aphids, the role of plant odours in orientation and landing has been denied for a long time, but nowadays evidence is accumulating in support of the substantial function of plant odours in host-plant selection by aphids (Pickett *et al.*, 1992).

The antennae of aphids bear olfactory sensilla, called rhinaria, which respond to pheromones as well as plant odours. Except for a few studies (see refs. in Pickett *et al.*, 1992), we hardly know which plant odour components are perceived by aphids and, thus, release particular behaviours. For that reason we studied the responses of the vetch aphid, *Megoura viciae* Buckton, to a collection of volatiles representing a wide range of plant odours. From isolated heads, we recorded electroantennogram responses (EAGs) to plant volatiles. The EAG reflects the summation of receptor potentials which arise on stimulation of the olfactory sensilla. In addition, we measured the different shape characteristics of EAGs in order to quantify the events underlying sensory transduction.

MATERIAL AND METHODS

Insects. The *M. viciae* rearing was started with one virginopara from the Lees clone (UK). Aphids were continuously reared on broad bean plants *Vicia faba* L. *cv.* Minica at a day-night cycle of 17 hours light, 19°C, 60% RH, and 7 hours dark, 16°C, 60% RH, and reproduced parthenogenetically in these conditions. From this colony apterous virginoparae were used in the experiments.

Plant volatiles. Chemicals were obtained from commercial sources, *i.e.*, Aldrich, Fluka and Roth, and were ≥ 97% pure, except allyl isothiocyanate from Aldrich (95% pure). Chemicals were diluted in paraffin oil (Merck, Uvasol) to 1% v/v.

EAG recording technique. The head of an aphid was cut behind the eyes. One antenna was removed. The tip of the 6th antennal segment of the remaining antenna was cut, leaving the distal primary rhinarium untouched. The recording electrode contacted the cut tip of the antenna while the indifferent electrode was inserted in the base of the remaining head. The electrodes were glass capillaries filled with 0.1 M KCl. Ag-AgCl wires connected the preparation with the recording instruments: Grass HIP16A input probe, Grass P16D amplifier (rise time: 30 ms), Philips PM3302 oscilloscope, Krenz TRC 4010 transient-recorder and Estate PC AT386.

Odour stimuli were prepared by applying 25 µl of the dilution onto a piece of filter paper (60 x 8 mm, S&S 589^2), which subsequently was placed into a Pasteur pipette. Through a tube (internal diameter: 10 mm) air, which was purified and humidified, passed continuously over the preparation at a rate of 40 cm/s (30 ml/s). At a distance of 20 cm from the preparation, odour stimuli were injected into the main stream by passing air at a rate of 1 ml/s through the Pasteur pipette for 2 s. The position of stimulus injection in the main flow caused a delay in arrival of odour stimuli at the preparation of 0.5 s. The onset of stimulus injection triggered the storage of the recording. Data were digitized and stored by the transient-recorder and transferred to the PC. Each antenna was stimulated with a series of plant volatiles at regular intervals of 30 s. In order to compensate for the decrease in responsiveness of the antennal preparation in the course of time, the antenna was stimulated by a standard, *i.e.*, a Pasteur pipette containing 25 µl of (*E*)-2-hexenal diluted in paraffin oil to 1% v/v, prior and past each stimulation by a plant volatile.

Data analysis. Software in ASYST was developed in order to analyse the digitized EAG recording. The recording was corrected for DC drift and smoothed. From each recording the following values were extracted in time periods starting with the onset of stimulus injection at second 0.0: (a) *Peak* is the largest deflection in 0.0-2.5 s (in µV), (b) *Mean Response* is the mean deflection in 1.5-2.0 s (in µV), (c) *Rise* is the mean deflection in 0.0-1.0 s relative to the Mean Response (in %), and (d) *Decay* is the mean decrease of deflection in 2.5-3.5 s relative to the Mean Response (in %). The foregoing time periods were selected in order to counterbalance the 0.5 s delay of stimulus arrival at the preparation after injection onset as well as to quantify the different shape charactistics of EAG recordings. *Normalized Peak* responses were calculated relative to the responses of adjacent standards (in %).

RESULTS AND DISCUSSION

Specificity. The normalized EAG peak responses to the array of plant volatiles shows the specificity of the olfactory receptor system in *M. viciae* (Figure 1). In the class of the general green leaf volatiles (Visser *et al.*, 1979), hexanal and (*E*)-2-hexenal elicit the strongest responses, followed by, in decreasing order, hexanol-1, (*Z*)-3-hexenyl acetate, (*E*)-2-hexenol-1, (*Z*)-3-hexenol-1 and 2-hexanone. From this response profile, it is concluded that aldehydes elicit stronger responses than alcohols (hexanal *v.* hexanol-1, (*E*)-2-hexenal *v.* (*E*)-2-hexenol-1) and ketones (hexanal *v.* 2-hexanone), one double bond in the alcohols decreases the response (hexenol-1 *v.* hexanol-1), while an acetate group restores the response ((*Z*)-3-hexenyl acetate *v.* (*Z*)-3-hexenol-1).

The response profile (Figure 1) to the remaining of the plant volatiles, the group being chemically very diverse, shows an additional high sensitivity of the *M. viciae* antenna to *p*-anisaldehyde (4-methoxybenzaldehyde), (-)-(1*S*)-ß-pinene, linalool, citronellal, hexanonitrile and 1,6-hexanedithiol. Small changes in the molecular structure of plant volatiles strongly affect their perception by *M. viciae* olfactory sensilla (benzaldehyde *v. p*-anisaldehyde, geraniol *v.* linalool, (+)-citronellol *v.* citronellal). It is striking that the non-host-plant volatile hexanonitrile elicits the strongest response of all volatiles tested. Nitriles are present in the headspace of macerated plant parts of *Brassica* species (Tollsten & Bergström, 1988). Hexanonitrile is, therefore, expected to act as strong repellent for *M. viciae* analogous to the repellency of isothiocyanates for *Aphis fabae* (Isaacs *et al.*, 1992).

Sensory transduction. On hitting the antenna and entering the pores of the olfactory sensilla, the odour molecules are thought to be transported by carrier proteins to the receptor sites in the dendritic membrane. The molecule-receptor interaction initiates ion channels to open, which consequently depolarizes the dendritic membrane and produces the receptor potential. The sum of all receptor potentials in the population of olfactory sensilla, is reflected in the EAG. After stimulation, the odour molecules are inactivated by enzymes. This whole process, which is more complicated than described here, is called sensory transduction and proceeds at the subcellular level in a few seconds (see refs. in Dickens *et al.*, 1993). The shape of the EAG is affected by the three events underlying sensory transduction: (1) Rise of the EAG is proportional to the velocity of odour transport to receptor sites, (2) Peak relates to the number of molecule-receptor interactions, and (3) Decay reflects the speed of inactivation of odour molecules.

From the digitized EAG recordings of *M. viciae*, the mean Rise and the mean Peak values were calculated for each plant volatile (Figures 2 and 3). It is obvious that plant volatiles are differently processed in the course of sensory transduction. Rise can be divided into two classes: one for volatiles which are transported rapidly to receptor sites, and the second for those showing slow transport. Decay can be distinguished in three classes for respectively fast, medium and slow inactivation of volatiles after stimulation. Therefore, it is concluded that the olfactory sensilla of *M. viciae* express, in addition to the differential sensitivity for the array of plant volatiles, component-specific sensory transduction.

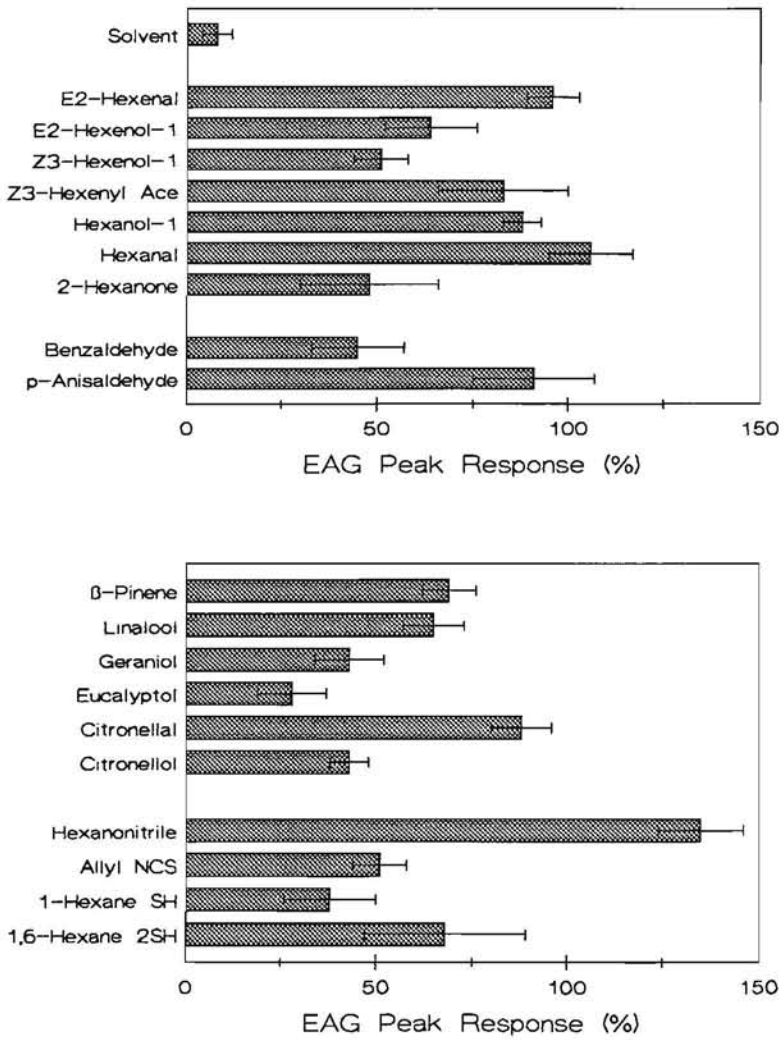

Fig. 1. Normalized EAG Peak responses of apterous virginoparae of Megoura viciae to plant volatiles diluted in paraffin oil (at the source: 1% v/v). Bars show means and 95% confidence intervals (n=9-14).

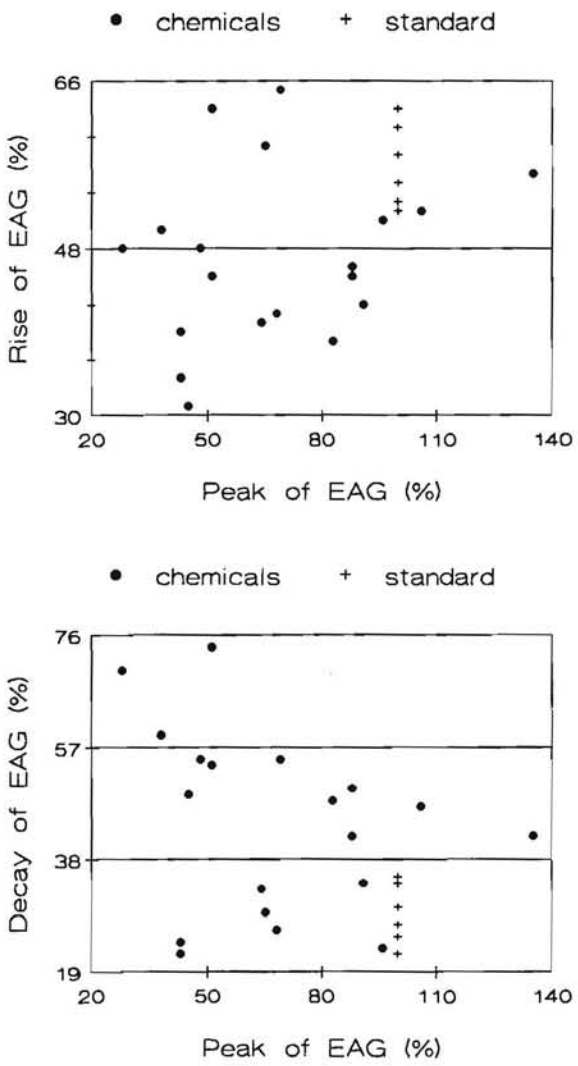

Fig. 2. Shape characteristics of EAG responses of apterous virginoparae of Megoura viciae to plant volatiles (volatiles listed in Figure 1). Top: mean normalized Peak versus mean Rise of EAG. Bottom: mean normalized Peak versus mean Decay of EAG.

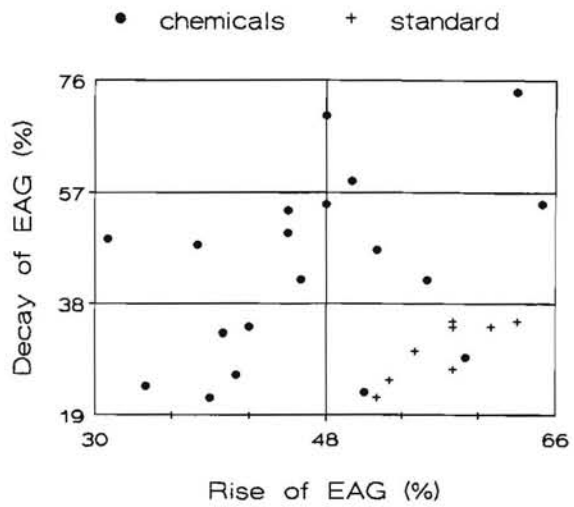

Fig. 3. Shape characteristics of EAG responses of apterous virginoparae of Megoura viciae to plant volatiles (volatiles listed in Figure 1). Mean Rise versus mean Decay of EAG.

Acknowledgements. We thank Marlies Vaz Nunes for correction of the English text.

REFERENCES

Dickens, J.C., J.H. Visser & J.N.C. van der Pers, 1993. Detection and deactivation of pheromone and plant odor components by the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *J. Insect Physiol.* **6**:503-516.

Isaacs, R., J. Hardie, A.J. Hick, L.E. Smart & L.J. Wadhams, 1992. Responses of the black bean aphid, *Aphis fabae*, to a non-host plant volatile in laboratory and field. In: *Proc. 8th Int. Symp. Insect-Plant Relationships* (S.B.J. Menken, J.H. Visser & P. Harrewijn eds):112-114. Kluwer Acad. Publ., Dordrecht, The Netherlands.

Pickett, J.A., L.J. Wadhams & C.M. Woodcock, 1992. The chemical ecology of aphids. *Annu. Rev. Entomol.* **37**:67-90.

Tollsten, L. & G. Bergström, 1988. Headspace volatiles of whole plants and macerated plant parts of *Brassica* and *Sinapis*. *Phytochemistry* **27**:2073-2077.

Visser, J.H., S. van Straten & H. Maarse, 1979. Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado beetle, *Leptinotarsa decemlineata*. *J. Chem. Ecol.* **5**:13-25.