Host odour recognition by the Colorado potato beetle

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STELLINGEN

1. Het is twijfelachtig op basis van elektroantennogrammen conclusies te trekken over taxonomische verwantschap van insekten.

2. De door Kramer beschreven precisie waarmee honingbijen geurconcentraties kunnen herkennen is mogelijk het gevolg van het gebruik van pure geurstoffen.
   Kramer, Physiol. Entomol., 1 (1976) 27-37

3. Concentratie-effecten van geurstoffen op leerprestaties van bijen kunnen de experimenten van Getz en Smith sterk beïnvloed hebben.
   Getz & Smith, J. Comp. Physiol. A, 160 (1987) 239-245

4. De op gevoeligheid voor twee feromooncomponenten te onderscheiden receptorcellen in een sensillum trichodeum van de vruchtbladroller hebben geen karakteristieke verhouding in aktiepotentiaal-amplitudo, in tegenstelling tot wat Den Otter veronderstelt.
   Den Otter, J. Comp. Physiol., 121 (1977) 205-222

5. Het streven elektroantennogram-responsen te vertalen naar gedragsreakties is vooral gebaseerd op optimisme.
   Tichenor & Seigler, J. Insect Physiol., 126 (1980) 309-314
   Dickens et al., J. Chem. Ecol., 9 (1983) 1383-1395

6. Het dragen van een toga tijdens promoties is uit het oogpunt van brandveiligheid af te raden.

7. Leptosomen hebben relatief de meeste ruggegraat.

Stellingen behorende bij het proefschrift:
"Host odour recognition by the Colorado potato beetle" door Ruurd de Jong.

Wageningen, 6 april 1988.

Ruurd de Jong

Host odour recognition by the Colorado potato beetle

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C. C. Oosterlee,
in het openbaar te verdedigen
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van de Landbouwuniversiteit te Wageningen

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CONTENTS

CHAPTER 1. INTRODUCTION - HOST ODOUR PERCEPTION IN THE COLORADO POTATO BEETLE

R. DE JONG, J.H. VISSER, W.H. FRENTZ & H.M. SMID

The importance of olfaction in the initial orientation phase in host selection by phytophagous insects is by now well-appreciated (Visser, 1986). It has been reported for several insect species that the odour of a host plant influences orientation behaviour. In the Colorado potato beetle, Leptinotarsa decemlineata Say, the odour of potato plants elicits an upwind locomotory response (Visser, 1976; Visser & Nielsen, 1977). This odour-conditioned anemotaxis increases the probability of the beetle to encounter its host (Visser, 1988).

The composition of most plant odours is very complex. Some phytophagous insects identify a host odour by the presence of a single specific compound. The flea beetle Phyllotreta cruciferae, for example, is attracted by allylisothiocyanate, a specific compound of its cruciferous host plants (Feeny et al., 1970). Other insects recognize host odours by the specific ratio of general odour components. Visser & Avé (1978) demonstrated that the so-called 'green odour' is important in the Colorado potato beetles' recognition of the odour of its host, potato. Green odour, which is composed of C-6 alcohols, aldehydes and the derivative acetate, forms a significant part of all leaf odour blends, but the proportions of its individual components show differences between plant species (Visser et al., 1979).

THE OLFACTORY SYSTEM

Insects receive olfactory information by activation of olfactory receptors. Most olfactory receptors are located in sensilla on the antennae. The number of receptors varies considerably between different insect species. The antennae of Manduca sexta and Periplaneta americana contain about 177,000 and 195,000 olfactory receptors respectively (Sanes & Hildebrand, 1976; Boeckh et al., 1984), while the antennal nerve of

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Drosophila _melanogaster_ is composed of 1800 axons (Venkatesh & Singh, 1984).

Virtually all the olfactory sensilla of the Colorado potato beetle's antenna are concentrated on the 5 distal segments (Schanz, 1953). An ultrastructural study demonstrated that about 430 olfactory sensilla are distributed over the third distal segment of the Colorado potato beetle's antenna (Boeckh & Selsam, unpublished data). This represents 28.5% of the whole population of the antennal olfactory sensilla (Schanz, 1953). Most of these sensilla, about 90%, showed 2 dendrites in cross section, while the remaining 10% showed 3 dendrites (Boeckh & Selsam, unpublised data). An estimate based on these data indicates that the Colorado potato beetle's antenna contains about 3200 olfactory receptors.

Electroantennogram recordings showed that the antennal olfactory receptor system of the Colorado potato beetle is tuned to the perception of general green leaf volatiles (Visser, 1979). These recordings reflect the summed receptor potentials of stimulated olfactory receptors. Individual receptors react differentially to the applied stimuli and show a continuum in their response spectra (Ma & Visser, 1978; Visser, 1983). The sensitivity of the receptors to green leaf volatiles indicates an important role of these compounds in host odour recognition by the beetle.

Information from the olfactory receptors is directly conducted to the brain via receptor axons. These axons terminate in glomeruli, spheroidal neuropile structures in the deutocerebrum, where synaptic contacts are made with interneurones. The number of glomeruli is usually invariable within species and sex (Rospars, 1983), and numbers have been reported varying from 19 in female _Drosophila_ _melanogaster_ (Stocker et al., 1983), to about 1000 in _Locusta_ _migratoria_ (Ernst et al, 1977). Projection patterns of antennal receptors of female Colorado potato beetles, obtained by cobalt chloride fillings of antennal nerves, reveal the presence of about 25-30 glomeruli in the antennal lobe (Fig. 1).

Projection patterns of sensilla of _Drosophila_ _melanogaster_, predominantly reflect the type of sensillum rather than its
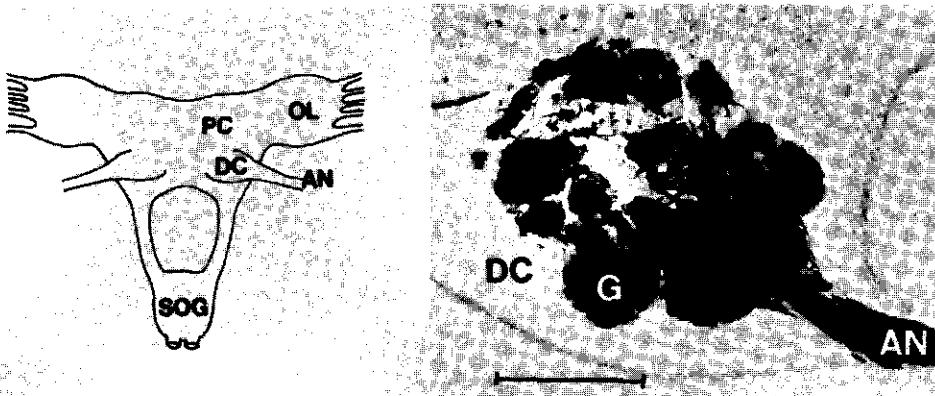
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Fig. 1 Diagram of the nervous system in the head of an adult
Colorado potato beetle (left), and section through the deutocerebrum of a
female Colorado potato beetle (right), showing the projection areas of sensory
afferents. Cut end of antenna was kept in a 1% cobalt chloride solution for 1
h, followed by treatment with ammonium sulfide, fixation in alcoholic Bouin's
solution and silver intensification (Timm's method). AN, antennal nerve; DC,
deutocerebrum; G, glomerulus; OL, optic lobe; PC, protocerebrum; SOG,
suboesophageal ganglion. Bar represents 50 μm.

location on the flagellum, suggesting that individual glomeruli
might represent functional units in the deutocerebrum (Stocker et
al., 1983). Interneurones which are stimulated as a result of the
perception of female sex pheromone components innervate the
macroglomerulus, a deutocerebral neuropile structure which is
found exclusively in males. It has been demonstrated for Manduca
sexta that a trans-sexually grafted male antenna induces the
formation of a deutocerebral structure resembling the
macroglomerulus in a gynandromorphic female (Schneiderman et al.,
1982). These gynandromorphic females show behavioural responses
to pheromones (Schneiderman et al., 1986).

A number of deutocerebral interneurones, the so-called output
neurones, have their axons running to the protocerebrum, where
they terminate in 2 neuropile structures, i.e. in the mushroom
bodies and in the lobus lateralis protocerebralis (Boeckh et al.,
1984; Matsumoto & Hildebrand, 1981). In these structures
olfactory information is integrated with other sensory input,
like visual, taste and mechanosensory information (Erber &
Menzel, 1977; Homberg, 1984; Schildberger, 1981). The number of

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deutocerebral output neurones is relatively small compared to the
number of input antennal fibres. In the cockroach <u>Periplaneta</u>
<u>americana</u>, for example, each antenna contains 195,000 olfactory
receptors, and only 260 output neurones leave the deutocerebrum
(Boeckh et al., 1984). The convergence of olfactory information
causes an increase in the sensitivity of deutocerebral neurones,
compared to the sensitivities of individual receptors. Boeckh &
Selsam (1984) reported a 100-fold amplification of the signal in
pheromone sensitive interneurones in the American cockroach. The
number of output neurones in the Colorado potato beetle is still
unknown, but the 100 to 1000-fold increase in sensitivity of
deutocerebral neurones (De Jong & Visser, 1988) originates from
the input convergence in its deutocerebrum.

BEHAVIOURAL RESPONSE TO OLFACTORY STIMULATION

    Visser & Avé (1978) reported a disruption in the odour-
conditioned anemotaxis of Colorado potato beetles, when the
ratios between components of the potato leaf odour were changed
artificially. We extended these experiments with 5 green odour
components, i.e. cis-3-hexen-1-ol, trans-2-hexenal, cis-3-hexenyl
acetate, trans-2-hexen-1-ol and 1-hexanol, and recorded the
beetle's response on a locomotion compensator in front of a wind
tunnel. The experimental set-up has been described previously
(Visser, 1976; Thiery & Visser, 1986), and a more detailed
description of this equipment will be presented elsewhere (Visser
& Thiery, in prep.).
    We used 2-day-old female beetles from our laboratory stock
culture, which were fed for 2 h on potato foliage and then
starved for at least 12 h prior to the experiments. Locomotory
responses to wind (situation A), wind + potato leaf odour
(situation B), and wind + potato leaf odour + 1 green odour
component (situation C), were recorded for 4 min each. Green
odour components were added by flowing air (1 l/min) over 1 ml of
the test chemical, which was diluted in paraffin oil (10^{-3} v/v),
and with a contact surface with the air of 1.23 cm^2. The
container with the test chemical was placed in the wind tunnel
between the 6 potato plants standing in the dark upwind section

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Table 1. Orientation responses of female Colorado potato beetles to (A) wind, (B) wind + potato plant odour, and (C) wind + potato plant odour + 1 green odour component. Upwind orientation expressed as the quotient of upwind displacement and the total length of the walking track; data represent group means.

Experiment		A: Wind	B: A + Host odour	C: B +	Compound
1	N=35[1]	0.611 a[2]	0.792 b	0.463 a	cis-3-Hexen-1-ol
2	N=34	0.496 a	0.787 b	0.484 a	trans-2-Hexenal
3	N=35	0.546 a	0.731 b	0.445 a	cis-3-Hexenyl acetate
4	N=35	0.562 a	0.794 b	0.382 a	trans-2-Hexen-1-ol
5	N=35	0.608 a	0.862 b	0.750 a	1-Hexanol

[1] Number of beetles.
[2] Different letters on a line indicate statistical differences between treatments of an experiment at $P < 0.02$ (2-tailed, Wilcoxon matched-pairs signed-ranks test; Siegel, 1956).

of the wind tunnel. The air flow leaving the wind tunnel was set at 80 cm/s (4320 1/min). Upwind orientation is expressed as the quotient of upwind displacement and the total length of the walking track.

Addition of green odour components prevented the release of a positive anemotactic response of the beetle, demonstrating the importance of these 5 compounds in the recognition of host plant odour. No significant differences were observed between responses to situations A and C (Table 1). Thiery & Visser (1986) obtained similar results when odour of potato plants was combined with odour of wild tomatoes or cabbage. Towards the latter odour blends, the beetles did not show odour-conditioned anemotaxis, their responses were identical with those towards odourless wind. These results illustrate the specificity of the chemical message emanating from the host plants.

PRESENT ANALYSES

The composition of plant odours is analyzed by the Colorado potato beetle's olfactory system. Behavioural evidence suggests that this system can distinguish the characteristic ratios of

green odour components in potato plant odour from those in other plant odours. The study presented in this thesis was undertaken to investigate the coding mechanism and central processing underlying host odour recognition in the Colorado potato beetle. Responses of neurones to stimulation with volatiles were recorded at 2 levels. Chapter 2 describes responses of deutocerebral neurones to stimulation of the antenna with 5 green odour components and the odour of a paraffin oil extract of potato leaves. Chapters 3 and 4 report on effects of odour mixtures on the olfactory receptors. The effects of feeding experience on the perception of olfactory information is described in Chapter 5.

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CHAPTER 2. INTEGRATION OF OLFACTORY INFORMATION IN THE COLORADO POTATO BEETLE BRAIN

R. DE JONG & J.H. VISSER

The processing of olfactory information in the Colorado potato beetle, Leptinotarsa decemlineata Say, was studied by recording responses of olfactory neurones intracellularly in the deutocerebrum. Response characteristics of neurones in this first relay station of the olfactory pathway were measured when the antennae were stimulated with 5 general green leaf volatiles, i.e. cis-3-hexen-1-ol, trans-2-hexenal, cis-3-hexenyl acetate, trans-2-hexen-1-ol and 1-hexanol. These compounds are part of the so-called green odour of potato, whose defined composition is essential for the beetle's host plant finding. The response spectra of deutocerebral neurones can be divided roughly into 2 classes: one class containing neurones which are not very specific for the tested compounds, and another class with highly specialized neurones. Their different responses to a potato leaf extract suggest 2 channels for the processing of olfactory information in the antennal lobe: one channel for the detection of the presence of green leaf odour components, and an other one for an evaluation of the component ratios.

Anatomical studies of the olfactory system in insects revealed that axons of antennal receptors terminate in the antennal lobe of the deutocerebrum. This part of the insect brain is the first relay station in the olfactory pathway. Synaptic connections between receptor neurones and deutocerebral neurones are made in glomerular neuropile regions in the deutocerebrum (Boeckh et al., 1976 and 1984; Ernst et al., 1977; Stocker et al., 1983). These glomeruli are innervated by local interneurones and output neurones. Local interneurones remain with their processes within the deutocerebrum, while output neurones have an axon running to the protocerebrum via the tractus olfactorio globularis (Boeckh et al., 1976 and 1984; Ernst et al., 1977; Matsumoto & Hildebrand, 1981). The axons of the output neurones terminate in two neuropile structures in the protocerebrum, i.e. in the calyces of the mushroom bodies and in the lobus lateralis protocerebralis (Boeckh et al., 1976 and 1984; Ernst et al., 1977).

An important part of the physiological research on this olfactory system has been done with pheromones (Boeckh & Boeckh, 1976; Boeckh & Selsam, 1984; Burrows et al., 1982; Kanzaki &

Shibuya, 1986; Olberg, 1984; Schaller-Selzer, 1984; Waldow, 1977). Studies at the peripheral level of the nervous system showed that pheromone receptors are extremely narrowly tuned in their sensitivity to chemical compounds, and are specialized in the detection of certain pheromone components (Boeckh et al., 1965). Information from these receptors seems to be processed separately from other olfactory information in several insect species. Deutocerebral neurones sensitive to pheromone components innervate a macroglomerulus (Boeckh & Boeckh, 1979; Boeckh et al., 1984; Burrows et al., 1982; Matsumoto & Hildebrand, 1981; Olberg, 1984), a neuropile structure which is found exclusively in males.

The narrow tuning of pheromone receptors seems to be different from those of food odour receptors, which generally have broad and overlapping response spectra (Visser, 1986). A major problem in the investigation of the processing of food odours is that these stimuli usually have a very complex composition (Selzer, 1981; Visser, 1986; Visser et al., 1979), and that it is not clear which of their components are relevant for their identification. Relatively few electrophysiological studies on food odour processing in the deutocerebrum have been undertaken (Boeckh, 1974; Matsumoto & Hildebrand, 1981; Selzer, 1979; Waldow, 1977; Yamada, 1971). These studies involved food odours with an undefined chemical composition, like odours of fruit, cheese and bread, and compounds which are sometimes known as potent stimuli for receptors but which have an unknown behavioural significance.

The Colorado potato beetle, Leptinotarsa decemlineata Say, is an insect species with a very limited host plant range. Its most important host in Europe is potato, Solanum tuberosum L. The beetle can distinguish between a host plant and a non-host plant by differences in their odour composition (Visser & Nielsen, 1977). In previous work the potato plant odour has been analyzed (Visser et al., 1979; Visser, 1983) and the beetles' antennae have been tested for their sensitivity to its pure components (Ma & Visser, 1978; Visser, 1979 and 1983). The olfactory receptors are sensitively tuned to the perception of green odour (Ma & Visser, 1978), which is composed of C-6 alcohols, aldehydes and

the derivative acetate (Visser, 1983). The upwind locomotory response of the beetle which is induced by potato leaf odour, is prevented by artificial changes in concentration ratios of these green odour volatiles in the potato leaf odour (Visser & Avé, 1978). It was concluded, therefore, that the ratio of these C-6 compounds in the plant odour is decisive in the beetle's host plant finding.

In order to investigate the mechanism of potato leaf odour recognition in the beetle, physiological properties of neurones in the antennal lobe were studied. In this report, responses of deutocerebral neurones are described on stimulation with 5 behaviourally important C-6 compounds, namely cis-3-hexen-1-ol, trans-2-hexenal, cis-3-hexenyl acetate, trans-2-hexen-1-ol and 1-hexanol. Responses to the odour of an extract of potato leaves were also recorded. In some cases additional stimulations were performed with an artificial mixture consisting of the 5 C-6 compounds. The results indicate that information concerning stimulus quantity and quality are processed separately.

MATERIALS AND METHODS

One-week-old female beetles from the department stock culture were used in the experiments. An individual beetle was mounted in a stainless steel holder and its antennae were immobilized by tape (Fig. 1). The brain was exposed by removing the part of the head capsule between the eyes. Mouthparts, muscles, fat and the anterior part of the gut were removed. The latter was replaced by a plug of paper tissue. An insect pin through the head capsule helped further to stabilize the brain. The brain's tracheal air supply was not interrupted. The brain was constantly immersed in saline solution and the head was surrounded by vaseline to prevent leaking of this solution. The composition of the saline has been described by Khan et al. (1982). The sheath of the brain was treated with a 2% (w/v) solution of pronase (8 DMC-U/mg, Serva) in saline solution for 20 minutes, in order to enable penetration of the recording electrode. Capillary microelectrodes were made of filament glass. The tip was filled with 5% Lucifer Yellow and the shank with 1% Lithium Chloride. The measured
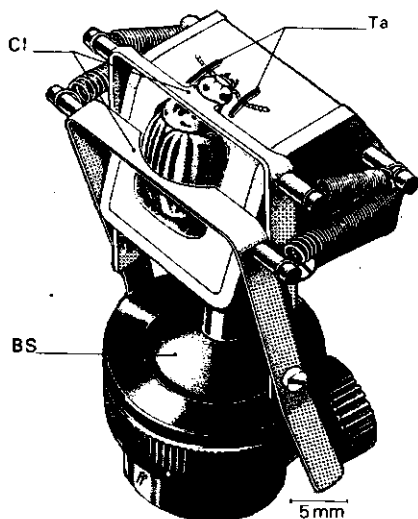
Fig. 1 Stainless steel holder for mounting the beetles during intracellular recordings of deutocerebral neurones. BS, ball-and-socket joint; Cl, clamps; Ta, tape.

resistance was 120-180 MΩ. Recordings were made with the aid of a Winston Electronics amplifier (Model 1090, with a BR-1 Bridge). After finishing intracellular recording, dye was injected by a direct current of 0.3-1.0 nA.

The animal was placed in a continuous and steady flow of air (40 cm/s, 30 ml/s) (Fig. 2). Antennal receptors were stimulated by injection of odour stimuli (1 ml/s for 2 s) into this airstream from pasteur pipettes. The time between 2 stimulations was 30-60 s. The delivery of an odour puff was controlled by an electromagnetic valve. This valve was operated through a timer which also provided a 50 Hz signal as a marker of stimulus application. The pasteur pipettes were loaded with 6.0 x 0.5 cm strips of filter paper on which a 25 μl paraffin oil solution of the test chemical was pipetted. Initially a dilution of 10^{-4} v/v was used, but this was substituted by a dilution of 10^{-5} v/v to prevent overstimulation.

The test chemicals were obtained from commercial sources (Roth, Koch-Light) and were > 97% pure. Five C-6 components of the potato leaf odour complex were used: cis-3-hexen-1-ol, trans-2-hexenal, cis-3-hexenyl acetate, trans-2-hexen-1-ol and 1-hexanol. In addition to these test chemicals, the cells were also stimulated with the odour of a paraffin oil extract of
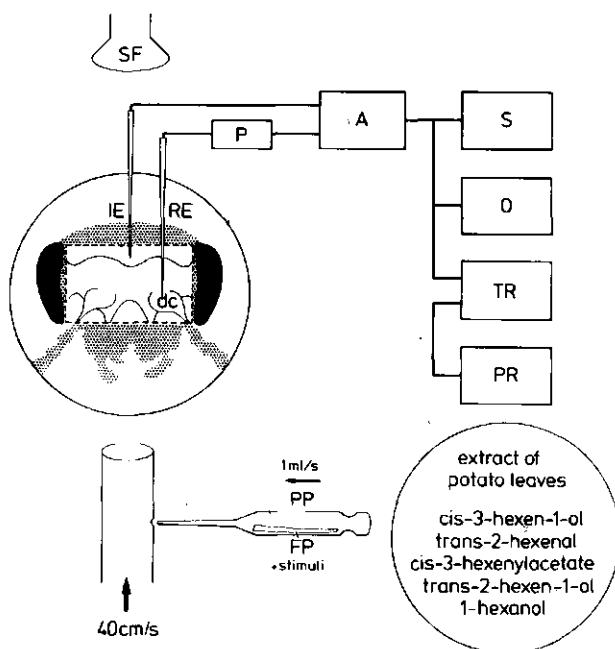
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Fig. 2 Schematic representation of the experimental set-up. A, amplifier; dc, deutocerebrum; FP, filter paper; IE, indifferent electrode; O, oscilloscope; P, probe; PP, pasteur pipette; PR, paper recorder; RE, recording electrode; S, speaker; SF, suction funnel; TR, tape recorder.

potato leaves. This extract was made by blending 40 g potato leaves in the presence of 30 ml water. The product was shaken in 20 ml paraffin oil. The paraffin oil was collected after centrifugation (6000 rpm, 15 min) and stored at 8 °C. This extract contained the potato leaf odour components (Visser, unpubl.). Some neurones were additionally tested with an artificial mixture consisting of the 5 test components in an 1:1:1:1:1 ratio (at a dilution of 10^{-5} v/v). Paraffin oil was used as control. The sensitivity of the neurones to a mechanical stimulus was tested by fluttering the airstream.

The number of spikes in the first reaction second (which corresponds with the stimulation time period t = 0.5-1.5 s) was counted and corrected for the spontaneous firing by subtracting the cell's average firing frequency in the 2 s prior to stimulation. The main change in a cell's activity due to a chemical stimulation was set on a 100%-level in order to obtain

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its relative reaction spectrum for the 5 general green leaf volatiles. Inhibition percentages were related to the mean spontaneous activity of the cell.

RESULTS

The activity of 22 neurones showing responses to the test odours used, was recorded from the deutocerebrum of the Colorado potato beetle. Most of these neurones had a background firing of 3-8 spikes/s, but in some cases it was under that level or as high as 25 spikes/s. The amplitude of recorded spikes was 3-7 mV. Most of the cell recordings lasted for 10-15 min. This was too short a period for an additional Lucifer Yellow staining to reveal morphological details of a cell. However, since the cell somata were marked, it was possible to identify the recorded neurones as deutocerebral neurones. Two fillings seemed to be complete and showed details of cell innervations. The recordings of these cells lasted for about 45 min.

The recorded neurones differed in their response specificity to the stimuli (Fig. 3). Some neurones were highly specific to 1 of the tested components (Fig. 3A,C) while other neurones had a more broadly tuned sensitivity (Fig. 3B,D). We classified the neurones (numbers 1-22) in 4 groups, based on their relative response spectra for the 5 C-6 compounds (Fig. 4).

Group I contains narrowly tuned neurones which were sensitive to cis-3-hexenyl acetate. They showed inhibition responses or relatively weak excitation responses to the other volatiles. The spontaneous spike frequency of those neurones hardly changed after a stimulation with the odour of the potato leaf extract. Mechanical stimulation of the antenna had no effect on these neurones.

Neurones of group II were more broadly tuned. They responded in an excitatory manner to most of the volatiles. The tested alcohols elicited the strongest responses in these neurones. All group II neurones showed a clear and excitatory response to the potato leaf extract. Cell number 8 was the only cell in this group which responded (inhibitory) to a mechanical stimulation of the antenna. This cell was identified as an output neurone by
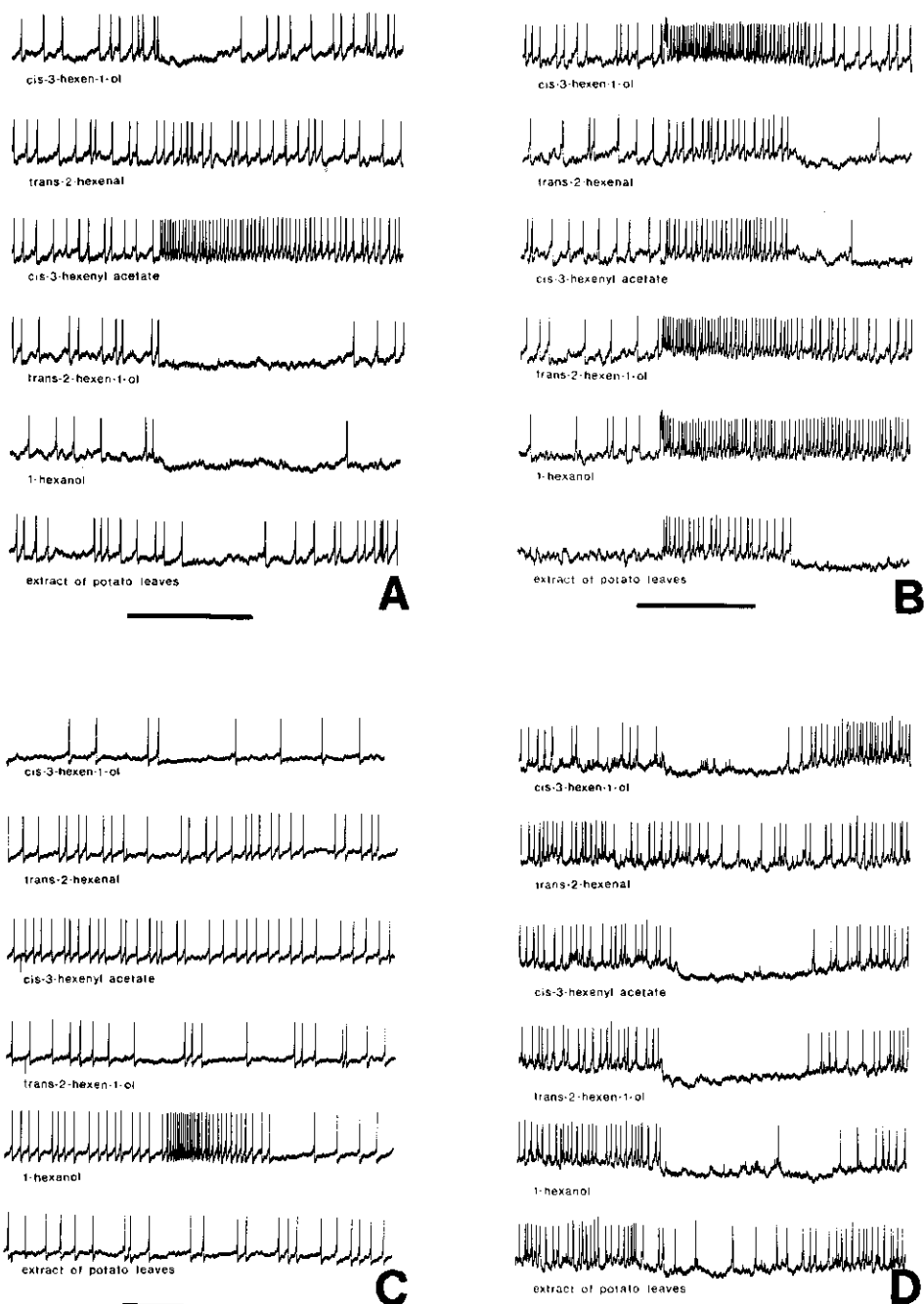
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Fig. 3 Responses of 4 different deutocerebral neurones (A-D) to 5 leaf odour components (at a dilution of 10^{-5} v/v) and to a paraffin oil extract of potato leaves. The stimulus duration (2 s) is indicated by bar. A-D correspond with spectra number 2, 5, 13 and 21 in Fig. 4.

Lucifer Yellow staining.

Group III neurones were sensitive to 1-hexanol. The other tested leaf odour components, including the other alcohols, elicited relative weak responses. The cells placed in this group showed no reaction, or a weak one, to stimulation with the odour of the potato leaf extract. An exception in this respect was cell number 9 which showed a clear excitatory response to the extract. The cells number 10 and 14 showed an exitatory response after mechanical stimulation of the antenna.

Neurones with an inhibition reaction as the most significant response were placed in group IV. The strongest reactions in group IV neurones were caused by stimulation with the alcohols. These cells showed a clear inhibitory response to stimulation with the odour of the potato leaf extract. Cell number 17 was the only neurone of this group which showed only a very weak inhibition to stimulation with the extract. Cell number 16 was identified as a local interneurone.

The neurones number 4, 5, 6, 13, 16, 18, 19, 20 and 22 were additionally tested with the artificial mixture. The neurones number 4, 5 and 6 of group II showed an excitatory response at levels of 80%, 74% and 50% respectively when compared with the response to their 'best' compound. The neurones number 16, 18, 19, 20 and 22 of group IV responded with a complete inhibition of their spontaneous activity. Neurone number 13 of group III showed an inhibition response to this mixture at a level of 24% when compared with the response to its 'best' compound.

The Colorado potato beetle's deutocerebral neurones can be divided roughly into 2 physiological classes: one class of neurones which are narrowly tuned and do not respond clearly to the extract of potato leaves, and another class of more broadly tuned neurones which show an evident response to the potato leaf extract.

DISCUSSION

The Colorado potato beetle's receptor cells investigated by Ma and Visser (1978) and by De Jong and Visser (1988) have been stimulated with higher stimulus concentrations than the neurones
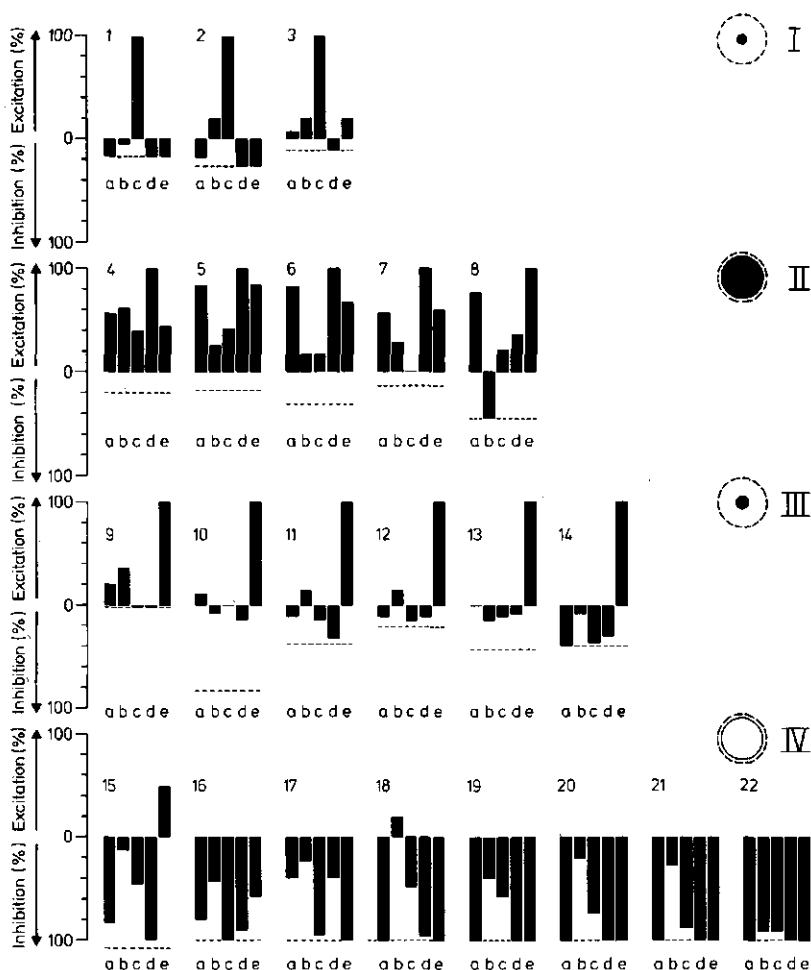
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Fig. 4 Relative reaction spectra of 22 neurones of the Colorado potato
beetles' deutocerebrum for 5 potato leaf odour components. a,
cis-3-hexen-1-ol; b, trans-2-hexenal; c, cis-3-hexenyl acetate; d,
trans-2-hexen-1-ol; e, 1-hexanol. At the stimulus source filter papers
contained the chemicals at a dilution of 10^{-5} v/v, except for spectra number
3, 9, 12, 14, 15 and 17, which were recorded with chemicals at a dilution of
10^{-4} v/v. The broken line indicates the relative level of maximum inhibition
(total inhibition of spontaneous activity). Areas of circles indicate the
average relative responses for each neurone group to a paraffin oil extract of
potato leaves. Filled, open and broken circles indicate excitation, inhibition
and the 100%-level respectively.

at the deutocerebral level in order to elicit clear responses
(10^{2}-10^{3} times higher). An increased sensitivity of deutocerebral
neurones as compared to receptor neurones is a common feature in

insects (Boeckh & Boeckh, 1979; Boeckh et al., 1984; Olberg, 1984). This effect is due to convergence, caused by the connection of a large number of receptors with the same interneurone (Boeckh et al., 1984).

Inhibitory responses in interneurones, like the responses of group IV neurones (Fig. 4), have been reported for other insect species as well (Boeckh, 1974; Matsumoto & Hildebrand, 1981; Yamada, 1971). It has been suggested that inhibition responses in the olfactory circuitry will lead to a better signal-to-noise ratio in the processing of olfactory information (Boeckh, 1974; Harrow & Hildebrand, 1982; Masson, 1977). Improvement of the signal-to-noise ratio might be the function of the group IV neurones from which we recorded. Their response spectra are more or less the mirror image of those of group II. Both groups consist of more broadly tuned interneurones. Most of these neurones gave the strongest responses when there was stimulation with an alcohol, and were sensitive to stimulation with the potato leaf extract.

Groups I and III (Fig. 4) contain neurones which were narrowly tuned to one of the potato leaf odour components. Neurones with similar response spectra as the interneurones of group I have been found recently at the periphery (De Jong & Visser, 1988). In lobsters highly specialized cells have been found at different neuronal levels (Derby et al., 1984). Narrow-spectrum interneurones in lobsters are thought to have an important function in coding, either by dominating the across-fibre pattern for that stimulus or by the formation of labelled lines (Derby & Ache, 1984; Derby et al., 1984). The highly specific responses of the Colorado potato beetle's group I and III interneurones suggest a similar role for these neurones. They might obtain information about the presence of a particular compound in a mixture. However, since there was no clear response to the potato leaf extract, such a role in the specific detection of components by these deutocerebral neurones is not very probable. Neurone number 13, a specialist for 1-hexanol (Figs. 3 and 4), did not show an excitatory response to the artificial mixture, but was stimulated by 1-hexanol in a pure form. Here the response of the cell to a single compound was apparently also inhibited in the

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presence of other compounds. Mixture suppression therefore could
be an important feature of these narrowly tuned interneurones.
Since the level of excitation in the narrowly tuned neurones
seems to depend not only on the presence of the stimulus to which
they are tuned, but also on the presence of other chemicals,
these neurones might process information about the composition of
an odour blend.

Our results suggest a warning function for this class of
narrowly tuned neurones in the Colorado potato beetle: there is a
response if certain stimulus component ratios differ from those
in potato leaf odour, and there is no response if these ratios
are similar.

A simplified hypothetical diagram for the mechanism of host
plant odour recognition in the Colorado potato beetle is
presented in Figure 5. The presence of a mixture of components
can be detected by the antennal receptors. These receptors
transfer information to the deutocerebrum where 2 classes of
neurones are present: class A neurones which are narrowly tuned
and whose response depends on the composition of the stimulus,
and class B neurones which respond when certain components are
present. There are 3 possible situations:

I: Both neuron classes do not respond. In this case important
leaf odour components are not present and there is no detection
of plant odour.

II: Only class B neurones respond. In this situation there is
a stimulation with green odour components. Since the class A
neurones do not respond, these components are in the correct
ratio, and the beetle detects the presence of potato leaf odour.

III: In situation III both classes respond to a stimulus. This
stimulus therefore contains green odour components in a ratio
which differs from the one in potato leaf odour. This is the
situation when there is stimulation from a plant odour other than
potato.

Evidence from other insect species also demonstrates the
existence of deutocerebral neurones with response levels that
depend on the ratio of certain food odour components, rather than
on the presence of one specific compound. In the hawk moth
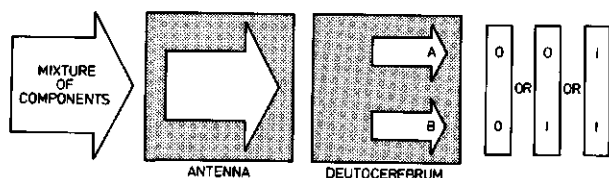Manduca sexta and in the locust Locusta migratoria deutocerebral

Fig. 5 Hypothetical diagram for the mechanism of host plant odour recognition in the Colorado potato beetle. Channels A and B act independently and show a response (1) or no response (0). In this way information concerning stimulus quality (channel A) and stimulus quantity (channel B) is processed separately (see text for further explanation).

neurones have been reported which responded to trans-2-hexenal, a common leaf-aldehyde, but not to the odour of tobacco leaf extract and grass, respectively (Boeckh, 1974; Matsumoto & Hildebrand, 1981). Furthermore, neurones have been found in the antennal lobe of the cockroach Periplaneta americana which were sensitive to hexanol, a constituent of lemon oil (Selzer, 1981), but not to lemon odour (Boeckh, 1974).

Mixture interaction in insect neurones has been described previously (Kaissling, 1979; O'Connell, 1985; Olberg, 1984), and suppression of the response to one chemical by another has been demonstrated to exist at different neuronal levels in the lobster (Derby et al., 1985). Such evidence implies an important role of mixture interactions in the coding of chemical cues. The importance of mixture interaction in the Colorado potato beetle's coding mechanism, could lie in the formation of an information channel with a response level depending on the quality of the stimulus. A study of the antennal receptor responses indicates that this mechanism is also present at the peripheral level of the Colorado potato beetle's nervous system (De Jong & Visser, 1988).

ACKNOWLEDGEMENTS

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CHAPTER 3. SPECIFICITY-RELATED SUPPRESSION OF RESPONSES TO BINARY MIXTURES IN OLFACTORY RECEPTORS OF THE COLORADO POTATO BEETLE

R. DE JONG & J.H. VISSER

Responses of antennal olfactory receptors of the Colorado potato beetle, Leptinotarsa decemlineata Say, to stimulation with 5 general green odour components, i.e. cis-3-hexen-1-ol, trans-2-hexenal, cis-3-hexenyl acetate, trans-2-hexen-1-ol and 1-hexanol, were recorded extracellularly. Response spectra derived from these recordings can not be classified into distinct reaction types. The spectra overlap in their sensitivity to individual stimuli, but there are differences in their degree of specialization with a gradual conversion from generalist to specialist receptors. Moreover, specialization is found to different stimuli. Receptor reactions to stimulation with binary mixtures of 3 of these compounds indicated that suppression of the response to one chemical by another is very common in olfactory receptor cells. The more a receptor is specialized, the stronger is this suppression. Suppression in narrowly tuned olfactory receptor neurones, therefore, is expected to play a fundamental role in the recognition of natural odour blends.

The invertebrate's coding mechanism for olfactory cues has been investigated by examining responses of neurones at various levels of the nervous system (Boeckh, 1974; Derby et al., 1984; Selzer, 1981). Responses of peripheral olfactory neurones in different insect species to various test stimuli have been studied in fair detail. They have been classified into reaction groups on the basis of similarities among the reaction spectra (Boeckh, 1976; Kafka, 1970, Ma & Visser, 1978; Mustaparta, 1975; Selzer, 1984; Vareschi, 1971). Receptors with differences in their degree of specificity and with specializations to different stimuli have been described. Examples of receptors which are extremely narrowly tuned to only one or a few compounds have been given for pheromone receiving neurones in several species (Den Otter, 1977; Kaissling, 1979; Mustaparta et al., 1984; O'Connell, 1985; Sass, 1983). Most food odour receptors, on the other hand, are more broadly tuned in their perception of stimuli (Boeckh et al., 1965; Kafka, 1970; Selzer, 1981; Vareschi, 1971).

Phytophagous insects exhibit specific behavioural responses either to a host odour specific component or to the mixture of

different non-specific host odour components (Visser, 1986). Flea beetles Phyllotreta cruciferae, for example, are attracted by allylisothiocyanate which is a specific component of their cruciferous host plants (Feeny et al., 1970). However, in the Colorado potato beetle, Leptinotarsa decemlineata, the ratio of several odour components is important for host odour recognition. 'Green' odour, which forms a significant part of all leaf odour blends, is composed of C-6 alcohols, aldehydes and the derivative acetate (Visser, 1983). Different plant species may show different proportions of the individual components of the green odour complex. Distortion of the composition of the green odour of potato leaf, Solanum tuberosum, by addition of small amounts of its components to the odour of potato plants, prevented upwind orientation of Colorado potato beetles towards the plants (Visser & Avé, 1978). Furthermore, the odour of their host plant, potato, is masked for the beetles when it is mixed with other plant odours (Thiery & Visser, 1986). Nevertheless, a general problem in studies on olfactory coding is the lack of knowledge about the precise composition of food odour blends. Although most biologically relevant odours consist of complex mixtures, an analysis of olfactory coding, therefore, has been based on neuronal responses to individual odour components (Derby & Ache, 1984; Selzer, 1981; Visser, 1986).

The olfactory receptors in the antennae of Colorado potato beetles are sensitively tuned to C-6 compounds (Ma & Visser, 1978; Visser, 1979). In previous work we used 5 green odour components, i.e. cis-3-hexen-1-ol, trans-2-hexenal, cis-3-hexenyl acetate, trans-2-hexen-1-ol and 1-hexanol, to study the responses of neurones in the deutocerebrum of the Colorado potato beetle (De Jong & Visser, 1988). In this first relay station, synaptic connections between receptor neurones and interneurones are formed. Characterization of deutocerebral neurones revealed the existence of 2 groups: one group (A) of neurones showed specific responses to individual leaf odour components, and another group (B) of less specific responding neurones. After stimulation with a potato leaf extract, group B neurones responded, while group A neurones hardly changed from their spontaneous firing activities.

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Since the potato leaf extract is a mixture of odour components, the lack of response in group A to this mixture implies an important role of suppression as a mixture effect. A coding mechanism for the odour of potato leaf has been pr posed on the basis of this suppression (De Jong & Visser, 1988).

The present study was carried out to characterize the receptor responses to stimulation with the same 5 C-6 components. In order to examine if mixture interactions like those in specialized deutocerebral neurones also exist at the peripheral level, we additionally characterized the olfactory receptors by their responses to some artificial mixtures.

MATERIALS AND METHODS

Two-day-old female Colorado potato beetles were obtained from the laboratory stock culture and used for the experiments. Recordings from antennal olfactory receptor cells were made as described previously (Ma & Visser, 1978). We modified the method of stimulus delivery by placing the preparation in a continuous airflow (40 cm/s, 30 ml/s) in which odour stimuli were injected by flowing air through a pasteur pipette (1 ml/s, 2 s). The pasteur pipette contained a piece of filterpaper (6.0 x 0.5 cm) loaded with a stimulus solution (25 μl). We used as stimuli 5 C-6 components of the potato leaf odour, namely cis-3-hexen-1-ol, trans-2-hexenal, cis-3-hexenyl acetate, trans-2-hexen-1-ol and 1-hexanol (at a dilution of 4 x 10^{-2} in paraffin oil, v/v). The test chemicals were obtained from commercial sources (Roth, Koch-Light) and were > 97% pure. Paraffin oil alone was used as the control. An interstimulus time of at least 1.5 min was used.

The receptors were recorded extracellularly. In most of the recordings, only one spike amplitude was visible. In a few cases we used recordings with two clearly distinguishable spike amplitudes. The number of spikes in the first reaction second was counted in order to obtain the response level to a stimulus.

The relative response spectrum for each cell was obtained by setting its 'best' component on a 100%-level. The degree of specialization of a receptor cell (DS) was calculated by:

$$DS = \frac{500 - \sum_{i=1}^{5} R_i}{4}$$

R_i represents the cell's relative response to component i. This calculation expresses DS as a percentage which, theoretically, ranges between 0 for cells without any specialization, and 100 for cells responding to only one of the 5 test stimuli. Once the degree of specialization of a cell was known, its responsiveness to mixtures was determined. This was done by measuring its reaction to three 1:1 binary mixtures, each at two dilutions of the total amount of volatiles (4×10^{-2} and 8×10^{-2}, v/v). The components used in mixtures were trans-2-hexenal, cis-3-hexenyl acetate and 1-hexanol. Each mixture response was expressed as a percentage of the response to the 'best' component that was also present in the mixture, and from these 6 values an average mixture response (AMR_6) was calculated for each cell. Stimulation sequences started with the lowest concentration stimuli. Variables were compared using the non-parametric Spearman rank correlation test (Siegel, 1956).

RESULTS

Thirty-nine receptor neurones on the antennae of Colorado potato beetles were recorded. Most of these neurones revealed a low background firing rate of 3 ± 4 spikes/s (mean ± sd) which increased to 26 ± 16 spikes in the first reaction second after stimulation with the cell's 'best' component. The response spectra of the receptors were classified in 5 groups, according to their 'best' stimulus (Fig. 1A-E). Some spectra (Fig. 1 nos. 29, 30, 36) do not show a 'best' stimulus and, therefore, can be classified in more groups. Within a group the spectra were arranged according to their DS-values. The degree of specialization in one group increases from left to right. The degree of specialization of the receptors for the 5 stimuli ranges from DS-values of 19 to 94 (Fig. 1, nos. 1 and 28 respectively). We did not find a correlation between the sensitivity of cells to their 'best' component (in number of
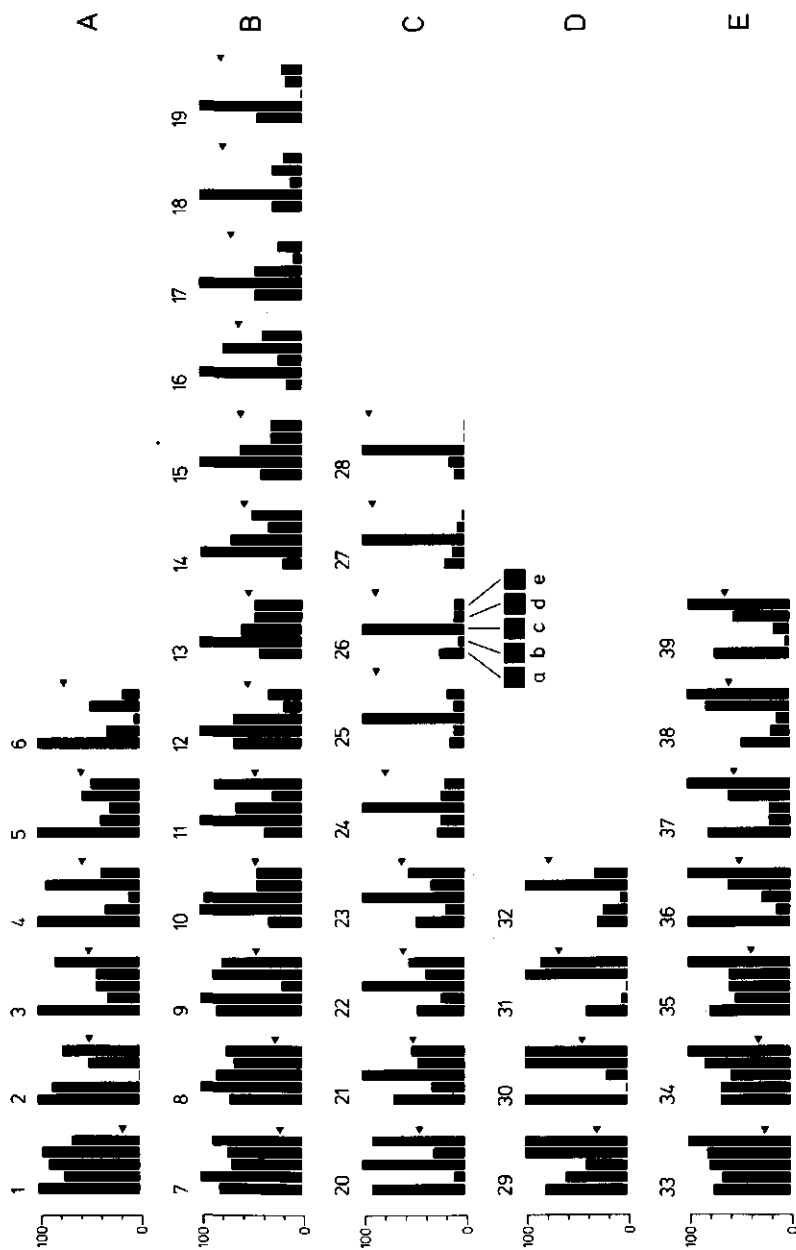
Fig. 1 Relative response spectra of 39 olfactory receptor cells in Colorado potato beetle antennae for 5 leaf odour components. The receptor's 'best' component is 100%. a, cis-3-hexen-1-ol; b, trans-2-hexenal; c, cis-3-hexenyl acetate; d, trans-2-hexen-1-ol; e, 1-hexanol. Chemicals were pipetted on filterpaper at a dilution of 4 x 10^{-2} v/v. Triangle indicates DS value.
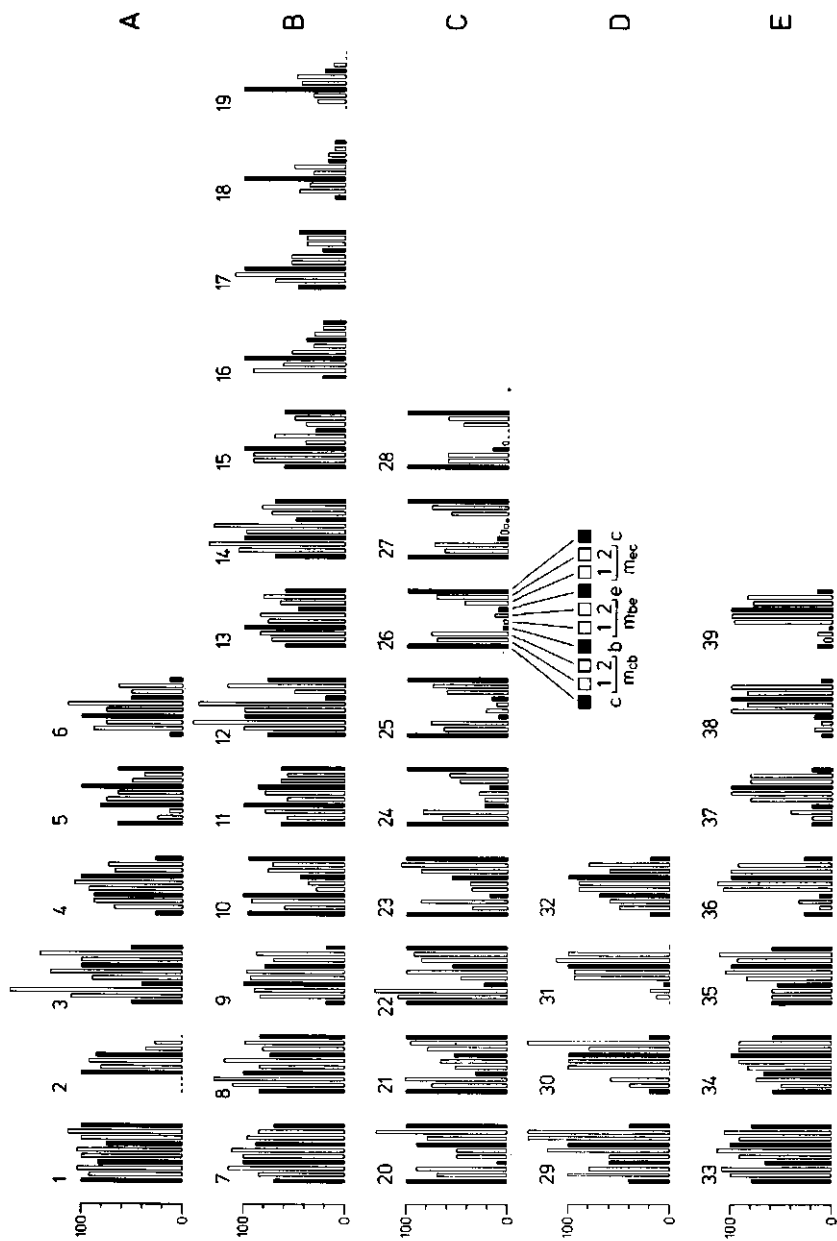
Fig. 2 Relative response spectra of the receptors shown in Fig. 1 for 3 binary mixtures at 2 concentrations, and their individual components. The 'best' component is 100%. c, cis-3-hexenyl acetate; b, trans-2-hexanal; e, 1-hexanol; m_{cb}, 1:1 mixture of c and b; m_{be}, 1:1 mixture of b and e; m_{ec}, 1:1 mixture of e and c; 1, mixture at a dilution of 4 x 10^{-2} v/v; 2, mixture at a dilution of 8 x 10^{-2} v/v. Pure compounds were at a dilution of 4 x 10^{-2} v/v.

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spikes in the first reaction second) and the DS, nor between the background frequency and the DS. The response spectra in Figure 1 show that there are no distinct receptor types to the 5 stimuli. The recorded population of olfactory receptors responded differentially to the set of stimuli and individual spectra show overlap. Nevertheless, considerable differences in receptor responses are found. Some receptors showed non-specific responses, while others were specialized to particular stimuli. In 13 cases out of 39 we were able to repeat the stimulation series demonstrating the consistent character of the spectra. Newly calculated DS values, then, did not differ significantly from previous values (Wilcoxon matched-pairs signed-ranks test) ($\overline{\Delta DS}$ = 5.8).

The responses of the cells in Figure 1 to the binary mixtures are represented in Figure 2, and, in addition, are related to the response to the 'best' mixture component. The classification of these responses in groups corresponds with the one in Figure 1. In 63 of the 117 cases, one component alone elicited a stronger response than the mixture in which the same concentration of that component was present. On the other hand, only in 6 cases the sum of the responses to individual components was smaller than the responses to the corresponding mixtures. Suppression, therefore, is found to be the most common mixture effect. The strength of this suppression varied in the different receptors and was sometimes found to be very pronounced. Figure 3 shows a recording in which clear responses to trans-2-hexenal and 1-hexanol were strongly reduced in the presence of cis-3-hexenyl acetate. The response spectrum of this neurone is represented in Figure 2 no. 2.

The AMR_6 values, which are used as indices for receptors' responses to mixtures, range between 35 and 170 and correlate significantly with receptor DS (r_s = -0.52; N = 39; P < 0.001, 2-tailed). Highly specialized cells show a considerably stronger suppression than the more broadly tuned cells. A scattergram for the AMR_6 and the DS of the receptors is presented in Figure 4.
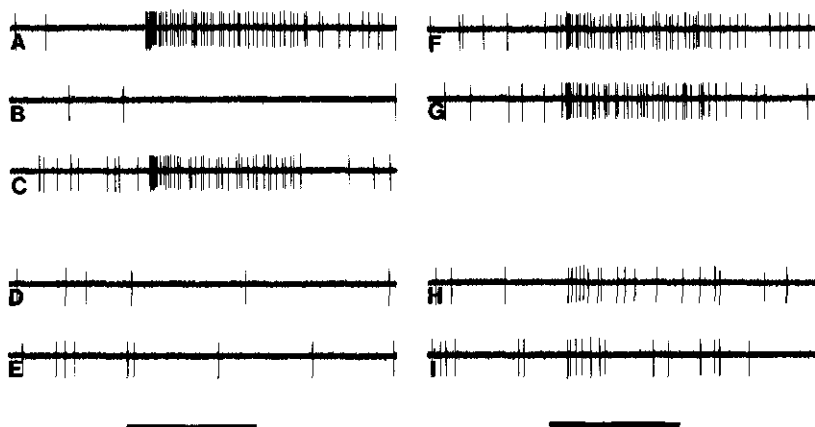
Fig. 3 Responses of one olfactory receptor cell in the Colorado potato beetles' antenna on stimulation with 3 compounds, and their 1:1 mixtures at 2 concentrations. Bar indicates stimulation period (2 s). A, stimulation with trans-2-hexenal (4×10^{-2} v/v); B, stimulation with cis-3-hexenyl acetate (4×10^{-2} v/v); C, stimulation with 1-hexanol (4×10^{-2} v/v); D and E, stimulation with an 1:1 mixture of trans-2-hexenal and cis-3-hexenyl acetate, respectively at 4×10^{-2} and 8×10^{-2} v/v; F and G, stimulation with an 1:1 mixture of trans-2-hexenal and 1-hexanol, respectively at 4×10^{-2} and 8×10^{-2} v/v; H and I, stimulation with a 1:1 mixture of 1-hexanol and cis-3-hexenyl acetate, respectively at 4×10^{-2} and 8×10^{-2} v/v.
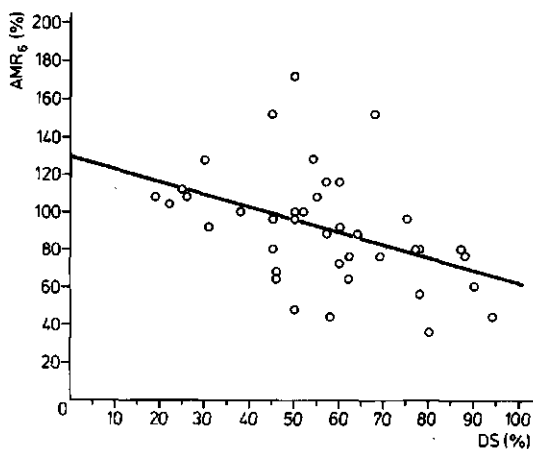

Fig. 4 Scatter diagram for degree of specialization (DS) and average mixture responses (AMR_6) in olfactory receptor cells of the Colorado potato beetle. $r_s = -0.52$; $N = 39$; $P < 0.001$, non-parametric Spearman rank correlation test, 2-tailed (Siegel, 1956).

DISCUSSION

Previous work on deutocerebral neurones indicated that mixture interaction is essential in the coding of olfactory information in the Colorado potato beetle. Here we studied response spectra of receptor neurones to pure compounds, and also examined the perception of more complex odours at the receptor level. Therefore, we additionally stimulated with simple 1:1 binary mixtures. These mixtures do not resemble natural food odour blends, but should be considered as a first step in the study of mixture effects.

Qualitative responses as presented in spectra are thought to give more reliable information about neuronal reactions than quantitative responses (Kafka, 1970) and, therefore, are used for unraveling the coding of olfactory input. Grouping of responses according to spectra have been applied often to characterize an olfactory receptor population. However, since classification of receptors in this way is not only dependent on the treatment of electrophysiological data but also on the set of stimuli used in the experiments, this approach has its limitations (Selzer, 1984). Within groups rather varied spectra can be present while each spectrum shows significant features. The response spectra of olfactory receptors of the Colorado potato beetle (Fig. 1) show varying degrees of receptor specialization and no clear separation in response types for the 5 stimuli. Our data reveal that suppression is an important feature of the response characteristics of receptor cells. The responses of receptors to the binary mixtures suggest a function of mixture effects in the perception of olfactory information. Such mixture interaction has been discussed previously for the processing of information in the antennal lobe of the Colorado potato beetle (De Jong & Visser, 1988).

Olfactory coding is generally thought to be realized either by labelled lines, or by across-fibre patterns. The labelled line coding theory was proposed for insect pheromone receptors which possess narrow, non-overlapping chemosensitivities (Boeckh et al., 1965). Each behaviourally relevant compound is thought to have its own receptor type for the detection of its presence. In
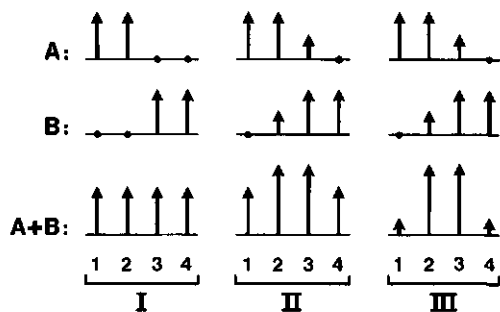
Fig. 5 A schematic diagram representing a population of 4 receptor cells. Receptor cells are thought to respond to compounds A and B applied singly, and the 1:1 mixture of A and B. I: labelled line concept in a cell population with solely specialist receptors. II: across-fibre pattern concept in specialist and generalist receptors. III: as II, involving suppression. The arrow length stands for response intensity.

this theory, the blend composition is determined by the activities in particular types of sensory neurones. A simplified illustration of this situation is presented in Fig. 5(I). Observations on food odour receptors, showing that most receptors have broad and overlapping response spectra (Boeckh et al., 1965; Visser, 1986) led to a second theory, the across-fibre pattern hypothesis. This concept holds that each perceptible odour is represented by a unique pattern of activity across the array of sensory neurones (Fig. 5(II)).

Highly specialized chemoreceptors are supposed to serve as inputs for specific detection systems (Ache & Derby, 1985; Derby et al., 1984; Johnson et al., 1984). In the Colorado potato beetle, the negative correlation between DS and AMR_6, however, shows that the level of excitation in narrowly tuned olfactory receptors in response to mixtures does not solely depend on the presence of their 'best' stimulus (Figs. 3 and 4). As suppression is correlated with the degree of specialization, other chemicals to which these cells do not show a pronounced reaction when applied singly, may contribute to the mixture response. Therefore, the activities of olfactory receptors depend on the total composition of a mixture, and the response pattern differs especially in narrowly tuned neurones from what is expected on the basis of their responses to single compounds (Fig. 5(III)). Similar observations have been made of olfactory receptor responses of other insect species and lobsters. 'Ionine' and 'alcohol' receptor types of the cockroach Periplaneta americana showed a smaller response to odour of lemon oil than was expected

on the basis of summation of responses to odour of lemon oil fractions (Selzer, 1984). Responses of taurine-sensitive receptors on the antennules of the spiny lobster _Panulirus argus_ were suppressed when taurine was presented in mixture with certain amino acids (Gleeson & Ache, 1985). Enhancement and suppression has been demonstrated in responses of narrowly tuned chemoreceptors in the American lobster, _Homarus americanus_ (Johnson et al., 1985). Although pheromone receptors are generally believed to function as labelled lines, synergism and suppression have also been reported in responses of pheromone receptors in the red-banded leaf roller _Argyrotaenia velutinana_ (O'Connell, 1971) and the cabbage looper _Trichoplusia ni_ (O'Connell et al., 1986). Such mixture interactions could explain why sometimes certain behaviourally important compounds, when applied in a pure form, fail to elicit clear activity on any of the identified receptor neurones (O'Connell, 1975; O'Connell et al., 1983).

The response levels in the group of highly specific neurones in the deutocerebrum of the Colorado potato beetle are thought to depend on the ratio of certain odour components, rather than on the presence of their 'best' component (De Jong & Visser, 1988). The other group containing less specific deutocerebral neurones did not show this sensitivity to different ratios. The reactions of receptors presented in this study suggest that peripheral responses are at least partially responsible for this effect. In general, specialized receptors and specialized deutocerebral neurones show suppression as a mixture effect, unlike the other receptors and deutocerebral neurones. The presumed separation of olfactory information by these groups is more obvious at the deutocerebral level than at the peripheral level.

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CHAPTER 4. SUPPRESSION-RELATED MIXTURE PERCEPTION IN OLFACTORY RECEPTORS

R. DE JONG and J.H. VISSER

It is known from psychophysical and electrophysiological research on olfaction that suppression is a common effect of mixture interaction. In a previous study on olfactory receptors of the Colorado potato beetle, a correlation has been found between the specialization of a receptor cell and the amount of suppression in its response to a mixture. Therefore, it has been suggested that suppression leads to a more quality-dependent and less quantity-dependent response in the receptors. Here, the effects of suppression on the perception of mixtures in olfactory receptors of the Colorado potato beetle are further described. It is demonstrated that the amount of suppression in a receptor cell, which is positively correlated with its degree of specialization, depends on the individual receptor rather than the specific stimulus mixture. Angles between mixture components derived from 2 psychophysical models, the vector model and the U model, are characteristic for receptors, and correlate with the receptors' degree of specialization. Suppression correlates with a decreased sensitivity for a concentration difference, and, in theory, provides receptor responses with a buffer for some variations in stimulus quality.

Most natural olfactory stimuli consist of complex mixtures. Odour perception, therefore, depends on the simultaneous reception of a variety of components. However, most research concerning the ways the information about such stimuli is deciphered by an organism, has been restricted to their pure constituents. The major problem in the interpretation of these studies is that mixtures are often perceived differently than one would expect from addition of receptor responses to the individual components.

In psychophysical experiments the perceived intensities of mixtures are usually lower than would be predicted from summing responses to the single components. The principle of odour interaction at the perceptual level has been described by Berglund et al. (1973). They introduced the vector model to describe intensity summation:

$$\psi_{AB} = \sqrt{\psi_A^2 + \psi_B^2 + 2\psi_A\psi_B\cos\alpha} \qquad (1)$$

The perceived intensity of the binary mixture AB is represented

by ψ_{AB}, and of its pure constituents A and B by ψ_A and ψ_B respectively. In this model, the angle α is thought to depend on the qualitative similarities between components of a mixture. The more similar these components are, the smaller is the angle α. This psychophysical model has been tested for humans for 2-component mixtures by Cain & Drexler (1974), Cain (1975), Moskowitz & Barbe (1977), Laffort & Dravnieks (1982) and Laing & Willcox (1983). Although the vector model performs very well for binary mixtures, it has shortcomings when applied in an extended form for more complex mixtures. Such an extended vector model has been tested by Berglund (1974), Moskowitz & Barbe (1977) and Laffort & Dravnieks (1979), and may not be able to predict the odour intensities of 3-component and higher-order mixtures when using α's which are completely determined in binary mixture experiments (Moskowitz, 1979).

The so-called 'U model' which has been proposed by Patte & Laffort (1979) does not suffer from problems for higher-order mixtures:

$$\psi_{AB} = \psi_A + \psi_B + 2\sqrt{\psi_A\psi_B}\cos\alpha \tag{2}$$

The U model has been tested in an extended form for ternary and quaternary mixtures by Laffort & Dravnieks (1982). Moreover, it fits the results for binary mixtures slightly better than the vector model, and includes cases where the perceived intensity of a mixture is stronger than that expected from simple additivity among the components (Laffort & Dravnieks, 1982). Suppression is, nevertheless, by far the most frequently encountered mixture effect in psychophysical studies.

Central mechanisms seem to be involved in mixture suppression. Gillan (1983) reported suppression of perceived odour intensities in odour-taste mixtures. Odour-odour mixtures, however, produced greater suppression than did the odour-taste mixtures. Furthermore, Cain (1975) demonstrated that 2 substances presented separately to each nostril of humans at the same time, produced suppression, but to a lesser degree than the suppression when the 2 substances were mixed in the vapour phase. This suggests that besides effects in the central nervous system, receptors also

contribute to mixture suppression.

Such interactive effects in the perception of odour mixtures at the level of individual receptor neurones have been described for invertebrates. Gleeson & Ache (1985) recorded from taurine-sensitive chemoreceptors on the antennule of the spiny lobster. They found suppression in the receptor responses to taurine when it was applied in combination with certain amino acids. In some cases these responses were even blocked completely.

Suppression and enhancement in the responses of pheromone receptors of different insect species have been reported by O'Connell (1971) and O'Connell et al. (1986). Etcheto et al. (1982) applied psychophysical models to electroantennogram (EAG) recordings from honey bee workers. These recordings are thought to reflect summated responses of the population of olfactory receptors in an antenna. These authors also demonstrated synergy and suppression in the EAG responses to mixtures. De Jong & Visser (1988) described for the Colorado potato beetle a positive correlation in the degree of specialization of the olfactory receptors with the amount of suppression in their responses to binary mixtures.

Electrophysiological experiments indicate that suppression is the most common mixture effect, and that some psychophysical effects originate from peripheral processes. Mayer et al. (1984) suggested that an insect's behavioural response to odour stimulation, reflects the intensity of olfactory sensation in the central nervous system, which in turn would be a measure for the summed responses of receptors. Psychophysical models, therefore, may be relevant to receptor responses by interpreting the psi in the models as the response magnitude.

Little is known about the function of mixture suppression in the perception of olfactory information. Bartoshuk (1975) postulated that the mixture suppression observed in psychophysical taste experiments, plays an important role in encoding a potentially large stimulus concentration range into a much smaller psychological range. Such a mechanism which encodes a broad range of concentrations into a fairly small range of response intensities may be important for lobsters as well (Johnson et al., 1985; Carr & Derby, 1986). De Jong & Visser

(1988) proposed an important qualitative role of mixture
suppression in the recognition of potato plant odour by the
Colorado potato beetle. According to their hypothesis, the coding
of olfactory information involves 2 separate channels. One
channel contains neurones which are highly specialized in the
perception of particular odour components, and at the same time
show strong suppression in their responses to mixtures. The other
channel consists of neurones which are more broadly tuned and
respond to mixtures with less suppression. Broadly tuned
receptors are supposed to detect the presence of compounds, while
information about their ratios in a stimulus mixture is obtained
by narrowly tuned receptors. The response level to mixtures in
narrowly tuned receptors, therefore, is expected to be more
quality-dependent and less quantity-dependent than in broadly
tuned receptors.

    In this report we evaluate data obtained from single cell
recordings of Colorado potato beetles' antennal neurones, in
order to investigate whether the suppression in a receptor
depends primarily upon certain mixture combinations or on
receptor characteristics. Furthermore, the effects of a change in
stimulus concentration on the responses of these receptors are
studied. The role of suppression in narrowly tuned receptors,
with respect to olfactory coding, is discussed with use of the
psychophysical models (1) and (2).

ELECTROPHYSIOLOGICAL DATA

    The data used in this report represent the responses of 39
olfactory receptors of the Colorado potato beetle to different
stimuli (De Jong & Visser, 1988). The degree of specialization
(DS) of an olfactory receptor, was expressed as the
specialization in the cell's responses to 5 pure compounds, i.e.
cis-3-hexen-1-ol, trans-2-hexenal, cis-3-hexenyl acetate,
trans-2-hexen-1-ol and 1-hexanol (at a source dilution of 4 x
10^{-2} in paraffin oil, v/v). These compounds are part of the
so-called 'green odour' of potato leaves (Visser, 1983), and are
thought to be essential for plant odour recognition by the beetle
(Visser & Avé, 1978). A receptor's relative response spectrum was

obtained by setting the cell's 'best' component on a 100%-level.
DS was calculated by (De Jong & Visser, 1988):

$$DS = \frac{100n - \sum_{i=1}^{n} R_i}{n - 1}$$

R_i represents the cell's relative response to compound i. This
calculation expresses DS as a percentage which ranges between 0
for cells without any specialization, and 100 for cells
responding to only one of the test compounds. The number (n) of
different compounds that was tested is 5.

The mixture effect was quantified as the average mixture
response of a receptor (De Jong & Visser, 1988). This was done by
measuring the cell's responses to three 1:1 binary mixtures, each
at 2 dilutions of the total amount of volatiles (4×10^{-2} and 8×10^{-2}, v/v, at the source). Compounds used in the mixtures were:
trans-2-hexenal, cis-3-hexenyl acetate and 1-hexanol. Each
mixture response was expressed as a percentage of the response to
the mixture's 'best' pure component (at a dilution of 4×10^{-2},
v/v, at the source). The average mixture response (AMR_6) was
calculated for each cell as the average of the 6 values.
Variables were compared using the non-parametric Spearman rank
correlation test (Siegel, 1956). A negative correlation between
AMR_6 and DS has been described previously (De Jong & Visser,
1988).

APPLICATION OF THE MODELS

We evaluated the electrophysiological data with respect to the
vector model (1), and were able to calculate an α for 82 of the
117 cases (Fig. 1A-C). In 6 cases, the responses to mixtures were
stronger than the summed responses of the corresponding
components, and since the vector model can not handle cases of
synergism, these α's could not be determined. In the remaining 29
cases, the responses to mixtures showed stronger suppression than
can be explained by the vector model. Using the U model (2), 106
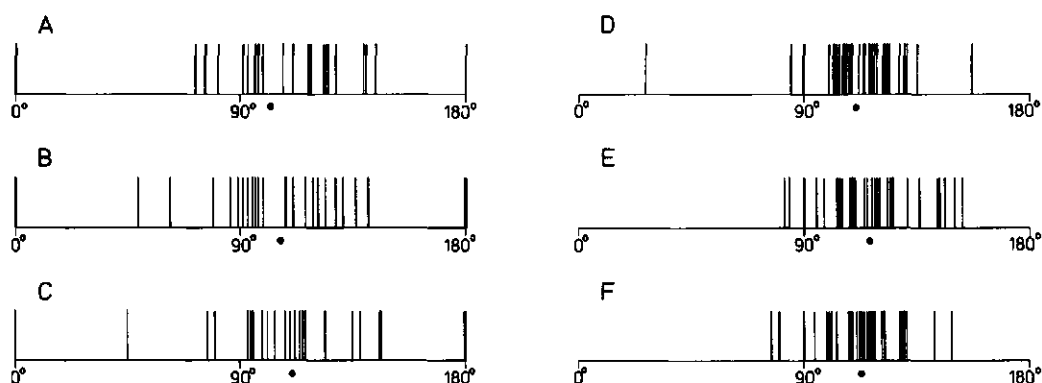of the 117 α's could be calculated (Fig. 1D-E). No synergism was

Fig. 1 Distribution of calculated angles for binary mixtures using the vector model (A-C) and the U model (D-F). Angle values are represented by bars, and mean values by filled circles. A and D, mixtures of trans-2-hexenal and cis-3-hexenyl acetate; B and E, mixtures of trans-2-hexenal and 1-hexanol; C and F, mixtures of cis-3-hexenyl acetate and 1-hexanol.

found to be too strong for this model. In 11 cases we calculated cosα values which were < -1. Besides a greater number of α's, the results of the U model show less variation in the α values. The mean values of the α's for the 3 mixtures and for both models do not differ very much (Fig. 1).

Some α's could not be calculated because some mixtures showed stronger suppression than either model can handle. This may be due to the fact that most receptors showed a background firing rate which was too low to reveal possible inhibitory responses. As a result the magnitude of an inhibitory response could not be expressed as a decrease in frequency. Therefore, instead of expressing the receptor responses by their spike frequencies, the receptor potential might be a better response criterion, but this would require intracellular recordings.

When the α-values for different pairs of mixture components are compared, it appears that α-values for the same receptor are correlated with each other. Receptors with a high or a low α-value for one mixture combination subsequently showed high or low values for other mixture combinations. α-Values, therefore, are characteristic for these olfactory receptors. Table 1 gives for both models an overview of correlations between different pairs of α's for the same receptor population. For the U model we found

Table 1. Correlations between different pairs of α's for the same receptor population. Variables were compared using the non-parametric Spearman rank correlation test (Siegel, 1956).

	Vector model	U model
α_{ab}[1] vs α_{ac}	r_s[2] = 0.72; N = 21; P < 0.001	r_s = 0.60; N = 33; P < 0.001
α_{ab} vs α_{bc}	r_s = -0.21; N = 23; P = 0.343	r_s = 0.47; N = 33; P < 0.010
α_{ac} vs α_{bc}	r_s = 0.29; N = 23; P = 0.180	r_s = 0.50; N = 34; P < 0.005

[1] a, trans-2-hexenal; b, cis-3-hexenyl acetate; c, 1-hexanol. α_{ab}, α_{ac} and α_{bc} stand for angles between a and b, a and c, and b and c, respectively.
[2] r_s, correlation coefficient; N, number of data pairs; P, probability (2-tailed).

significant correlations for all 3 combinations, while for the vector model this was the case for only one combination. The latter result may be due to a smaller number of data pairs.

The correlations between pairs of α's indicate that the correlation between AMR_6 and DS does not depend on the presence of one specific compound in the mixture, i.e. the most stimulating compound. In other words, the amount of suppression in a receptor cell, which is positively correlated with its degree of specialization, depends on receptor cell characteristics, rather than on the presence of one specific stimulus.

The correlation between AMR_6 and DS, therefore, is not due to the presence of the cell's 'best' stimulus in a mixture. Of the 39 cells tested, 31 showed the highest sensitivity to 1 of the 3 components used in the mixtures, and thus 4 of the 6 mixtures contained this compound. When the AMR_4 is calculated as the meanof these 4 mixtures containing the cell's 'best' compound, the correlation between AMR_4 and DS for these 31 cells is better (r_s = -0.70; N = 31; P < 0.001, 2-tailed; Fig. 2A) than that for AMR_6 and DS for the whole cell population (r_s = -0.52; N = 39; P < 0.001, 2-tailed). The AMR_2s of the same 31 cells, resulting
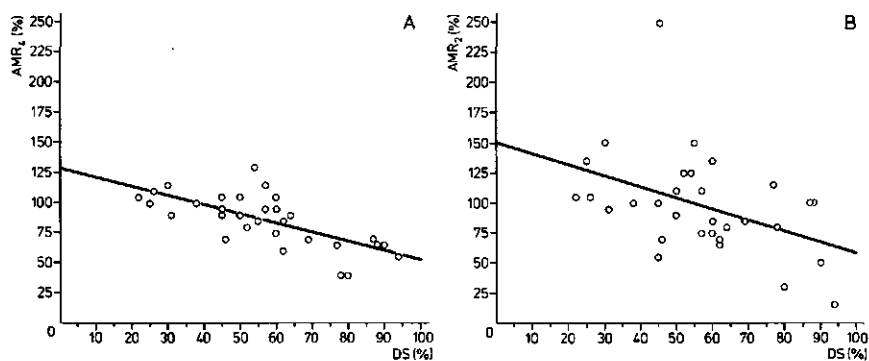
Fig. 2 Scatterdiagram for the average mixture response (AMR) and the degree of specialization (DS) in olfactory receptors of the Colorado potato beetle. A, mixtures containing the cell's 'best' compound (AMR_4) (r_s = -0.70; N = 31; P < 0.001, 2-tailed); B, same cells, mixtures not containing their 'best' compound (AMR_2) (r_s = -0.48; N = 31; P < 0.01, 2-tailed).

from the 2 mixtures not containing the receptor's 'best' component, still show a negative correlation with DS (r_s = -0.48; N = 31; P < 0.01, 2-tailed; Fig. 2B), even though the accuracy of measurements is less than in the foregoing procedure. This decrease in accuracy is caused by the weaker responses to mixtures lacking the 'best' compound, particularly in the more specialized cells, and to the smaller number of mixtures used for calculating the cell's AMR. With both models (1) and (2), α's are correlated with the receptor's DS. Using the vector model we found: r_s = 0.24; N = 82; P < 0.05 (2-tailed), and with the U model: r_s = 0.31; N = 106; P < 0.001 (2-tailed). These analyses demonstrate that suppression in response to binary mixtures is correlated with the extent of specialization of the receptor cells, and that suppression does not depend on the presence of one specific compound, e.g. the 'best' stimulus.

CONCENTRATION EFFECTS ON RESPONSES

Concentration shifts lead to changes in receptor responses. A change in mixture concentration is proportional to the changes in concentrations of its components. Assuming that this would change ψ_A and ψ_B by the same factor n to $n\psi_A$ and $n\psi_B$ respectively, the

response ψ'_{AB} to the new mixture concentration can be expressed for both models (1) and (2) as:

$$\psi'_{AB} = n\psi_{AB} \qquad (3)$$

Since, according to (3), $\psi'_{AB}/\psi_{AB} = n$, concentration shifts should lead to relatively similar changes in the responses of different receptors. However, the relative change in response to an increase in stimulus concentration (from 4×10^{-2} to 8×10^{-2}, v/v, at the source), averaged for the 3 mixtures, is significantly correlated with AMR_6: $r_s = 0.40$; $N = 39$; $P < 0.005$, 2-tailed, (Fig. 3). Thus, at strong suppression, the relative increase of receptor response caused by an increase in mixture concentration is small.

Differences between receptors in their dose-response relations can explain this effect. Dose-response relations are characterized by sigmoid-shaped curves when plotted on semi-logarithmic scales. The response intensity levels off at high stimulus concentrations. Visser (1979a) found, using the EAG recording technique, sigmoid-shaped dose-response curves for the Colorado potato beetle's antenna to the 'green odour' components. EAG recordings reflect the responses of the complete receptor cell population and, therefore, do not reveal differences between the receptors within this population. When, for instance, specialized neurones are more sensitive than generalized neurones, test concentrations sometimes lie at the right asymptotic region of the dose-response curves for specialized neurones, and in the steep region for the generalized neurones. This would cause relatively smaller effects by concentration shifts in specialized receptors. Although we did not find a relation between the absolute response levels of receptors to their 'best' compound and their DS (or between absolute response levels to their 'best' compound and AMR_6), this possibility cannot be excluded completely.

Another explanation could be differences between the steepness of dose-response curves for cells with high and low AMR_6 values. The steeper the curve, the smaller the concentration range in which the cell's response intensity is affected. A relationship
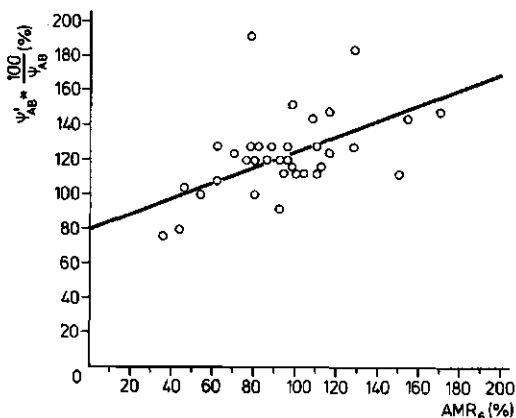
Fig. 3 Scatterdiagram for the relative change in receptor response to an increase in mixture concentration (ψ'_{AB} at 8×10^{-2}, v/v) related to responses to low mixture concentrations (ψ_{AB} at 4×10^{-2}, v/v) and the average mixture response (AMR_6). $r_s = -0.40$; $N = 39$; $P < 0.005$, 2-tailed.

between the receptor's AMR_6 and the steepness of its dose-response curves, thus explains the observed correlation between AMR_6 and the relative change in response to a concentration increase (Fig. 3). Specialized receptors may have steeper curves than more generalized ones. This could cause synergy at low concentrations and suppression at high concentrations, and thus would reduce the concentration dependency of the responses. When the test concentrations fall in the steep region of their dose-response curves, the situation would be reversed of course, since steeper curves then show stronger effects. However, since the test concentrations used in our experiments, are relatively high compared with the EAG dose-response curves (Visser, 1979a), the latter possibility does not seem very likely.

Concentration dependency of mixture interaction in receptor cells, has been described by Johnston et al. (1985) for some types of chemoreceptors in lobsters. These receptors showed synergy when mixtures were applied at low test concentrations, and suppression at high test concentrations.

Nevertheless, in contrast to the assumption made earlier that $\psi'_A/\psi'_B = \psi_A/\psi_B$, the concentration shift may have changed ψ_A and ψ_B in a dissimilar way. Since suppression may provide receptors with an additional buffer against small changes in the ratio of ψ_A and ψ_B, as is discussed later, the response levels of

specialized receptors then would be less influenced by
concentration changes. This effect could have contributed to the
observed correlation in Figure 3.

QUALITY BUFFERED RESPONSES

According to De Jong & Visser (1988), the response level in
narrowly tuned receptors depends on odour quality. The chemical
composition of a food odour, however, is not always exactly the
same, e.g. due to differences in age of the food sources.
Although the odour compositions of young and old potato plants
differ in their ratios of components (Visser, 1979b), both odours
are attractive to Colorado potato beetles (Visser, 1976). This
indicates that some tolerance exists in the mechanism of odour
quality coding. Potato leaf odour, however, loses its
attractiveness to Colorado potato beetles when small quantities
of its component odours are added artificially (Visser & Avé
1978; De Jong & Visser, unpubl.), which demonstrates that this
tolerance is limited. Nevertheless, a certain buffer capacity in
the perception of quality might enable an organism to distinguish
between biological important signals and background noise in
olfaction.

Fig. 4 illustrates for both the vector model (Fig. 4A) and the
U model (Fig. 4B), the change in response level ψ_M of an
olfactory receptor to a stimulus M when stimulus C is added.
Stimulus M alone elicits a response level ψ_{Mo}. The response
levels elicited by stimulus C (ψ_C) and combinations of stimuli M
and C (ψ_M), are expressed relatively to ψ_{Mo}. The relation between
ψ_M and ψ_C is given for several angles between M and C. Simple
addition of the response levels ψ_{Mo} and ψ_C occurs at $\alpha = 0°$ with
the vector model and at $\alpha = 90°$ with the U model. The response
intensity ψ_{Mo} can be suppressed by stimulus C at high α-values.
At $\alpha = 180°$, for example, there can be a severe suppression of
ψ_{Mo}. Cases where the response to a mixture is stronger than
predicted from adding responses to the single components are only
included in the U model and depend on the α-value ($0°\leq\alpha<90°$).
Both models show that within a certain range ψ_M can be relatively
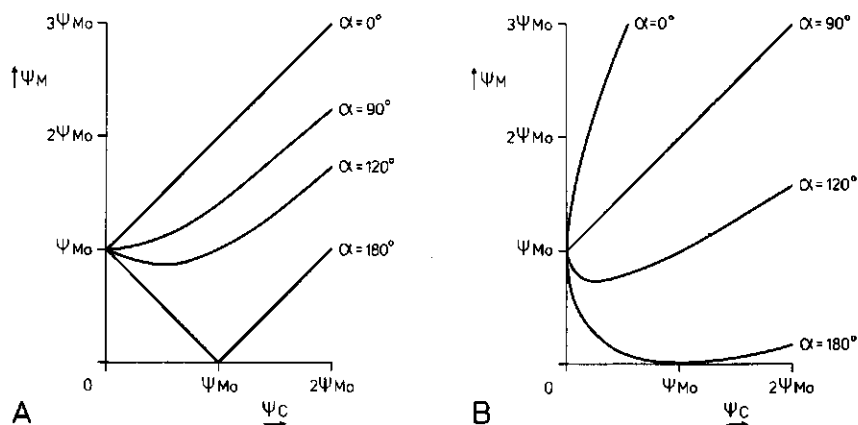unaffected on addition of stimulus C. With the vector model at α

Fig. 4 Response level ψ_M of a receptor after addition of a stimulus C (with response level ψ_C) to a stimulus M with a response level ψ_{Mo}. The relation between ψ_M and ψ_C is given for several angles between C and M in the vector model (A) and the U model (B). See text for further explanation.

= 90°, for instance, addition of stimulus C with a relatively small response level ψ_C does not affect ψ_{Mo} significantly. The capacity of this buffer is in both models determined by the particular angle between M and C, and by the ratio of response intensities ψ_C and ψ_{Mo}. Suppression, therefore, in theory provides receptor responses with a buffer for some variations in stimulus quality.

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CHAPTER 5. EFFECTS OF FEEDING EXPERIENCE ON HOST ODOUR PERCEPTION IN THE COLORADO POTATO BEETLE

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Effects of feeding experience on olfactory orientation of the Colorado potato beetle, Leptinotarsa decemlineata Say, towards different plant odours were studied using a locomotion compensator in front of a wind tunnel. Non-experienced beetles are attracted solely by the odour of potato plants. Potato plant odour is attractive to the beetles regardless of their feeding experience. Upwind orientation towards the odour of bittersweet and tomato plants is not significantly enhanced by a feeding experience on these plants. The odour of bittersweet plants however, is attractive to beetles with experience on tomato plants. Electroantennogram recordings demonstrate that feeding experience affects responses of olfactory receptors in a quantitative and qualitative way. This plasticity in receptor responses may be partly responsible, in addition to the central nervous system, for modifications in the olfactory orientation of the Colorado potato beetle.

The Colorado potato beetle, Leptinotarsa decemlineata Say, has a limited range of host plants, all of which are solanaceous species. The beetle was originally found in the eastern part of the United States where it fed on buffalo bur, Solanum rostratum. Nowadays, the beetle is a serious pest of the United States' potato and tomato crops. The beetle has also invaded continental Europe where its most important host plant is potato.

Bongers (1970) conducted experiments to test the suitability of 5 different Solanum species as hosts for Colorado potato beetles from the Wageningen laboratory stock culture. Bittersweet, Solanum dulcamara, proved to be a suitable foodplant, although on this plant species the beetles' mortality was higher than on the best host, potato, Solanum tuberosum. On tomato plants, Lycopersicon esculentum, there was considerable mortality, but still could be used as a food plant. The black night shade, Solanum nigrum, and the woolly night shade, Solanum luteum, were unsuitable as hosts.

Studies on behavioural responses of Colorado potato beetles showed that the beetles can distinguish between odour of a host plant and of non-host plants (Visser & Nielsen, 1977). Odour of potato plants elicits upwind walking behaviour in the beetle

(Visser, 1976; Visser & Nielsen, 1977; Thiery & Visser, 1986).
The ratios of 'green odour' components in leaf odours play a key
role in the beetle's olfactory discrimination (Visser & Avé,
1978).

Visser (1979) and Ma & Visser (1978) demonstrated that the
Colorado potato beetle's antennal receptor system is sensitively
tuned to the perception of green odour components. More detailed
studies on the beetle's mechanism of olfactory coding have been
conducted by De Jong & Visser (1988a and b). They recorded
neuronal responses at the receptor level and at the deutocerebral
level to stimulation of the antenna with green odour components.

Visser & Thiery (1986) demonstrated that prior feeding
experience on potato leaves enhances the upwind responses of
Colorado potato beetles towards potato plant odour. The
underlying mechanism of this phenomenon remained unknown, but it
indicates that the beetle's response to a host odour is not
fixed.

EFFECTS OF FEEDING EXPERIENCE ON ORIENTATION

It has been demonstrated for different species that adult
insects are able to modify their responses to certain odour
stimuli as a result of experience. Non-experienced females of the
parasitoid Asobara rufescens, for example, are not responding to
the odour of yeast. After experience with host larvae feeding in
yeast, however, these wasps are attracted by yeast odour (Vet &
Van Opzeeland, 1984). The present study was undertaken to see
whether the orientation behaviour of Colorado potato beetles
towards different plant odours is influenced by feeding
experience.

Newly-emerged female beetles were collected from the
laboratory stock culture (reared under 10 h photophase), and kept
isolated in petri dishes. Prior to the experiments, 1 group of
beetles was starved for at least 12 h (non-experienced beetles),
while the other beetles were fed for 4 h on 1 of 3 different food
plants before being starved for at least 12 h. The plants used as
food plants were: potato, bittersweet and tomato. Behavioural
responses of the beetles to plant odours were studied using a

Table 1. Effects of feeding experience on the orientation responses of female Colorado potato beetles to wind and wind carrying plant odour. Upwind responses expressed as the quotient of upwind displacement and the total length of the walking track; data represent group means.

Stimuli	Non-experienced Beetles	Beetles experienced on		
		Potato	Bittersweet	Tomato
Wind	0.671 a[1]	0.613 a	0.595 a	0.592 a
Wind + Potato odour	0.793 b	0.768 b	0.809 b	0.743 b
	(N=40)[2]	(N=39)	(N=42)	(N=39)
Wind	0.563 a	0.469 a	0.596 a	0.478 a
Wind + Bittersweet odour	0.670 a	0.652 a	0.671 a	0.700 b
	(N=42)	(N=39)	(N=41)	(N=41)
Wind	0.699 a	0.623 a	0.662 a	0.523 a
Wind + Tomato odour	0.653 a	0.608 a	0.659 a	0.534 a
	(N=40)	(N=40)	(N=40)	(N=40)

[1] Data were compared in each pair of stimuli and different letters indicate statistical differences at $P < 0.02$ (2-tailed, Wilcoxon matched-pairs signed-ranks test; Siegel, 1956).
[2] Groups of beetles were tested for only 1 pair of stimuli; N is number of beetles per group.

locomotion compensator in front of a wind tunnel (Visser, 1976; Thiery & Visser, 1986). In this way, walking tracks were recorded for 4 min each, and responses of beetles to wind were compared with responses to wind carrying 1 test plant odour. The test plants, i.e. 6 potato, 6 bittersweet or 8-9 tomato plants, were standing in the dark upwind section of the wind tunnel. The air flow leaving the wind tunnel was set at 80 cm/s (4320 l/min). The quotient of upwind displacement and the total length of the walking track was used as the parameter to describe the intensity of the beetles' upwind response.

The results presented in Table 1 show that non-experienced beetles are attracted solely by the odour of potato plants. For these beetles, the odour of bittersweet plants or tomato plants did not elicit a response different from the response to wind. The results indicate further that feeding experience did not change dramatically the innate odour preference of Colorado

potato beetles: potato plant odour was attractive to the beetles, regardless of their feeding experience. Feeding experience on bittersweet or tomato plants, did not significantly enhance the upwind response of the beetles towards the odour of the plant species with which they were experienced. However, feeding experience on tomato plants did influence the beetle's orientation behaviour. The odour of bittersweet plants was attractive to beetles with experience on tomato plants and not to beetles with experience on other plants (Table 1).

The results of the experiments conducted by Visser & Thiery (1986) show that potato plant odour recognition is enhanced by a 2 h experience on potato leaves. Experience on other plants, then, may adjust the innate odour preference in the direction of that plant odour. The data in Table 1 show that this possible change of preference does not include attraction towards odour emanating from the plants on which the beetles obtained prior feeding experience.

Visser & Nielsen (1977) analyzed behavioural responses of Colorado potato beetles on a walking plate to odour stimuli, by the division of walking tracks into 5 categories (criterion A) and by measuring the time required for the beetles to reach the upwind edge of the walking plate (criterion B). They found, using criterion A, that beetles fed with potato for 48 h and subsequently starved for 24 h showed similar responses to potato odour and bittersweet odour. With criterion B, however, responses were significantly different. Beetles fed on bittersweet responded to both odours equally by both criteria, indicating an effect of feeding experience on the beetle's behaviour. In our experiment, we did not observe significant differences between responses to wind and to wind carrying odour of bittersweet plants in beetles fed with potato or bittersweet (Table 1). The discrepancy between the results of Visser & Nielsen (1977) and our results may be due to certain limitations in the recording of behavioural responses on a walking plate.

According to Bongers (1970), the potato plant is the most suitable host for the beetles, followed by bittersweet. Tomato is a far less suitable host. For the beetles, therefore, bittersweet may be more similar to potato than tomato, which also may be

Table 2. Orientation responses of non-experienced female Colorado potato beetles to wind and wind carrying plant odour. Upwind responses expressed as the quotient of upwind displacement and the total length of the walking track; data represent group means.

Stimuli	Non-experienced Beetles[1]
Wind	0.563 ab[2]
Wind + Bittersweet odour	0.670 b
Wind + Tomato odour	0.454 a

[1] One group of 42 beetles was tested.
[2] Different letters indicate statistical differences at $P < 0.02$ (2-tailed, Wilcoxon matched-pairs signed-ranks test; Siegel, 1956).

reflected in the respective plant odour qualities. Non-experienced beetles indeed show a significant more intense upwind response to the odour of bittersweet plants than to the odour of tomato plants (Table 2). Assuming that the beetle's innate odour preference shifts towards the odour of the experienced plant, the experience on tomato, then, could change the beetle's preference more than the experience on bittersweet. When these changes are in the same direction, this may explain why the odour of bittersweet plants is attractive to beetles with experience on tomato plants (Table 1).

EFFECTS OF FEEDING EXPERIENCE ON OLFACTORY RECEPTORS

It is generally assumed that behavioural modifications are controlled by the central nervous system. Erber et al. (1980) demonstrated that in honey bees memory formation for odours can be disrupted by cooling the antennal lobes and the mushroom bodies in the protocerebrum. Coss et al. (1980) described changes in the morphology of interneurones in these mushroom bodies, which were associated with cumulative nursing and foraging experiences of the bees.

There is, nevertheless, also evidence that the sensitivity of insect chemoreceptors is flexible. Food quantity and quality can affect the sensitivity of peripheral chemoreceptors involved in feeding behaviour (Blaney et al., 1986). Response levels in

Table 3. Correlations between relative EAGs of left and right antennae from the same newly-emerged beetles to green odour components. Data represent Spearman rank correlation coefficient r_s and probability P (2-tailed, non-parametric Spearman rank correlation test; Siegel, 1956).

Compound[1]	Females[2]	Males[2]	Total
cis-3-Hexen-1-ol	$r_s = 0.05$ $P = 0.881$	$r_s = 0.02$ $P = 0.928$	$r_s = 0.13$ $P = 0.578$
trans-2-Hexenal	$r_s = 0.51$ $P = 0.130$	$r_s = 0.93$ $P = 0.001$	$r_s = 0.76$ $P = 0.001$
cis-3-Hexenyl acetate	$r_s = 0.68$ $P = 0.029$	$r_s = 0.73$ $P = 0.017$	$r_s = 0.71$ $P = 0.001$
trans-2-Hexen-1-ol	$r_s = 0.60$ $P = 0.019$	$r_s = -0.12$ $P = 0.764$	$r_s = 0.43$ $P = 0.063$
1-Hexanol	$r_s = 0.95$ $P = 0.001$	$r_s = 0.48$ $P = 0.233$	$r_s = 0.70$ $P = 0.001$

[1] Compounds were at a dilution in paraffin oil of 10^{-1} (v/v), except for the standard which is cis-3-hexen-1-ol at a dilution of 10^{-3} (v/v).
[2] Groups of 10 female and 10 male beetles were tested.

maxillary taste sensilla in <u>Manduca</u> <u>sexta</u> caterpillars, for example, depend on the diet (Schoonhoven, 1967 and 1969; Städler & Hanson, 1976), and this may influence host discrimination (Schoonhoven, 1969; Städler & Hanson, 1976). Little is known about the mechanism underlying this type of receptor modulation. It has been suggested that receptor sensitivity is controlled by one or several hormones, through effects on the ionic composition of the dendritic liquor, or by peripheral regulation of the spike generating process (Blaney et al., 1986). In order to examine whether the responses of olfactory receptors in the Colorado potato beetle are influenced by feeding experience, the following experiments were conducted.

Electroantennogram (EAG) recordings were made from newly-emerged female beetles which were collected from the laboratory stock culture (reared under 18 h photophase). The EAG method has been described by Visser (1979). We modified the method of stimulus delivery by using a 2 s stimulation time (1 ml/s). EAGs

of different antennae from 1 beetle tend to correlate to their relative sensitivities (Table 3). After the determination of EAG responses of 1 antenna of each beetle to a set of test chemicals, the beetles were numbered and kept isolated. For 2 days, these beetles were either starved, fed with leaves of 1 of the following plant species: potato, bittersweet, or tomato. Following this, EAGs of the beetles' other antennae were recorded. The test chemicals, i.e. cis-3-hexen-1-ol, trans-2-hexen-1-ol, cis-2-hexen-1-ol, trans-3-hexen-1-ol, cis-3-hexenyl acetate, trans-2-hexenal, hexanal, 1-hexanol, 2-hexanol, 3-hexanol and 1-octen-3-ol (each at a dilution in paraffin oil of 10^{-1}, v/v), are known to give clear EAG responses (Visser, 1979). These compounds are distributed in various plant species including solanaceous species, except for cis-2-hexen-1-ol, trans-3-hexen-1-ol, 2-hexanol and 3-hexanol, which are geometric isomers of plant compounds. The response to cis-3-hexen-1-ol was used as the standard response (equals 100%).

Figure 1 shows the relative EAG spectra of the newly emerged female beetles. The effects of the different treatments on beetles' EAG responses are represented in Figure 2. The sensitivities of antennae are affected by prior feeding experience. The absolute EAG value for the standard was significantly increased for beetles fed on potato and on bittersweet. These sensitivity changes affect the responses to the other test chemicals in the same direction but in a disproportionate way (Fig. 2). Beetles which were starved showed a decrease in their relative EAG responses to trans-2-hexen-1-ol, cis-2-hexen-1-ol, hexanal, 1-hexanol, 2-hexanol and 3-hexanol, and an increase to cis-3-hexenyl acetate and trans-2-hexenal. Feeding experience on potato induced a decrease in relative EAG responses to cis-3-hexenyl acetate, trans-2-hexenal, hexenal, 2-hexanol and 3-hexanol. Beetles fed with bittersweet and tomato showed decreased relative EAG responses to hexanal, 1-hexanol, 2-hexanol and 3-hexanol, and to trans-3-hexen-1-ol, 2-hexanol and 3-hexanol, respectively.

Receptor sensitivities in a number of insect species have shown a direct relationship to age and feeding history (Rees, 1970; Roelofs & Comeau, 1971; Davis & Takahashi, 1980). The
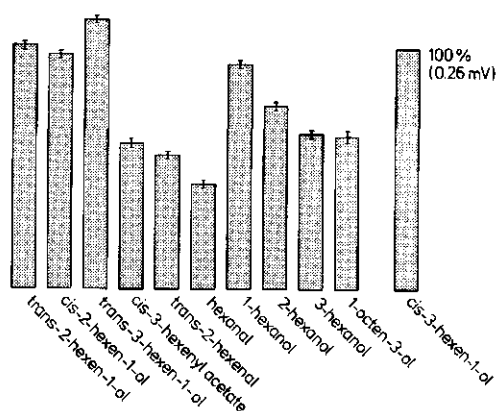
Fig. 1 Relative EAG spectra of newly-emerged non-experienced female Colorado potato beetles (N = 161). Chemicals were at a dilution of 10^{-1} in paraffin oil (v/v). cis-3-Hexen-1-ol was used as the standard. Bars at top of columns indicate 99%-confidence intervals.

responses of receptors can change at varying amounts of ingested food (Bernays & Chapman, 1972) as well as at differences in food quality (Schoonhoven, 1969). The observed changes in sensitivity in the Colorado potato beetle's olfactory receptors may be due to a combination of these factors. Our results demonstrate that relative EAG spectra change in all 4 groups of beetles and in each group differently (Fig. 2). The beetle's feeding history, therefore, affects the responses of the population of olfactory receptors both qualitatively and quantitatively.

A few hours feeding experience can induce a change in the Colorado potato beetle's behavioural response (see above and Visser & Thiery, 1986). The observed effects on the olfactory receptors in the beetles were measured after a 2-day starvation period or a 2-day feeding experience, and demonstrate a certain plasticity in the antennal receptor system. It is unclear however, whether such peripheral changes can occur within a few hours. Moreover, it is unknown whether such changes are important for the modification of the beetle's behavioural response. Work on other insect species, however, indicates that even relatively small changes in receptor sensitivities affect behavioural performance significantly (Blaney et al., 1986). Changes at the peripheral level induced by feeding experience, therefore, may be partly responsible, in addition to the central nervous system, for modifications in the olfactory orientation of the Colorado potato beetle.
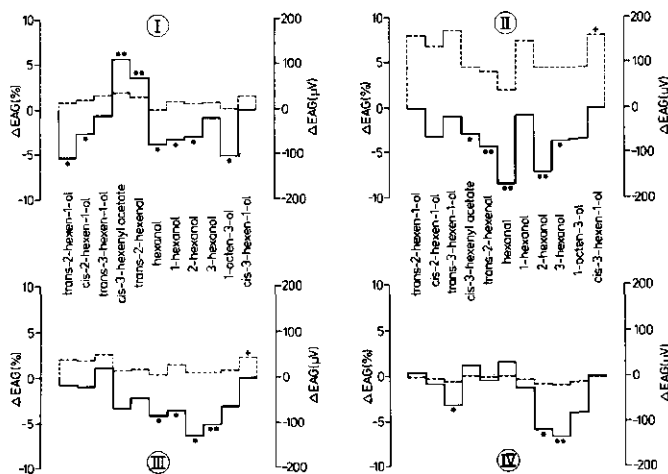
Fig. 2   Effects of prior feeding experience for 2 days on EAG responses of
female Colorado potato beetles. Solid lines represent changes in mean relative
EAG values, broken lines represent changes in mean absolute EAG values. I,
starved (N = 40); II, fed with potato leaves (N = 41); III, fed with
bittersweet leaves (N = 39); IV, fed with tomato leaves (N - 41).
cis-3-Hexen-1-ol is the standard. All chemicals were at a dilution in paraffin
oil of 10^{-1}, v/v. Statistical differences between relative EAG values before
and after treatments are indicated with 1 asterisk at P < 0.05 (2-tailed) and
with 2 asterisks at P < 0.005 (2-tailed, Wilcoxon matched-pairs signed-ranks
test; Siegel, 1956). Statistical differences between absolute EAG values of
the standard before and after treatments at P < 0.05 (2-tailed) are indicated
by +.

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SAMENVATTING

Dit proefschrift beschrijft de wijze waarop de Coloradokever een waardplantgeur kan herkennen. De geur van een waardplant wekt bij de Coloradokever een windopwaarts gericht loopgedrag op. Essentieel hierbij zijn de verhoudingen tussen een aantal algemene plantegeur-komponenten die samen de zogenaamde 'groene geur' van een plant vormen (Hoofdstuk 1).

Geurreceptoren in de antenne van de Coloradokever vertonen graduele verschillen in de mate van specialisatie op bepaalde groene geurkomponenten. De receptorrespons op een geurkomponent kan onderdrukt worden door de aanwezigheid van een tweede geurkomponent (Hoofdstuk 3). Dit fenomeen, suppressie genaamd, manifesteert zich in een mate die eerder karakteristiek is voor een receptor dan voor bepaalde kombinaties van geurkomponenten (Hoofdstuk 4). Doordat suppressie sterker optreedt naarmate een receptor meer gespecialiseerd is, ontstaan er binnen de receptorpopulatie zekere verschillen in gevoeligheid voor de hoeveelheid en de samenstelling van een groene geur (Hoofdstukken 3 en 4).

De scheiding van informatie betreffende geurkwantiteit en - kwaliteit wordt in de antennale lob van de hersenen verder aangescherpt. Met deze twee informatiekanalen kan het centraal zenuwstelsel van de kever op een eenvoudige wijze de waardplantgeur koderen (Hoofdstuk 2; Fig.1).

Een voedingservaring kan tot op zekere hoogte de waardering van de Coloradokever voor plantegeuren beïnvloeden. Dit verschijnsel is mogelijk voor een deel te verklaren uit een veranderde receptorgevoeligheid (Hoofdstuk 5).
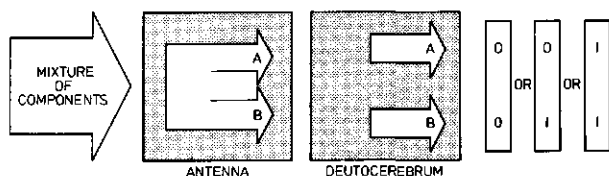
Fig. 1 Schema van het hypothetische mechanisme waarmee een waardplantgeur door de Coloradokever wordt herkend. Olfactorische informatie wordt verwerkt via de kanalen A en B, die onafhankelijk van elkaar wel (1) of geen (0) respons kunnen vertonen. Er zijn 2 groepen neuronen in de antennale lob (deutocerebrum) van de hersenen betrokken bij het verwerken van olfactorische informatie: een groep gespecialiseerde neuronen (A) die geen respons vertoont op een stimulatie met de geur van een aardappelloof extract, en een andere groep niet-gespecialiseerde neuronen (B) die daarop wel reageert. De situatie waarbij alleen kanaal B reageert, wordt verondersteld karakteristiek te zijn voor een stimulatie met waardplantgeur (Hoofdstuk 2). Onderzoek aan de geurreceptoren in de antenne wijst erop dat er op receptornivo al een begin wordt gemaakt met de informatiescheiding via de kanalen A en B (Hoofdstuk 3).

CURRICULUM VITAE

Ruurd de Jong werd op 31 mei 1956 te Jubbega-Schurega (gemeente Heerenveen) geboren. Na het behalen van het eindexamen Atheneum B aan de Gemeentelijke Scholengemeenschap Zuid te Enschede in 1975 begon hij in hetzelfde jaar zijn studie Planteziektenkunde aan de Landbouwuniversiteit te Wageningen. In 1983 studeerde hij af met entomologie als verzwaard hoofdvak en virologie als hoofdvak. Van 1983 tot 1987 werd aan de vakgroep Entomologie van de Landbouwuniversiteit het in dit proefschrift beschreven onderzoek verricht. Het onderzoek is gedurende drie jaar gefinancierd door BION (ZWO) en gedurende een half jaar door de Landbouwuniversiteit.