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Selective receptor neurone responses to $E$-$\beta$-ocimene, $\beta$-myrcene, $E,E$-$\alpha$-farnesene and homo-farnesene in the moth *Heliothis virescens*, identified by gas chromatography linked to electrophysiology

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Abstract An important question in olfaction is for which odorants receptor neurones have evolved. In the present study, olfactory receptor neurones on the antennae of the tobacco budworm moth *Heliothis virescens* were screened for sensitivity to naturally occurring plant-produced volatiles by the use of gas chromatography linked to electrophysiology. Volatiles of host as well as non-host plants collected by headspace techniques were used for stimulating the neurones, sequentially via two columns, one polar and one non-polar installed in parallel in the gas chromatograph. Three types of neurones presented in this paper responded to one, two or three compounds for which the retention times were determined in both column types. The chemical structures of the active components were determined on the basis of mass spectrometry linked to gas chromatography, indicating $E$-$\beta$-ocimene and $\beta$-myrcene as stimulants for neurone type 1, $E,E$-$\alpha$-farnesene for neurone type 2 and homo-farnesene for neurone type 3. Re-testing authentic materials verified the identifications for the type 1 neurones. The results demonstrate a high specificity for the three types of neurones by strong responses to one or two structurally similar compounds out of hundreds present in a large variety of plants. The study exemplifies plant odour detection by narrowly tuned receptor neurones in a polyphagous moth species.

Key words Insect-plant interactions · Olfactory receptor neurones · Plant odours · Gas chromatography · Single-cell recordings

Abbreviations DB-5 fused silica gel GC column, methylsilicone, nonpolar type · DB-WAX fused silica gel GC column, polyethylenglycol, polar type · $E$ entgegen (trans-configuration) · EAG electroantennogram · FID flame ionisation detector · GC gas chromatograph · GC-EAD gas chromatography-electro-antennographic detector · GC-MS gas chromatography-mass spectrometry · GC-SCR gas chromatography-single cell recording · TEM transmission electron microscopy · Z Zusammen (eis-configuration)

Introduction

Identification of biologically relevant odorants used by herbivorous insects to locate suitable hosts is a challenging task since plants can produce hundreds of volatile compounds. Furthermore, many are minor constituents that may easily undergo changes. Gas chromatography linked to electrophysiology has been employed in order to determine which of the naturally produced plant compounds are detected by insect olfactory receptor neurones. In an early study, the gas chromatograph (GC) was linked to recordings of electroantennograms (EAGs) which resulted in the identification of six plant substances important for the carrot fly *Psila rosae* (Guerin et al. 1983). In other studies of host odours, the GC has been linked to recordings from single receptor neurones (GC-SCR) of several species (Tømmerås and Mustaparta 1987; Blight et al. 1995; Wibe and Mustaparta 1996; Barata 1997; Wibe et al. 1997, 1998; M.H. Bichão et al., unpublished observations). In the pine weevil (*Hylobius abietis*) 30 different types of receptor neurones were classified, each specialized for one or two compounds present in conifer trees, and 47 active substances were identified by linked gas chromatography-mass spectrometry (GC-MS) (Wibe et al. 1997). In these previous studies as well as in an earlier investigation of the moth *Heliothis virescens* (Røstelien 1995), stimulation of the neurones was carried out using one GC column only. In the present
study of host and non-host odours in *H. virescens*, the GC-SCR technique has been elaborated by the installation of two columns in parallel in the GC-oven, with different separation properties. This allowed testing a single receptor neurone for the same odour mixture via both types of columns. It greatly helped to determine which GC-peaks elicited responses. The tobacco budworm moth *H. virescens* is found on a broad variety of plant species including sunflower, tobacco, cotton and tomato (Fitt 1991). The female moths, selecting host plants for egg laying, are assumed to use odours in addition to other sensory cues for locating a suitable host. The identification of the host plant volatile, germacrene D, as a specific cue for one receptor neurone type on the antenna of *H. virescens* females was recently reported by Røstelien et al. (2000). The results presented in this paper include identification of three other functional types of receptor neurones: one type specialized for two structurally similar plant compounds, *E*-β-ocimene and β-myrcene, a second type for *E,E*-α-farnesene, and a third type for a *homo*-farnesene isomer.

**Materials and methods**

**Insects**

The adult females of *H. virescens* used in the present study originated from a laboratory culture at Novartis Crop Protection Rostental (Switzerland). The insects, received as pupae every week, were kept at constant temperature (26.8 °C) with a photoperiod of 14:10 h, opposite to the natural day and night cycle. Emerged adults were placed in separate boxes, and 3- to 5-day-old individuals were used in the experiments.

**Plants**

Several host plant materials were tested, including different strains of sunflower (*Helianthus annuus* L.), from fields and greenhouses in Portugal, Arizona and Norway. Wild tobacco (*Nicotiana tabacum*) was collected in Arizona, tomato (*Lycopersicon esculentum*) from fields in Portugal and from greenhouses in Norway, and wild brier (*Rosa dunali*) from fields in Norway. In addition, volatiles from other plant materials available in the lab were tested as non-host odours. These included samples obtained from sawdust of spruce (*Picea abies*), branches of juniper (*Juniperus communis*; Wibe and Mustaparta 1996) and cut materials of pine (*Pinus pinea*; provided by M.H. Bichão), in addition to samples made of peel from commercially available orange fruits. Several fractions of commercial turpentine from thermomechanical pulp (TMP) were also tested.

**Isolation of plant volatiles**

Naturally produced plant volatiles were isolated using a headspace procedure. Air, filtered through active charcoal, was blown or sucked (40–250 ml min⁻¹) through a glass container covering one or two intact plants or cut plant materials. The lower part of the stem and the pot were not exposed to aeration. A heat-resistant plastic bag was initially used in a few experiments instead of the glass container. Volatile compounds were adsorbed on Porapak-Q (80–100 mesh). The adsorbent was packed in glass-columns.

**Fig. 2** Gas chromatograms of host plant volatiles (A, B) and authentic materials (C) recorded simultaneously with spike activity of a single receptor neurone during stimulation with the eluted compounds via the polar column. Responses of the same neurone to component I and II during elution of volatiles collected from tomato (A) and from sunflower (B). C Responses of the same neurone to β-myrcene and *E*-β-ocimene during elution of a standard solution containing β-myrcene (I), *Z*- and *E*-β-ocimene (IIa and II, ratio 1:2), and α- and γ-terpinene (α−, γ−). A weak response to *Z*-β-ocimene was also obtained. To the right is shown an expanded section of Fig. 2C, including the molecular structures of the activating compounds. Horizontal arrows indicate the maximum number of spikes per second.