

OLFACTION IN THE
COLORADO BEETLE
AT THE ONSET OF HOST PLANT SELECTION

CENTRALE LANDBOUWCATALOGUS



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vergelijkende dierfysiologie.

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J.H. VISSER

**OLFACTION IN THE COLORADO BEETLE
AT THE ONSET OF HOST PLANT SELECTION**

PROEFSCHRIFT TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE LANDBOUWWETENSCHAPPEN, OP GEZAG
VAN DE RECTOR MAGNIFICUS, DR. H.C. VAN DER PLAS,
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STELLINGEN

I

Het complex van C-6 alcoholen en aldehyden speelt in de waardplantselectie van waarschijnlijk vele fytofage insecten een essentiële rol, en kan hierbij als een algemene bladgeur-faktor met soortspecifieke elementen worden aangemerkt.

Dit proefschrift.

II

Het getuigt van een dubieuze vanzelfsprekendheid als attractie van insecten door vluchtige plantestoffen wordt vastgesteld aan de hand van ovipositie.

Finch, S. (1978). *Ent. exp. & appl.* 24:350-359.

Vernon, R.S., Pierce, H.D., Jr., Borden, J.H. &

Oehlschlager, A.C. (1978). *Envir. Entomol.* 7:728-731.

III

De conclusie en aanbeveling "We need more information on the quantitative correlation of the receptor cell and the behavioural responses to a pheromone stimulus (e.g. the receptor activities underlying the threshold response of an animal)" gaat aan de grondslag van de grote zintuiggevoeligheid voorbij.

Advanced Research Institute on Chemical Ecology (1979). *Chemical ecology: odour communication in animals*. F.J.Ritter (ed.), Elsevier/North-Holland, Amsterdam, p. 407.

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IV

Tegen het door Feeny geïntroduceerde begrip "plant apparentcy" in relatie tot de beschermende werking van secundaire plantestoffen tegen insecten, is in te brengen dat juist ten aanzien van deze factoren geen wezenlijk onderscheid tussen "apparent" en "unapparent" bestaat.

Feeny, P. (1976). In *Biochemical interaction between plants and insects*. J.W. Wallace & R.L. Mansell (eds.), Plenum Press, New York, pp. 1-40.

V

Hoewel in het taalgebruik "repellent" het antoniem is voor "attractant", is een "insect repellent" niet het tegenovergestelde van een "insect attractant". De terminologie dient daarom op dit punt te worden aangepast.

Dethier, V.G. (1947). *Chemical insect attractants and repellents*. Blakiston, Philadelphia, 289 pp.

VI

Met uitzondering van de eerste stelling en de laatste stelling, is elke stelling één stelling teveel.

VII

De argumenten tegen het gebruik van kringlooppapier voor offsetdruk kunnen door stoffelijke bewijzen weerlegd worden.

Wageningen Hogeschoolblad 2 (1979), no. 32:3.

Dit proefschrift.

J.H. Visser,

Olfaction in the Colorado beetle at the onset of host plant selection. Wageningen, 1 juni 1979.

**Aan Claartje
Aan mijn moeder**

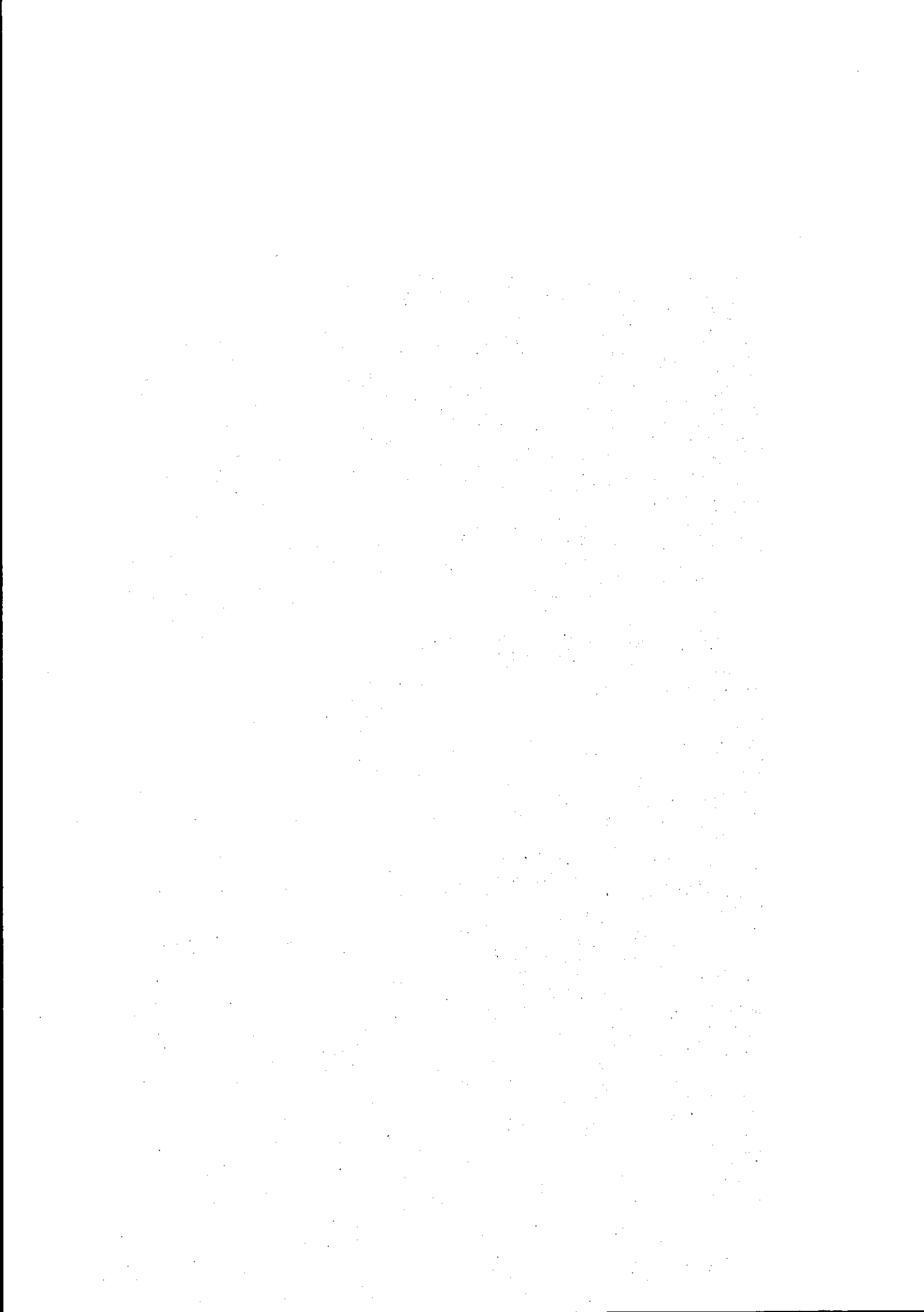
VOORWOORD

Bij het verschijnen van dit proefschrift is een dankwoord op zijn plaats aan allen die speciaal tot de totstandkoming hebben bijgedragen. Mijn promotor Prof. Dr. J. de Wilde ben ik erkentelijk voor de metamorfose die ik op het Laboratorium voor Entomologie mocht ondergaan. Zijn vertrouwen en ondersteuning hebben mij zeer gestimuleerd. Mijn co-promotor Prof. Dr. L.M. Schoonhoven ben ik dank verschuldigd voor zijn kritische opmerkingen die mij tot een concrete formulering van gedachten noopten. Het was een voorrecht om juist samen met Frans Dieleman het college Insekt-Plant Relaties te mogen verzorgen, hierdoor werd ik meer bekend met dit intrigerende onderwerp. De confrontatie met de Aerodynamica doorstond ik door de hulp van Ir. A. Kortleven (Technische Hogeschool Twente), die mij zeer veel van zijn tijd gunde en van waardevolle adviezen voorzag bij het ontwerpen van de windtunnel. De samenwerking met de Aroma-afdeling van het Centraal Instituut voor Voedingsonderzoek TNO was van essentieel belang voor de chemische analyse van de bladgeur-komponenten. De inzet van Jens Nielsen, Dick Avé en Wim Ma, mag niet onvermeld blijven. De service van alle Gecombineerde Diensten van het Binnenhaven Complex verdient mijn waardering. Het typewerk van de manuscripten werd verzorgd door de administratie, Ria Cuperus en Marian Flinterman. Tenslotte wil ik Claartje mijn dank betuigen voor haar steun en geduld.

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CHAPTER 1 INTRODUCTION

HOST PLANT SELECTION IN THE COLORADO BEETLE

BY

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More than one century ago the Colorado beetle *Leptinotarsa decemlineata* Say, was an innocuous feeder of buffalo bur *Solanum rostratum*, on the foothills of the Rocky Mountains in North America. At that time the cultivated potato *Solanum tuberosum*, was introduced into its habitat. This new food resource turned out to be quite acceptable, initiating an extension of the beetle's territory towards the east; even across the Atlantic Ocean, towards Europe where the Colorado beetle was reported as early as the 1920's in the Bordeau region of France. As the dispersal of this new insect pest over Europe brought about substantial damage to potato production, the beetle's specialized feeding habits soon caught attention.

Initially one basic question was focused on: - what makes potato foliage acceptable and/or, why are nonsolanaceous as well as a number of solanaceous plant spp¹ rejected for feeding and oviposition? Nowadays, in observing the complex interactions of insects and plants, it is realized that any attempt to give an unambiguous answer to this question, should oversimplify the process of host selection. Through a long period of association of the genus *Leptinotarsa* with a number of solanaceous plant spp, and under the constant action of natural selection, both the nutritional requirements and feeding habits of the present-day Colorado beetle have been adapted to the chemical characters of its food plants. With its introduction the potato proved to fulfil the beetle's physiological requirements - being suitable and acceptable -, and at present potato crops are the main food resource for maintaining the existence of this insect pest in the major part of its territory.

Nevertheless, from time to time the beetles are faced with lack of food, e.g. post-diapause beetles which because of crop rotation emerge in nonhost plant crops and have to search for suitable plants. Henceforth these beetles have to rely on their own abilities to locate their host plants, in directing their feeding and oviposition act towards particular plant spp. Besides behavioural components - exploration in space -, time is an essential aspect in host selection, since in the course of time the proportion and constitution of successive generations are affected by the various degrees to which the vegetable food resource meets the physiological requirements of both larvae and adults.

¹/spp : species

The present paper focuses attention on the restrictive elements in space and time, as deduced from the series of investigations carried out in The Netherlands over a period of thirty years. The sequence of discrete events in host selection follows rather the historical than the functional line, in order to do justice to the progress of our understanding of this insect-host plant relationship. In this way it is emphasized that every single event is a decisive element in the establishment of the Colorado beetle on a particular plant, and it endeavours to expose some of the foundations in the associations of phytophagous insects with food crops.

Larval feeding and growth

The larvae of the Colorado beetle exhibit remarkable discriminative abilities, apparent from the extreme preference for a limited number of solanaceous plant spp. The larvae starve to death in the absence of such "palatable" food plants. For the proper understanding of the expression "palatable", the observations on the behavioural patterns as released by discernable plant spp, are briefly summarized (Chin, 1950; De Wilde, 1958).

The odour of the resistant plant *Solanum demissum* is as attractive to the larvae as the odour of potato foliage. After coming into contact with the leaves, the larvae will bite this new substratum, their biting response being released by the odour. However, contrary to the feeding action performed on potato foliage, the larvae do not feed continuously and spend most of their time resting and wandering. For feeding *S. demissum* is rejected by the larvae. Besides *S. demissum*, a number of solanaceous spp do not match one of the essential qualities of the normal foodplants, i.e. an acceptable taste. The selective feeding habits of the larvae are caused by avoidance of deterrent factors present in a range of solanaceous plant spp. The nature of these factors has been attributed to members of a typical class of secondary plant substances distributed in Solanaceae, i.e. alkaloids and their parent alkaloid-glycosides.

Petunia hybrida is an example of solanaceous plant spp which causes adverse effects in another way. Larvae are attracted to the odour of *Petunia* leaves and when they meet these leaves, a relatively large amount of the foliage is consumed. However, *Petunia* is poisonous and feeding leads to the death of the larvae. Unlike the avoidance of feeding on *S. demissum*, release of the feeding action is not prevented in this particular case. Obviously the primary advantage resulting from rejection of "unpalatable" plant spp, consists in avoidance of toxic principles. The antibiotic nature of secondary plant substances seems to be the predominant reason for the selective feeding habits of the larvae.

It is self-evident that the nutritive ingredients of plants are crucial for feeding and growth of phytophagous insects. The nutritional requirements of the Colorado beetle instars have been studied by using artificial diets (Wardojo, 1969). These requirements do not differ essentially in the qualitative respect from the requirements of many other phytophagous insects, feeding on a diversity of plant spp. However, the suitability of the diet for supporting growth, strongly depends on

the concentration ratios of the individual nutrients. In addition to this metabolic aspect, the feeding act involves behavioural components as reflected in the amount of consumption, being affected by the proportions of several nutrients (Ritter, 1967).

Through dual discrimination of both secondary plant substances and nutrients, poisoning and malnutrition of the larvae are prevented. In general young larvae hatch from the egg pod, deposited by the female Colorado beetle on the proper food plant. Even so, there is no question about the advantage in having discriminative abilities on closer inspection of the casualties impending over the larval life, when they fall to the ground by heavy rains or run out of food. Larvae removed from their food plant search in a "random" fashion until they are guided by olfactory and optic stimuli. They moreover bite the substrate and thereby are informed of its chemical and physical properties.

Adult feeding, oviposition and exploration

In the acceptance of suitable feeding and oviposition sites the adults are even more selective than the larvae. The principles underlying the acceptance of their solanaceous hosts seem to involve discrimination of the very chemical plant characters inducing the selective feeding habits in the instars. The be-all and end-all of adult life is attained in reproduction. The number of eggs deposited on plants suitable for larval feeding and growth, reflects the various degrees to which individual beetles were able to overcome the restrictive elements in space and time.

Disalignment of adult feeding and oviposition has been observed in the oviposition preference for *Solanum luteum* (Bongers, 1970). When female Colorado beetles, previously fed on potato plants, are given the choice between the foliage of potato on the one hand and leaves of *S. luteum* on the other, they show a marked preference for ovipositing on the latter plant sp. However, in the course of time the beetle's fecundity is suppressed since feeding on *S. luteum* is avoided. Though *S. luteum* is preferred to potato as an oviposition substratum, reproduction on this plant sp - the very succession in time - is impeded.

Exploration in space is thought to be essential for the localization of host plants. The odour of solanaceous plant spp releases an upwind locomotory response in Colorado beetles, which brings the beetles in the vicinity of these plants (see Visser and Avé, 1978). In other words, through olfactory discrimination over a long-range, exploration is to some extent confined to a relevant part of the vegetation. Chemical analysis of potato plant odour revealed the complex of "general green leaf volatiles", like cis-3-hexen-1-ol, cis-3-hexenylacetate, trans-2-hexenal and trans-2-hexen-1-ol. The concentration ratios of these volatile components are decisive for the release of a positive anemotactic response in the Colorado beetle. When the particular ratio in this complex is changed artificially, the release of the upwind locomotory response is prevented. Complexes of the "general green leaf volatiles" form a predominant aspect of all leaf odours, discernable in the different and

particular concentration ratios. In diversified vegetations interactions of the different leaf odours might prevent the release of long-range olfactory orientation, in this way hiding plants from insects. This conception might give a new dimension to the onset of host plant selection, as the beetle's potentiality of exploration in space is not only affected by the host plant's spatial distribution, but also by the extent of plant odour heterogeneity of the vegetation.

Conclusions

The sequential order in the process of host selection is illustrated in Fig. 1. The interspaces of the solid lines visualize the very instant a plant character fulfils one of the physiological requirements of a particular insect. As demonstrated in the larval feeding act on *S. demissum* and *P. hybrida*, and in the oviposition preference for *S. luteum*, nonhost plant spp occasionally meet some of the physiological requirements of the Colorado beetle, however, these plants are devoid of other crucial qualities. The scheme indicates that both nutrients and secondary plant substances -antibiotics - are the predominant barriers for feeding, growth and reproduction of insects on the majority of plant spp. In the Colorado beetle successive behavioural patterns have been evolved to evade nonhost plant spp, the unfitness of which becomes

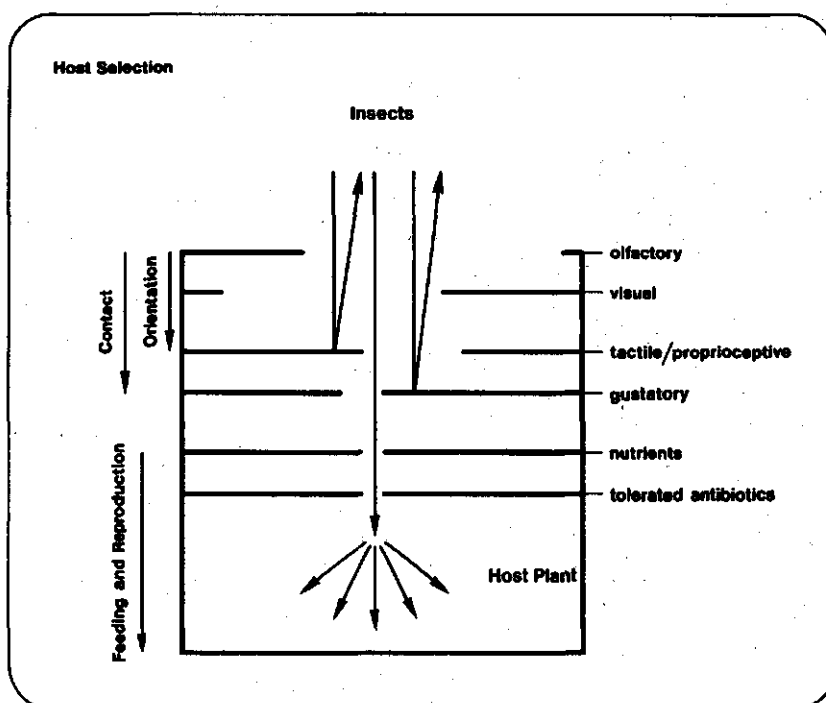


Fig. 1. General scheme of host selection in phytophagous insects.

apparent in the course of time. Through this, the selection process involves a second major aspect, i.e. exploration in space. The alignment of events in space and time, of successive behavioural patterns and plant characters favourable for reproduction, determines the process of host selection.

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CHAPTER 2

THE DESIGN OF A LOW-SPEED WIND TUNNEL AS AN INSTRUMENT FOR THE STUDY OF OLFATORY ORIENTATION IN THE COLORADO BEETLE (*LEPTINOTARSA DECEMLINEATA*)

BY

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Aerodynamic phenomena relevant to the olfactory orientation of walking adult Colorado beetles are discussed and a low-speed wind tunnel suitable for the study of olfactory orientation is described. It is concluded that in this wind tunnel the beetles respond to attractive plant volatiles by odour-conditioned anemotaxis. The presence of potted potato plants in the air stream of the wind tunnel causes positive anemotactic responses of male and female Colorado beetles. The maximum positive anemotactic response of the male beetles is obtained after 6 hours of starvation. Fully grown potato plants are more attractive to male beetles than young plants.

Two approaches are available for studying the responses of insects to olfactory stimuli. One involves presenting the odour in still air, limiting the range of the signal to very short distances. In still air the larvae of the Colorado beetle, *Leptinotarsa decemlineata* Say perceive olfactory stimuli of their host plant effectively at a distance of up to a few millimetres (Chin, 1950). The second approach makes use of wind-borne odours, which can be perceived at longer distances (Bossert & Wilson, 1963; Bossert, 1968).

In the past, tests with wind-borne volatiles in Y-tube olfactometers have shown olfactory attraction of Colorado beetles to their host plant potato (McIndoo, 1926; Schanz, 1953; Jermy, 1958). In experiments of this type negative results can be misleading because the two air streams mix at the junction, interfering with the insects' discrimination between them. Moreover, there is little room for the animal to behave normally.

Low-speed "wind tunnels" have therefore been employed in the study of olfactory orientation to plant volatiles (Haskell *et al.*, 1962; Kennedy & Moorhouse, 1969; de Wilde *et al.*, 1969; de Wilde, 1974; Coaker & Hawkes, 1974), pheromones (Farkas & Shorey, 1972; Mayer, 1973; Kennedy & Marsh, 1974) and host vapours (Gatehouse & Lewis, 1973).

It is generally held that long-range olfactory orientation in insects is brought about by an anemotactic response (Grubb, 1973). In this way, the wind acts not only as the dispersal medium of volatiles, but also guides the insects to the source of the stimulus. Hence the reliability of data on olfactory orientation depends on a

number of aerodynamic phenomena of the air flow. This paper describes these phenomena which are important in designing a wind tunnel for olfactory orientation of Colorado beetles to host plant vapours.

Wind Tunnel Theory

The turbulent components of air flow are usually important (Bradshaw, 1971). Outdoors, a high degree of turbulence tends to distribute the vapours more uniformly through the atmosphere (Bossert & Wilson, 1963) and is likely to interfere with directional cues for the searching insect.

The flow of air in a tunnel may be turbulent or laminar, depending on the diameter of the tunnel and the air velocity under a given set of conditions. In the main stream of a wind tunnel, the transition of laminar to turbulent flow occurs at a critical air flow velocity U_{crit} given by the following formula (Schlichting, 1955; Ower & Pankhurst, 1966):

$$U_{crit} = \frac{2300 \cdot \nu}{D_H}$$

2300 being the critical Reynolds number; ν is usually termed the kinematic coefficient of viscosity of the medium; D_H is named the hydraulic diameter, $D_H = 4A/C$; A denotes the cross-sectional area and C denotes the perimeter of the wind tunnel. The relationship between the critical velocity U_{crit} of the main stream in a wind tunnel and its hydraulic diameter D_H is illustrated in Fig. 1. Obstructions in the air stream, e.g. plants, other odour sources, air filters and the ventilator of the wind tunnel, increase the amount of turbulence, which declines with the distance. Low turbulence can be obtained in the following manners: (1) by increasing the distance between the obstructions and the test section of the tunnel ("setting region") and (2) by keeping the initial section of the tunnel, containing the obstructions, wider than the terminal test section (Pankhurst & Holder, 1952; Pope, 1954); in such a low velocity compartment of the wind tunnel, obstructions will give rise to little turbulence, and (3) damping screens will reduce its intensity. By the action (4) of a tunnel contraction, the turbulent velocity components of the air stream, passing through this contraction into the narrower test section, decrease mainly in the longitudinal direction.

In a thin layer near the wall the air velocity, u , is lower than in the main stream, U , as shown in Fig. 2. The thickness of this boundary layer δ is defined as the distance from the wall where $u/U = 0.99$ (Schlichting, 1955), and increases along the plate in a downstream direction. The boundary layer near the leading edge of the plate is laminar and becomes turbulent further downstream. In case of a flat plate with a sharp upwind edge the transition of laminar to turbulent flow occurs at a distance from the leading edge, the region of transition x_t , given by the following formula:

$$R_{crit} = \frac{U \cdot x_t}{\nu} = 3.2 \cdot 10^5 \text{ to } 5 \cdot 10^5$$

$3.2 \cdot 10^5$ to $5 \cdot 10^5$ being the critical Reynolds number, depending on the intensity of

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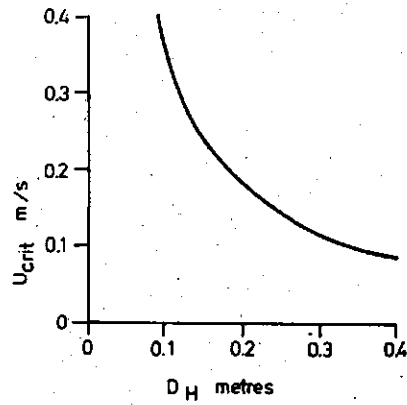


Fig. 1. The relationship between the critical velocity U_{crit} of the main stream in a wind tunnel and its hydraulic diameter D_H at 25° and 760 mm Hg ($\nu = 0.1544 \cdot 10^{-4} \text{ m}^2/\text{s}$). Exceeding the critical velocity implies that the air flow will be turbulent.

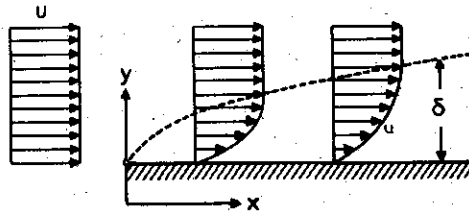


Fig. 2. Sketch of boundary layer on a flat plate in parallel flow. It is shown that there is a thin layer near the wall in which the air velocity u is smaller than in the main stream, U . The thickness of this boundary layer δ increases along the plate in a downstream direction.

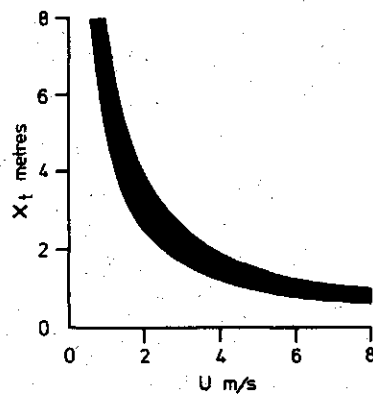


Fig. 3. The transition from a laminar to a turbulent boundary layer on a flat plate occurs at regions x_t from the leading edge, depending on the air velocity in the free stream, U ($\nu = 0.1544 \cdot 10^{-4} \text{ m}^2/\text{s}$).

WIND TUNNEL FOR STUDY OF OLFACTORY ORIENTATION

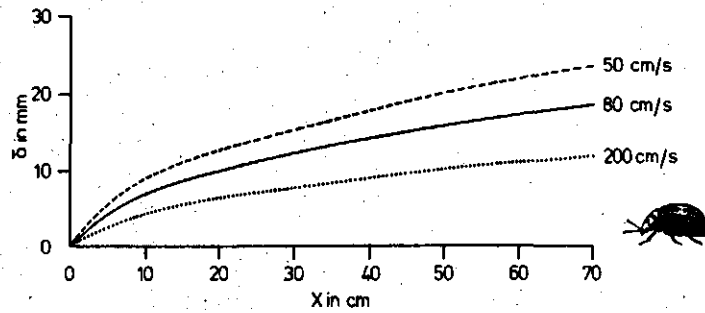


Fig. 4. The thickness of the laminar boundary layer δ along a flat plate at several distances x from the upwind edge and with different velocities of the main stream (at 25° and 760 mm Hg). For comparison, a Colorado beetle in real size is shown.

the turbulence in the free stream. The relationship between the main stream velocity U and the region of transition x_t is shown in Fig. 3. The thickness of the laminar boundary layer can be calculated, as in Fig. 4, by applying the formula given by Blasius (Schlichting, 1955):

$$\delta = 5 \sqrt{\frac{\nu x}{U}}$$

where x denotes the distance from the leading edge. The thickness of the boundary layer might have important effects on the olfactory orientation of walking insects. As small walking insects move in the boundary layer, the possibility of their detecting the air flow and its direction can be limited by the thickness of the boundary layer.

Wind Tunnel Design

Diagrams of the low-speed wind tunnel are given in Figs. 5 and 6. A centrifugal ventilator (Fischbach D770/E650-4; c.v.) sucks air through two air filters (A.A.F. Amer-glass Blue and Varicel 60 PS; a.f.1.-a.f.2.). The resistances of a 80 mesh screen (s.) and the activated charcoal filter (a.c.f.) raise the air pressure in the pressure section (pr. s.), allowing pressure equilibration. The air leaves the filter at a more uniform flow rate across the cross-section, leaving most unwanted volatiles behind. The activated charcoal filter consists of folded perforated metal plates, spaced 17 mm apart and filled with activated charcoal pellets. In experiments on olfactory orientation undamaged potted plants are placed in the next section (pl.s.) through a door (d.) at one side of the wind tunnel. Four 18 mesh damping screens 0.34 mm wire (d.s.1-d.s.4), the setting region (s.r.) and the contraction (t.c.) decrease the turbulence intensity of the air flowing into the narrower insect test section (i.t.s.). By the contraction, the air velocity is raised ten times in the insect test section as compared with the preceding region of low velocity in the wind tunnel. The wind speed can be set by the power control of the ventilator. The test section is enclosed by walls ("closed throat" type), equipped with several glass windows (g.w.) which can be opened. A glass walking plate (w.p.) 70 cm long, 23.5

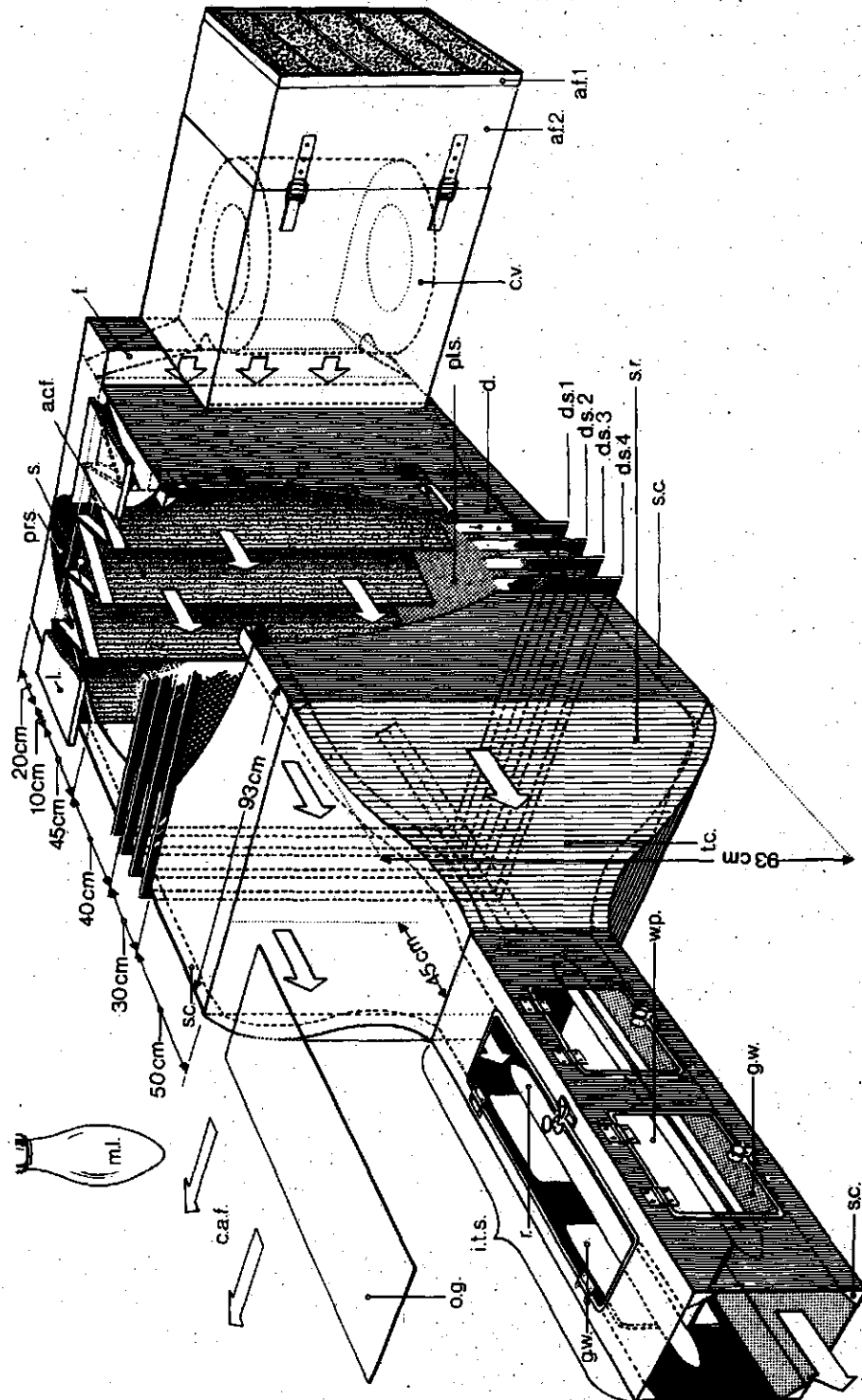


Fig. 5. Diagram of the low-speed wind tunnel. a.c.f.: activated charcoal filter; af.1.-af.2.: air filters; c.a.f.: cooling air flow; c.v.: centrifugal ventilator; d.: door; ds.1.-ds.4.: four damping screens; f.: funnel; g.w.: glass windows sealed with silicone tubing; l.i.s.: insect test section; l.: lid of activated charcoal filter sealed with Ethafoam; m.l.: high-pressure mercury bulb; o.g.: opaline glass; p.r.s.: plant section; p.r.s.: pressure section; r.: streamlined rails; s.: screen; s.c.: streamlined corners; s.r.: setting

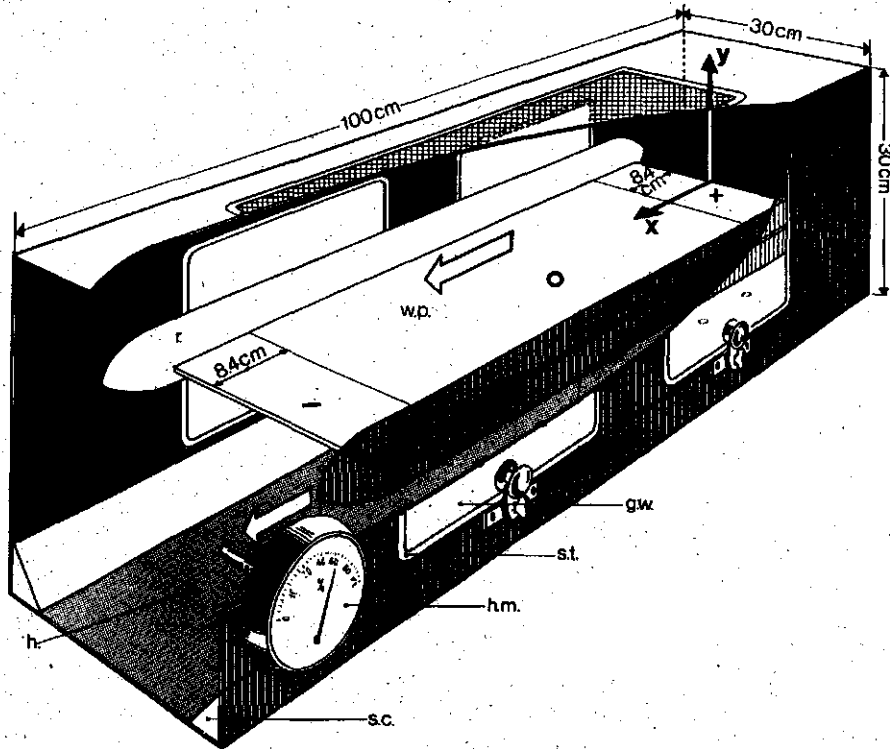


Fig. 6. Detailed diagram of the insect test section. h.: holes; h.m.: hygrometer; s.t.: silicone tubing; x,y: ordinates; + : positive, 0 : indifferent and - : negative regions of the walking plate. Arrows indicate the direction of the air flow. See Fig. 5 for further explanations.

cm wide and 3 mm thick, scoured with Carborundum, is fixed at half height in the test section with streamlined rails (r.). The side glass windows are darkened with black paper on the outsides and the walking plate is illuminated from above by a high-pressure mercury bulb (Philips HPL 400W; m.l.), cooled by outside air (c.a.f.). In the bottom of the insect test section 36 holes (h.) are available for the introduction of hot-wire probes of the anemometer, normally these holes are closed. The wind tunnel is housed in a controlled environment room; at least 90% of the total air volume of this room is replaced by fresh air every 30 minutes. Air is taken from the room and returned again by this "open circuit" tunnel. Contamination problems like those described by Browne *et al.* (1974), have been avoided by using clean air and "non-emitting" materials in the construction of the wind tunnel; mainly zinc, but also brass, iron, glass, silicone tubing and Ethafoam (Dow Chemical).

MATERIAL AND METHODS

The measurement of air flow

A hot-wire anemometer (Disa 55D05) with temperature-compensated hot-wire probes (Disa 55P81) was used in the measurements of air flow velocities in the

boundary layer of the rough glass walking plate in the insect test section. In combination with an r.m.s. voltmeter (Disa 55D35), a Digitest voltmeter and an oscilloscope (Tektronix 502A), the intensity of turbulence of the main stream was defined in the insect test section. The measurements were performed at 20°.

The olfactory orientation of Colorado beetles

After emergence the male and female Colorado beetles of the laboratory stock culture were separated and reared on greenhouse-grown potato plants (cultivar Eigenheimer) at an 18 hour photoperiod at 25° and 60% relative humidity. Before the experiments, individual beetles were isolated in vials and starved for several hours. In the insect test section of the wind tunnel the responses of these individuals to a "control air stream" were examined, pots with wetted soil and sticks standing in the plant section, and also their responses to the air stream plus potato plant volatiles by the introduction of four greenhouse-grown potted potato plants (cultivar Eigenheimer) into the plant section. Care was taken not to bruise the leaves and stems. Small potato plants were raised, so that the foliage was positioned at half height in the plant section. The streamlined rails on both sides of the walking plate were painted with Fluon (Polytetrafluoro-ethylene dispersion, Imperial Chemical Industries) to prevent beetles from climbing up. The upwind and downwind edges were also painted with Fluon, thus causing beetles reaching these edges to fall off.

In each experiment, one beetle was placed carefully in the centre of the walking plate and was allowed to walk around. Five minutes after it started locomotion, the position of this individual on the walking plate was recorded. The response was noted as positive when the beetle fell off the upwind edge or was situated within 8.4 cm from the upwind edge, and as negative when the animal fell off the downwind edge or was situated within 8.4 cm from the downwind edge. Otherwise the response was noted as indifferent (see also Fig. 6). The results were analysed statistically by the χ^2 test (Siegel, 1956), positive choices versus combined negative and indifferent choices.

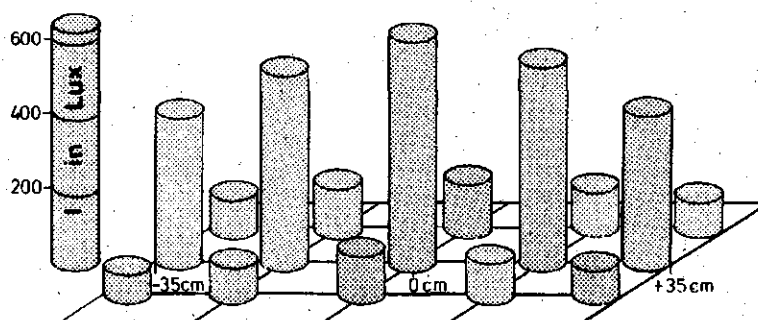


Fig. 7. The distribution of light intensities over the walking plate in the insect test section. Ordinate: intensity. Abscissa: +35 cm = upwind edge, 0 cm = centre and -35 cm = downwind edge of the walking plate.

The velocity of the main stream was set at 80 cm/s in the insect test section. The wind tunnel was housed in a controlled environment room at 25° and 60% R.H. The four damping screens in the low velocity compartment of the wind tunnel were removed. At the in- and outstream ends of the insect test section 18 mesh screens were fixed in order to obtain a more uniform light distribution over the walking plate. This light intensity distribution was measured by a Hartmann & Braun EBLXI photometer, fitted with a circular detector of 66 mm diameter, and is shown in Fig. 7.

RESULTS AND DISCUSSION

The measurement of air flow

The hydraulic diameter of the insect test section is 0.3 m, and as a consequence, wind exceeding a speed of 12 cm/s will be turbulent in the main stream of the test section (Fig. 1). The percentage of turbulence, an indication the amount of turbulence, can be defined (Schlichting, 1955) as:

$$\% T = 100 \cdot \frac{\sqrt{\bar{u}^2}}{U}$$

where $\sqrt{\bar{u}^2}$ denotes the mean velocity fluctuations of the air flow and U is the mean air flow rate. Without damping screens in the low velocity compartment of the wind tunnel, the percentage of turbulence in the main stream of the insect test section is 3% at a wind speed of 1 m/s. With the four damping screens installed,

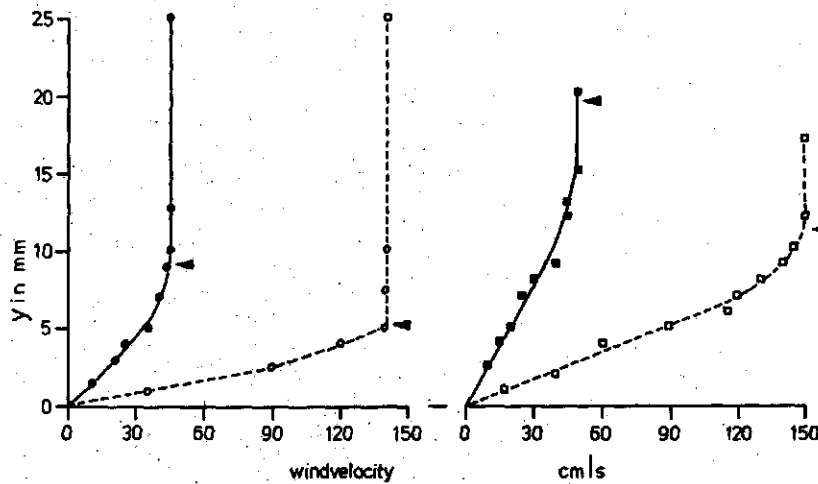


Fig. 8. Measurements of the wind speeds at several heights y above the walking plate and with different main stream velocities U in the insect test section. 10 cm downwind the leading edge: U = 45 cm/s (●—●) and U = 140 cm/s (○---○); 50 cm downwind the leading edge: U = 50 cm/s (■—■) and U = 150 cm/s (□---□). Arrows indicate the laminar boundary layer thickness, calculated by applying the formula of Blasius (at 20° and 760 mm Hg, $\nu = 0.1499 \cdot 10^{-4} \text{ m}^2/\text{s}$).

J. H. VISSER

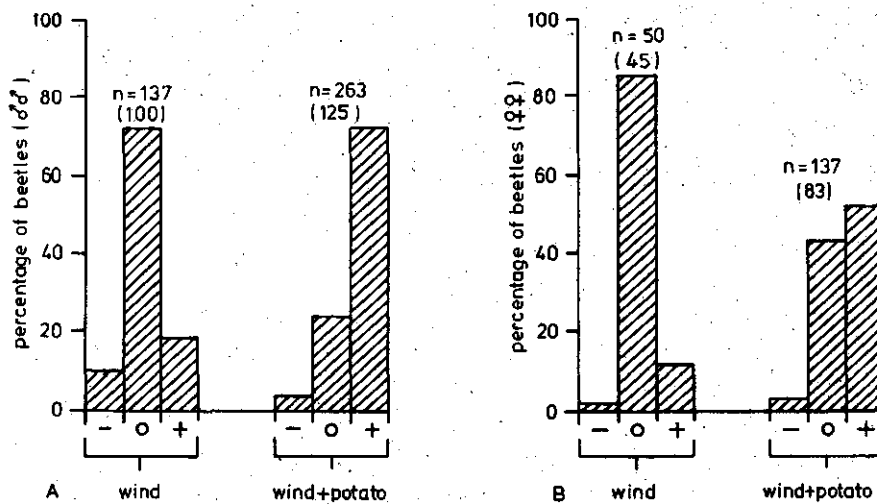


Fig. 9. The responses of Colorado beetles to wind and to wind plus potato plant volatiles. Undamaged potted potato plants 2-8 weeks old were used. Abscissa: - = negative, 0 = indifferent and + = positive choices. A. male beetles 0-4 weeks after emergence and starved for 1-17 h. B. female beetles 0-2 weeks after emergence and starved for 1-19 h. In parentheses the number of individuals; n is the number of tests.

measurements revealed that this percentage of turbulence was reduced to 1% at a wind speed of 1 m/s. Because the amount of turbulence in the test section proved to be limited, the damping screens were omitted in the olfactory orientation studies. Air flow measurements and the introduction of smoke behind the activated charcoal filter showed many eddies, a high degree of turbulence originating from the folded perforated metal plates of the filter. This turbulence appeared to decrease in the setting region and in the tunnel contraction. Volatiles of plants placed directly behind the activated charcoal filter, are thus distributed over the total cross-sectional area of the wind tunnel, resulting in a uniform vapour concentration in the insect test section. Therefore, in this wind tunnel, while it is not possible for the beetles to react to concentration gradients (e.g. osmotropotaxis, see Haskell *et al.*, 1962), they will respond to attractive plant volatiles by an odour-conditioned anemotaxis. The main stream of the test section exhibited a uniform air flow rate.

The total boundary layer of the walking plate will be laminar, when the main stream does not exceed 7.1 m/s in the insect test section (Fig. 3). At several positions on the walking plate and at different free stream velocities, air flow measurements in the boundary-layer, as shown in Fig. 8, revealed values in good agreement with the formula of Blasius. In the olfactory orientation studies the main stream velocity was set at 80 cm/s in the insect test section, the resulting laminar boundary layer over the walking plate being illustrated in Fig. 4.

The olfactory orientation of Colorado beetles

The responses of walking individual male and female Colorado beetles to wind and to wind plus potato plant volatiles were examined, as shown in Fig. 9.

WIND TUNNEL FOR STUDY OF OLFACTORY ORIENTATION

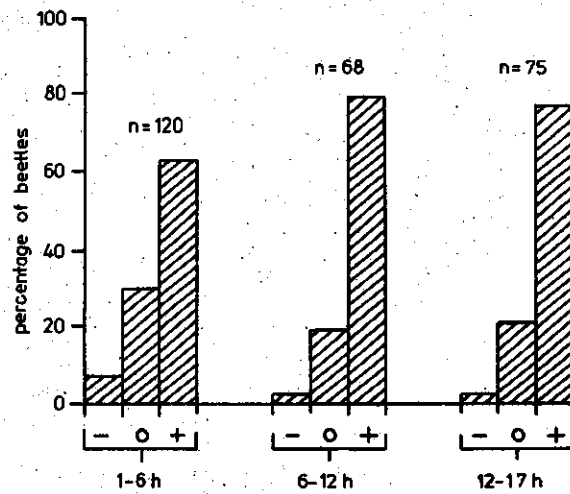


Fig. 10. The effects of prolonged starvation on the responses of male Colorado beetles to wind plus potato plant volatiles. Abscissa: — = negative, 0 = indifferent and + = positive choices. The male beetles were starved for periods of 1-6 h, 6-12 h and 12-17 h; n is the number of tests.

Subsequently the figures obtained in these tests with male Colorado beetles were re-arranged in order to differentiate between various starvation periods (Fig. 10) and various potato plant ages (Fig. 11). Confusing the effects of these last-mentioned conditions was thereby avoided. As preliminary experiments with groups of Colorado beetles showed that the individuals affected one another's behaviour, it was decided to use single individuals.

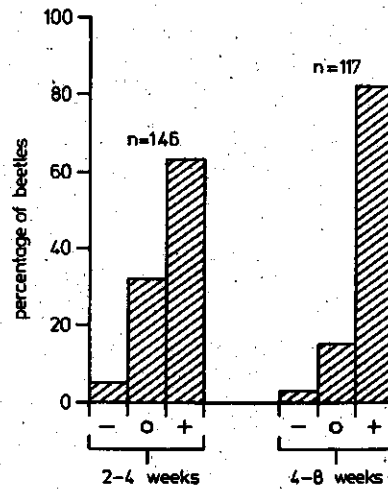


Fig. 11. The effects of the age of potato plants on the responses of male Colorado beetles to wind plus potato plant vapours. Abscissa: — = negative, 0 = indifferent and + = positive choices. Undamaged potted plants 2-4 weeks and 4-8 weeks old were used; n is the number of tests.

Without potato plant vapours, starved beetles tended to move slightly upwind (Fig. 9). This probably does not result from the distribution of light intensity over the walking plate (see Fig. 7). The responses to wind were not altered by different starvation periods. The introduction of potato plants into the air stream caused substantial positive anemotactic responses of male and female beetles ($p < 0.001$). The nature of the responses did not change remarkably with the age of the beetles. The maximum positive anemotactic responses of male beetles to potato plant volatiles were obtained after 6 hours of starvation (Fig. 10); 1-6 h compared with 6-12 h: $p < 0.02$. Moorhouse (1969) showed that, in locusts, this response was maximal after they had been starved for 4 hours or longer. Potato plants grown for 4-8 weeks, 60-100 cm high, were more attractive to male beetles than 2-4 weeks old plants, 20-60 cm high (Fig. 11, $p < 0.001$). This is probably caused by a lower amount of attractive volatiles being emitted by the young plants.

In long-range olfactory orientation of insects, the wind dispersing the volatile sign-stimuli through the atmosphere directs the insects to the source of the stimulus. Bossert & Wilson (1963) have demonstrated the effect of wind speed on the distribution of the signal. In the atmosphere the amount of turbulence is increased at higher wind velocities, "diluting" the attractant in the air flow.

Turbulence is also likely to interfere with anemotactic orientation of insects in another way. The scale of turbulence, the magnitude of turbulent eddies may affect the ability of insects to detect the main direction of the air flowing downwind from the odour source. The size of an insect is small compared to the big eddies which arise from obstructions in the air flow, like the vegetation. Walking insects are faced with a second obscuring factor; the boundary layer in which the air velocities are less than in the free stream. Linsenmair (1969, 1970, 1973) showed that several walking coleopterous species not subjected to attractants are able to use pure anemomenotactic orientation. In these experiments, the lowest wind velocities which will evoke anemomenotactic orientation were about 0.15 m/s for *Geotrupes* and 0.4 m/s for *Pimelia* species. In the field, the size of the boundary layer over the ground will be much larger than the laminar one over the walking plate in the test section. It is therefore likely that the low air velocities in the boundary layer will impair the anemotactic responses of walking insects. Vogel (1969) has discussed the design of low-speed wind tunnels for biological investigations and he, too, did not mention the boundary layer phenomena. Outdoors, it is observed that Colorado beetles searching for potato plants frequently climb tiny hills obviously thus "escaping" the boundary layer.

In view of this it is likely that the statement of Jermy (1958), that the olfactory responses of Colorado beetles to potato plants are limited to a range of a few dm, is not in conflict with the results obtained by McIndoo (1926), Schanz (1953), de Wilde *et al.* (1969) and de Wilde (1974). Furthermore, the age of the potato plants may have affected the outcomes of his field experiments.

Linsenmair (1969) observed preference for upwind angles in the anemomenotaxis of *Pimelia*, *Tenebrio* and *Geotrupes* species. In addition, he mentioned (1973) anemomenotactic orientation of *Leptinotarsa decemlineata*.

We observed that in a clean air flow the Colorado beetles spent much time on the sides of the walking plate; the positive anemotaxis of the beetles in wind without potato plants, distinct in the experiments of de Wilde *et al.* (1969) and de Wilde (1974), and less marked in the present investigation, seems to be a transformed anemomenotaxis. The findings of de Wilde *et al.* (1969) and de Wilde (1974) that starvation enhances the anemotactic response in "pure" air contradict with my own observations and the results of Linsenmair (1969, 1973) with other coleopterous species. It is advantageous for walking insects searching for food to move by an anemomenotactic orientation (Linsenmair, 1969). Wind-borne attractants will then shift the angle of their orientation to the upwind direction, guiding the insects to the source of the stimulus.

In 1926 McIndoo showed that Colorado beetles can be attracted by emanations from their living host plants. In the experiments of Schanz (1953) humidity was excluded as a causal factor in the attraction of Colorado beetles. Jermy (1958) did not believe that low concentrations of potato plant volatiles were sufficient to play a rôle in host plant finding from large distances. He concluded that contact chemoreception is the principal factor in host plant selection by Colorado beetles.

Our present observations reveal that unbruised potato plants can also attract Colorado beetles at a longer range, by inducing positive anemotactic responses. More extended experiments, using the actual tracks of the beetles showing that these responses were not caused by a kinetic effect, will be published in a subsequent paper.

I wish to acknowledge the valuable advice on the design of the wind tunnel given by Prof. W. Draijer and Ir. A. Kortleven, and to thank Ir. C. Keizer for his help. I am indebted to our technicians Mr. G. van Surksun, Mr. G. van den Brink, Mr. O. van Geffen, Mr. G. G. Schuurman and Mr. B. Tollenaar for constructing the wind tunnel, and to Mr. W. C. Th. Middelplaats and Mr. F. J. J. von Planta for drawing the figures. I am grateful to Prof. Dr. J. de Wilde and Prof. Dr. L. M. Schoonhoven for criticizing the manuscript, and I thank Prof. Dr. K. N. Saxena for reading the manuscript.

ZUSAMMENFASSUNG

BESCHREIBUNG EINES WINDTUNNELS FÜR GERINGE WINDGESCHWINDIGKEIT ALS GERÄT ZUM STUDIUM DER GERUCHSORIENTIERUNG DES KARTOFFELKÄFERS (LEPTINOTARSA DECEMLINEATA)

Aerodynamische Phänomene werden diskutiert, die für die geruchliche Orientierung der adulten Kartoffelkäfer von Bedeutung sind, nämlich der Übergang von wirbelfreier zu turbulenter Strömung im freien Strom; die Grenzschicht; der Übergang von wirbelfreier zu turbulenter Strömung in der Grenzschicht und die Dicke der wirbelfreien Grenzschicht. Ein Windtunnel für niedrige Windgeschwindigkeit wird beschrieben, der sich für das Studium der geruchlichen Orientierung des Kartoffelkäfers als günstig erwies. Messungen der Windströmung in diesem Tunnel zeigten, daß der Prozentsatz von Turbulenz im Hauptstrom der Insektenprüfkammer niedrig ist und daß eine einheitliche Lockstoffkonzentration in dieser Kammer vorliegt. Es wird geschlossen, daß die Käfer auf

pflanzliche Duftstoffe mit Geruch-induzierter Anemotaxis reagieren. Die Dicke der wirbelfreien Grenzschicht über der Laufplatte in der Prüfkammer wurde ermittelt.

Die Einführung von getopften Kartoffelpflanzen in den Luftstrom des Windtunnels löst positive anemotaktische Reaktionen bei Männchen und Weibchen des Kartoffelkäfers aus. Die stärkste positive anemotaktische Reaktion der Männchen wird nach sechs Stunden Hunger erzielt. Ausgewachsene Kartoffelpflanzen sind attraktiver für Männchen als junge.

In der Diskussion wird vermutet, daß die Größe der Turbulenz und die Dicke der Grenzschicht unter Freilandbedingungen die geruchlich induzierte anemotaktische Reaktion der Insekten beeinträchtigt.

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CHAPTER 3

SPECIFICITY IN THE OLFACTORY ORIENTATION OF THE COLORADO BEETLE, *LEPTINOTARSA DECEMLINEATA*

BY

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Wind induces positive anemotactic responses of unfed newly-emerged female Colorado beetles. These responses are enhanced by the volatiles of several solanaceous species i.e. *Solanum tuberosum*, *S. nigrum*, *S. luteum*, *S. dulcamara*, *S. lycopersicum*, *Nicotiana tabacum*, *Capsicum annuum* and *Petunia hybrida*, and a non-solanaceous species, *Tropaeolum majus*. *Brassica oleracea*, *B. pekinensis*, *Raphanus sativus* and *Phaseolus vulgaris* are neutral in this respect. Unfed post-diapause Colorado beetles also react to the vapours of undamaged potted potato plants. It is concluded that the olfactory orientation will mainly lead the Colorado beetle towards solanaceous species. When contact results, further steps in host plant selection will occur.

In the host selection of phytophagous insects, the existence of an initial olfactory orientation phase has not been overlooked but, owing the difficulties in experimentation, has been poorly studied. The Colorado beetle, *Leptinotarsa decemlineata* Say, is one of the exceptions in this respect. McIndoo (1926), Chin (1950), Schanz (1953), Grison (1957), Jermy (1958), de Wilde *et al.* (1969), Bongers (1970) and de Wilde (1974) reported attraction of larvae and adult Colorado beetles by potato plant volatiles. However, the rôle of this attraction in the host selection of this oligophagous insect was estimated very unequally. Most of this divergence is related to the various methods of stimulus application. Visser (1976) described the design of a low-speed wind tunnel, and making use of this equipment, he showed attraction of adult Colorado beetles towards potted potato plants being caused by odour-conditioned anemotaxis. The wind, dispersing the plant volatiles through the atmosphere, guides the insects towards the source of the stimulus. Under field conditions, the scale of turbulence and the thickness of the boundary layer over the underlying substrate will impair anemotactic responses of insects.

The present study gives more extended information of the olfactory orientation of adult female Colorado beetles under laboratory conditions. Our observations concentrate on the specificity in this initial phase of host selection.

MATERIAL AND METHODS

Female Colorado beetles were obtained from the laboratory stock culture. After emergence they were separated from the males. Prior to the experiments they

were isolated in vials and starved for several hours at an 18 hour photoperiod, 25° and 60% relative humidity. In the wind tunnel described by Visser (1976), the responses of these individuals to wind-borne volatiles of several plant species were examined. This low-speed wind tunnel comprises two main parts, namely a low velocity compartment and the insect test section. In experiments on olfactory orientation, undamaged potted plants were placed in the low velocity compartment at a distance of 125 cm from the test section. Small plants were raised, so that the foliage was positioned at half height in this section. In the insect test section, a rough glass walking plate was fixed with streamlined rails, and was illuminated from above by six fluorescent lamps (Philips TL MF 40W/33RS). The distribution of light intensities over the walking plate was measured by a Hartmann & Braun EBLX3 photometer, fitted with a circular detector of 66 mm diameter, and is shown in Fig. 1. Six red lines, spaced equally at 10 cm intervals, were drawn on the underside of the walking plate (see Fig. 2).

In each experiment one female beetle was placed carefully in the centre of the walking plate and was allowed to walk around. After the beetle started locomotion, it was observed for 5 minutes, or until it reached one of the edges of

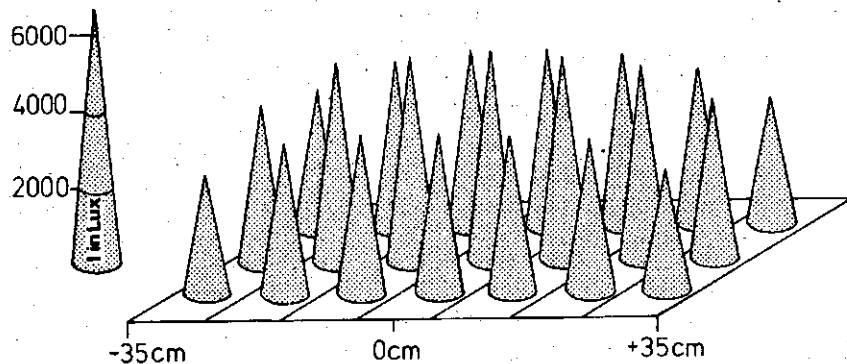


Fig. 1. The distribution of light intensities over the walking plate (represented in skew perspective). Ordinate: intensity. Abscissa: +35 cm = upwind edge, 0 cm = centre and -35 cm = downwind edge of the walking plate.

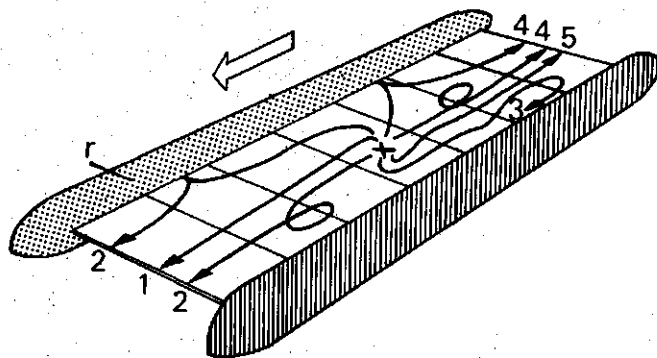


Fig. 2. Walking plate fixed with streamlined rails (r). Beetles were released at the cross. 1—5: category of response. Arrow indicates the direction of the air flow.

the walking plate. The tracks of the beetles were divided into five categories (Fig. 2):

1. straight to the downwind edge; crossing each of the three lines once and not touching the rails.
2. indirectly to the downwind edge; crossing a line more than once or touching the rails.
3. reaching neither of the edges.
4. indirectly to the upwind edge; crossing a line more than once or touching the rails.
5. straight to the upwind edge; crossing each of the three lines once and not touching the rails.

The same beetle was subjected to control and experimental situations. The results were analysed statistically by the Sign Test (Siegel, 1956); using criterion A, the categories of responses, and criterion B, the length of the periods required to reach the upwind edge.

Undamaged greenhouse-grown potted plants were used i.e. *Solanum tuberosum* L. (cultivar Eigenheimer), *Solanum nigrum* L., *Solanum luteum* Mill., *Solanum dulcamara* L., *Solanum lycopersicum* L., *Nicotiana tabacum* L., *Petunia hybrida* (Hook.) Vilm., *Capsicum annuum* L., *Phaseolus vulgaris* L., *Raphanus sativus* L., *Brassica oleracea* L. var. *gemmifera* DC., *Brassica pekinensis* Rupr. and *Tropaeolum majus* L.. The number of plants used per experiment was arranged to give approximately the same leaf area.

The experiments were conducted in a controlled environment room at 25° and 60% R.H.. In the insect test section the wind speed was set at 80 cm/s.

RESULTS AND DISCUSSION

Two groups of 42 female Colorado beetles each, one group starved for 24 hours and the other starved for 72 hours, were tested in still air and in wind without plant odour (Table I). Wind induced positive anemotactic responses of these unfed newly-emerged beetles (criterion A), and the individuals starved for 72 hours were

TABLE I

Individual responses of unfed newly-emerged female Colorado beetles, in still air and in wind without plant odour

		Category of response					Criterion	
		5	4	3	2	1	A	B
starved for 24 hours	no wind	0	5	26	11	0		
	wind	1	14	21	6	0	*	ns
starved for 72 hours	no wind	1	7	29	5	0		
	wind	4	19	16	3	0	*	*

ns: not significant at $P > 0.01$

*: significant at $P < 0.01$

faster in reaching the upwind edge (criterion B). Schanz (1953), de Wilde *et al.* (1969), de Wilde (1974) and Visser (1976) reported positive anemotaxis of Colorado beetles in an air flow devoid of plant vapours. However, it is plausible that this type of anemotaxis is in fact a transformed anemomenotaxis (Linsenmair, 1969 and 1973; Visser, 1976).

As the same individuals were used in different situations, it was important to make sure that the responses of individual beetles were not influenced by earlier experience in the test section of the wind tunnel. For this purpose two groups of female Colorado beetles were observed. Group A was tested successively three times in pure wind and once with wind-borne volatiles of four fully grown potato plants standing in the low velocity compartment of the wind tunnel. Group B was subjected alternately to a pure air flow and to potato plant odour (Table II). Wind-borne potato plant odour strongly attracted the unfed newly-emerged Colorado beetles. These responses and those to pure wind were not altered by previous tests. Visser (1976), making use of different criteria, showed attraction of fed male and female Colorado beetles by potato plant volatiles in this wind tunnel. In the present investigation, the light intensity over the walking plate was raised ten times (Fig. 1), without affecting the responses.

TABLE II

Effect of previous tests on the individual responses of unfed newly-emerged female Colorado beetles, starved for 24 hours

	Successive tests	Category of response					Criterion ²	
		5	4	3	2	1	A	B
group A 18 beetles	wind	1	7	8	2	0		
	wind	1	4	10	3	0	ns	ns
	wind	0	4	13	1	0	ns	ns
	wind + potato	9	9	0	0	0	**	**
group B 17 beetles	wind	0	6	8	3	0		
	wind + potato	11	6	0	0	0	**	**
	wind	0	8	4	5	0	ns	ns
	wind + potato	12	5	0	0	0	**	**

²) the levels of significance were compared with the first test pure wind

ns: not significant at $P > 0.01$

** : significant at $P < 0.001$

To investigate the specificity of the olfactory orientation, female Colorado beetles were subjected to the vapours of several plant species. On the same day, six different experimental situations were presented in random order. Results from three series are given in Table III. All the solanaceous species tested i.e. *S.tuberosum*, *S.nigrum*, *S.luteum*, *S.dulcamara*, *S.lycopersicum*, *N.tabacum*, *C.annuum* and *P.hybrida*, elicited substantially more and faster upwind movements of unfed newly-emerged Colorado beetles than a pure air flow. One non-solanaceous plant species, *Tropaeolum majus*, also attracted these beetles. *Brassica oleracea*,

B. pekinensis, *Raphanus sativus* and *Phaseolus vulgaris* were neutral in this respect. To a certain extent, unfed newly-emerged female Colorado beetles possess olfactory discriminative capacities, enabling them to select plants at a distance.

TABLE III

Individual responses of unfed newly-emerged female Colorado beetles, starved for 24 hours, to several plant species

	Number of plants	Category of response					Compared with wind criterion		Compared with potato criterion	
		5	4	3	2	1	A	B	A	B
wind		0	6	10	4	0				
<i>Solanum tuberosum</i>	4	5	15	0	0	0	**	**		
<i>Raphanus sativus</i>	12	2	7	10	1	0	ns	ns	*	*
<i>Brassica oleracea</i>	6	0	10	7	3	0	ns	ns	**	**
<i>Brassica pekinensis</i>	12	0	11	6	3	0	ns	ns	**	**
<i>Tropaeolum majus</i>	4	4	11	4	1	0	**	**	ns	**
wind		0	4	13	3	0				
<i>Solanum tuberosum</i>	4	7	12	1	0	0	**	**		
<i>Solanum nigrum</i>	6	3	12	5	0	0	*	*	ns	ns
<i>Solanum luteum</i>	6	6	12	2	0	0	**	**	ns	ns
<i>Solanum dulcamara</i>	4	3	14	3	0	0	**	*	ns	**
<i>Solanum lycopersicum</i>	5	4	14	2	0	0	**	**	ns	ns
wind		0	7	12	1	0				
<i>Solanum tuberosum</i>	4	6	14	0	0	0	**	**		
<i>Nicotiana tabacum</i>	4	9	11	0	0	0	**	**	ns	ns
<i>Capsicum annum</i>	6	3	15	1	1	0	*	**	ns	ns
<i>Petunia hybrida</i>	5	9	9	2	0	0	**	**	ns	ns
<i>Phaseolus vulgaris</i>	12	2	8	8	2	0	ns	ns	*	*

ns: not significant at $P > 0.01$

*: significant at $P < 0.01$

**: significant at $P < 0.001$

In order to examine whether previous feeding will affect the specificity of olfactory orientation, newly-emerged female Colorado beetles were fed on *S. tuberosum* or *S. dulcamara* for 48 hours. Subsequently, they were starved for 24 hours and subjected to wind-borne odours of several plant species (Table IV). The number of plants used in these series were the same as mentioned in Table III. *Brassica oleracea* and *Solanum nigrum* evoked no significant attraction of the fed beetles. *S. tuberosum*, *S. luteum* and *S. dulcamara* still elicited more upwind movements of beetles fed on potato or bittersweet than wind without plant odour. However, the responses of potato-fed beetles towards *S. dulcamara* were slower than towards *S. tuberosum*. Beetles fed on bittersweet reacted equally towards potato and bittersweet. De Wilde *et al.* (1969) reported olfactory orientation of potato-fed female Colorado beetles towards leaves of *S. nigrum* and *Apium*

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graveolens L.. Newly emerged female beetles were not attracted by the leaves of *A. graveolens*. It is concluded that the specificity of olfactory orientation is not distinctly affected by previous feeding of female Colorado beetles shortly after emergence.

TABLE IV

Responses of female Colorado beetles, previously fed on potato or bittersweet, to several plant species

	Category of response					Compared with wind criterion		Compared with potato criterion	
	5	4	3	2	1	A	B	A	B
fed on potato:									
wind	1	10	7	2	0				
<i>Solanum tuberosum</i>	7	13	0	0	0	**	**		
<i>Solanum nigrum</i>	1	14	5	0	0	ns	ns	*	**
<i>Solanum luteum</i>	3	14	3	0	0	**	*	ns	ns
<i>Solanum dulcamara</i>	3	16	1	0	0	*	ns	ns	**
<i>Brassica oleracea</i>	1	12	7	0	0	ns	ns	**	**
fed on bittersweet:									
wind	1	11	5	3	0				
<i>Solanum tuberosum</i>	9	11	0	0	0	**	**		
<i>Solanum nigrum</i>	4	9	6	1	0	ns	ns	*	**
<i>Solanum luteum</i>	7	13	0	0	0	*	*	ns	ns
<i>Solanum dulcamara</i>	9	11	0	0	0	**	**	ns	ns
<i>Brassica oleracea</i>	2	11	4	3	0	ns	ns	**	**

ns: not significant at $P > 0.01$

*: significant at $P < 0.01$

**: significant at $P < 0.001$

TABLE V

Responses of unfed female Colorado beetles, brought out of diapause 24 hours earlier

	Category of response					Compared with no-wind criterion		Compared with wind criterion	
	5	4	3	2	1	A	B	A	B
no wind	1	3	12	13	1				
wind	1	16	11	2	0	**	**		
potato	3	24	1	2	0			**	**

**: significant at $P < 0.001$

Jermy (1958) noted that post-diapause Colorado beetles reacted only to cut pieces of potato leaf, not to intact leaves. He concluded that olfactory stimuli play a minor rôle in host plant selection. However, experiments with post-diapause

beetles (Table V) demonstrated positive anemotactic responses in a pure air flow, which were enhanced by the introduction of four potato plants to the wind tunnel.

In his Y-tube olfactometer, McIndoo (1926) showed that adult Colorado beetles were directed more towards unbruised potato plants than towards the volatiles emanating from soil. In addition, he noted attraction towards water extracts and steam distillates of potato plant leaves, *Solanum carolinense* L., *Hyoscyamus niger* L., *S. lycopersicum* and *Datura stramonium* L.. However, these responses were liable to substantial variation. Chin (1950) performed screen test experiments with larvae and reported arresting effects of potato, *Solanum demissum* Lindl. and *P. hybrida*. No responses were elicited by *Doronicum pardalianches* L.. Schanz (1953) using a Y-tube olfactometer, established olfactory responses of adult Colorado beetles to potato plant leaves. Air humidity was excluded as the causal factor of this attraction. Grison (1957) mentioned that in screen tests, potato leaves were more attractive to adult beetles than leaves of neutral plant species like *Pisum sativum* L. and cabbage. Jermy (1958) noted that newly-emerged Colorado beetles needed first to have been fed on potato in order to prefer potato leaf odour to the vapours of lucerne, *Medicago sativa* L., in a Y-tube olfactometer. De Wilde *et al.* (1969) found that the leaves of potato, *S. lycopersicum*, *S. dulcamara*, *S. nigrum* and *Apium graveolens* enhanced the upwind movements of adult female Colorado beetles in a wind tunnel. Leaves of *Alnus incana* (L.) Vill. did not elicit olfactory responses,

TABLE VI

Plant species being attractive, neutral and repellent to *Leptinotarsa decemlineata*

Attractive	Neutral	Repellent
Umbelliferae i.e. <i>Apium graveolens</i> ^b	Corylaceae i.e. <i>Alnus incana</i> ^b	Compositae i.e. <i>Taraxacum officinale</i> ^b
Tropaeolaceae, i.e. <i>Tropaeolum majus</i> ³	Papilionaceae i.e. <i>Medicago sativa</i> ⁴ <i>Pisum sativum</i> ^c <i>Phaseolus vulgaris</i> ³	Gramineae i.e. <i>Poa annua</i> ^b
Solanaceae i.e. <i>Solanum tuberosum</i> ^{a-h,3} <i>dulcamara</i> ^{b,3} <i>luteum</i> ³ <i>nigrum</i> ^{b,3} <i>lycopersicum</i> ^{a,b,3} <i>carolinense</i> ^c <i>demissum</i> ^b	Cruciferae i.e. <i>Brassica sp.</i> ^c <i>oleracea</i> ³ <i>pekinensis</i> ³ <i>Raphanus sativus</i> ³	
<i>Nicotiana tabacum</i> ³ <i>Capsicum annuum</i> ³ <i>Petunia hybrida</i> ^{b,3} <i>Hyoscyamus niger</i> ^c <i>Datura stramonium</i> ^c	Compositae i.e. <i>Doronicum pardalianches</i> ^b	

^a: Bongers, 1970; ^b: Chin, 1950; ^c: Grison, 1957; ^d: Jermy, 1958; ^e: McIndoo, 1926; ^f: Schanz, 1953; ^g: Visser, 1976; ^h: de Wilde *et al.*, 1969; ³: present investigation

whereas the beetles were repelled by leaves of *Taraxacum officinale* Weber and *Poa annua* L. Bongers (1970) showed olfactory responses of adult beetles to potato leaves in screen tests and in an olfactometer. The information of these studies and of the present investigation is classed in Table VI.

It is obvious that all the solanaceous species tested evoked olfactory responses of *Leptinotarsa decemlineata*. Their odours probably include some identical components attractive to Colorado beetles. However, only *S.tuberosum*, *S.dulcamara*, *S.lycopersicum*, *S.carolinense* and *H.niger* are able to support feeding, growth and reproduction of Colorado beetles (Chin, 1950; Jermy, 1961; Hsiao & Fraenkel, 1968a,b; Bongers, 1970; Hsiao 1974). *S.luteum* and *S.nigrum*, plant species highly attractive for oviposition, are nevertheless not eaten (de Wilde *et al.*, 1960 Hsiao & Fraenkel, 1968b; Bongers, 1970). *S.demissum* is resistant and *P.hybrida* toxic to the larvae (Chin, 1950). *N.tabacum*, *C.annuum* and *D.stramonium* are unacceptable for support of growth and oviposition (Jermy, 1961; Hsiao & Fraenkel, 1968a,b). The non-solanaceous plants *A.graveolens* and *T.majus* also attracted Colorado beetles. *A.graveolens* is a non-host plant (Hsiao & Fraenkel, 1968b) and *T.majus* has never been recorded as being attacked by the Colorado beetle. The neutral and repellent plant species listed in Table VI cannot be regarded as natural host plants (Chin, 1950; Jermy, 1958, 1961; Hsiao & Fraenkel, 1968a,b).

The present investigation revealed that fed and unfed newly-emerged, as well as unfed post-diapause beetles responded to the vapours of several solanaceous species and one non-solanaceous plant. In these experiments, the stimulus was applied in the most natural way by using undamaged potted specimens, as cutting leaves changes not only the quantitative but also the qualitative composition of the plant odour in the air (Weurman, 1969). Possibly the observed repellent actions of *T.officinale* and *P.annua*, and the attraction by *A.graveolens* (de Wilde *et al.*, 1969) resulted from such effects.

Despite two exceptions, it is clear that the initial olfactory orientation will mainly lead the Colorado beetle in the field towards solanaceous species. Further steps in host plant selection will occur by the combined action of feeding incitants, feeding stimulants, feeding co-factors and inhibitors (de Wilde, 1958; Jermy, 1961, 1966; Hsiao, 1969, 1974).

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ZUSAMMENFASSUNG

SPEZIFITÄT BEI DER GERUCHSORIENTIERUNG DES KARTOFFELKÄFERS, LEPTINOTARSA DECEMLINEATA

Wind induziert positive anemotaktische Reaktionen von frisch geschlüpften, noch nicht gefütterten Weibchen des Kartoffelkäfers. Diese Reaktionen werden verstärkt durch flüchtige Verbindungen

verschiedener Solanaceen, nämlich *Solanum tuberosum*, *S. nigrum*, *S. luteum*, *S. dulcamara*, *S. lycopersicum*, *Nicotiana tabacum*, *Capsicum annuum* und *Petunia hybrida* und eine Nichtsolanacee, *Tropaeolum majus*. *Brassica oleracea*, *B. pekinensis*, *Raphanus sativus* und *Phaseolus vulgaris* waren in dieser Hinsicht neutral. Ungefütterte Postdiapause-Kartoffelkäfer reagieren ebenfalls auf flüchtige Verbindungen von ungeschädigten, getopften Kartoffelpflanzen. Es wird geschlossen, daß die geruchliche Orientierung den Kartoffelkäfer vorwiegend zu Solanaceen-Arten leitet. Sobald sich ein Kontakt ergibt, werden weitere Schritte in der Wirtspflanzenwahl erfolgen.

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CHAPTER 4

ISOLATION AND IDENTIFICATION OF VOLATILES IN THE FOLIAGE OF POTATO, *SOLANUM TUBEROSUM*, A HOST PLANT OF THE COLORADO BEETLE, *LEPTINOTARSA DECEMLINEATA*

BY

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The volatile compounds of *Solanum tuberosum* L., a host plant of the Colorado beetle, *Leptinotarsa decemlineata* Say, were isolated using successively vacuum steam distillation, freeze concentration and extraction. The main components are trans-2-hexen-1-ol, hexanol-1, cis-3-hexen-1-ol, trans-2-hexenal and linalool. The distribution of these compounds in a variety of plant families and their biosyntheses are reviewed. These leaf volatiles constitute a general green leaf volatile complex, being active in the olfactory orientation of the Colorado beetle and as such probably of importance to various phytophagous insects.

As contrasted with the well-studied actions of nonvolatile primary and secondary plant substances (Schoonhoven, 1968; Staedler, 1976), our knowledge is incomplete on the rôle of plant odours in host selection by phytophagous insects, in regard to host plant orientation and the initiation and continuation of feeding. The reported information forms a range of underlying thoughts concerning plant odours :

a. consisting of one or a group of chemically related, host plant specific "key" components, e.g. organic sulfur compounds to *Hylemya antiqua* (Matsumoto & Thorsteinson, 1968a,b) and isothiocyanates of cruciferous species to *Plutella maculipennis* (Gupta & Thorsteinson, 1960a,b);

b. being composed of less host plant specific substances like the large chemical class of terpenes and their derivatives acting on a variety of forest pest insects (see e.g. Werner, 1972; Staedler, 1974; Selander *et al.*, 1974);

c. existing as a complex, a mixture of generally distributed unspecific components (Rodriguez *et al.*, 1976). According to the latter view the total "essence" is required in performing the particular sequence of behavioural steps in host selection by phytophagous insects.

Visser and Nielsen (1977) have shown that adult Colorado beetles are attracted by the volatiles of their host plant, potato. Besides this, other members of the Solanaceae are attractive, while most of the nonsolanaceous plant species elicit neutral or repellent responses in Colorado beetles, except for *Apium graveolens* (de Wilde *et al.*, 1969) and *Tropaeolum majus*, which enhance positive anemotaxis. Host plant selection by this oligophagous insect is a catenary process, in which the initial olfactory orientation confines this selection process mainly to solanaceous plant species. Eventually, when contact results the aim is not achieved by a sole

"odd" substance, but attained by a combination of feeding incitants, feeding stimulants, feeding co-factors and inhibitors (Ritter, 1967; Hsiao, 1969). Analogously, a complex of volatiles composing the attractive plant odour probably exists. The present study elucidates the chemical complexity of host plant odour acting in the initial attraction of Colorado beetles.

MATERIAL AND METHODS

The chemical analysis of potato plant odour started with the isolation of the volatile components in sufficient quantities to allow identification with a mass spectrometer. Extraction can be applied as a first-step procedure, using relatively large quantities of low-boiling organic solvents. The extraction process entails two major disadvantages: impurities from the solvent may accumulate in the aroma concentrate and nonvolatile components are isolated along with the volatiles. Therefore, we preferred distillation as a first-step procedure for the isolation of all volatile material (see review of isolation procedures: Weurman, 1969). For the isolation of potato plant volatiles a series of methods were employed: vacuum steam distillation followed by freeze concentration in order to reduce the amount of solvent used in the final extraction.

One kilogram of fully grown potato plants (cultivar Pimpernel), i.e. mainly leaves, stems, some flowers and fruits equivalent to the overground parts of two plants, were harvested from the field. This amount of plant material was homogenized in a mixer with 5 litres of demineralized water at 3°C. The resultant slurry was passed through glass wool and the filtrate was steam distilled in two halves (Ahrenst-Larsen & Hansen, 1964) by the apparatus shown in Fig. 1. Having installed the flask containing the potato plant filtrate, nitrogen was flown through this equipment for 4 minutes, in order to minimize artefact formation during the distillation process (Nye & Spoehr, 1943). The steam distillation was carried out at reduced pressure (10-15 Torr near the vacuum pump, 20-40 Torr in the distillation system). At this reduced pressure the water in the steam generator, set in a water bath kept at 42°C, was boiling and the generated steam bubbled through the potato plant filtrate, at a constant 39°C. The volatiles were transferred by the steam, condensed and trapped into respectively the receiving flask and a cold trap in liquid nitrogen. One litre of distillate was produced in one run. This amount was precooled to 2°C and transferred to the freeze concentration arrangement (see Fig. 2). This technique had been reported to give high recoveries (> 90%) after 20-fold concentration (Kepner *et al.*, 1969). After a ten-fold concentration the combined concentrates of two distillation runs (200 ml) were extracted three times, in total with an equal volume of a mixture of diethylether and n-pentane, 1:2 by volume. These solvents (Merck, analytical grade) were distilled before use. The extract was dried with sodium sulphate, followed by calcium sulphate at 4°C (Nursten & Williams, 1966). The solvents were carefully removed by distillation.

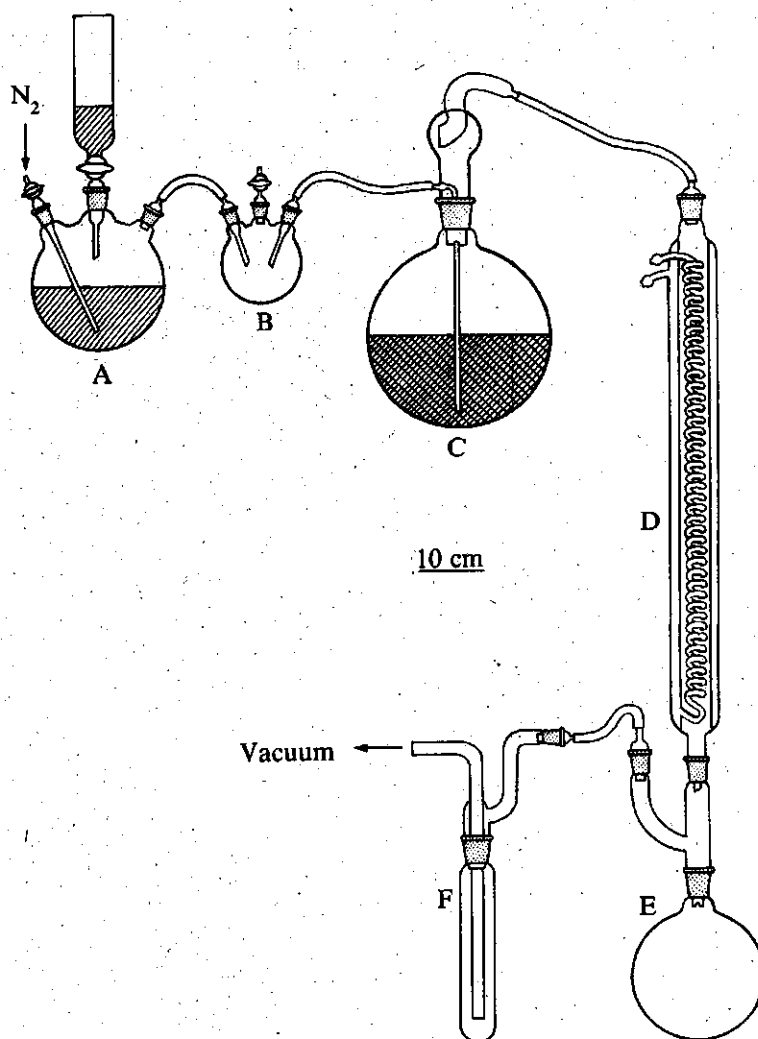


Fig. 1. Vacuum steam distillation apparatus. A: steam generator, filled with 1 litre of bidistilled water; B: steam drier; C: sample flask, containing 2.5 litres of potato plant filtrate; D: condenser; E: receiving flask, set in ice-water bath; F: cold trap, cooled in liquid nitrogen. Preceding the distillation nitrogen was administered into the steam generator.

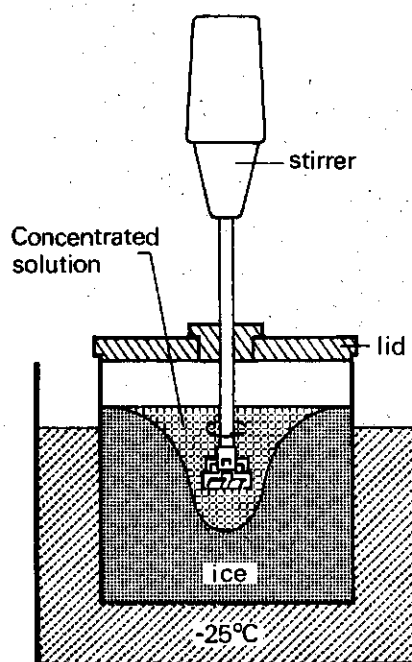


Fig. 2. Freeze concentration arrangement. A stainless steel jar containing one litre of precooled distillate is inserted in a methanol bath of -25°C . Performing simultaneously cooling and stirring, the water is selectively frozen out leaving the volatiles concentrated into a conical hole.

Analytical glc separations were made on a Becker gas chromatograph (with flame ionisation detector) fitted with packed polar (Carbowax 20M) and nonpolar (Apiezon L) columns. Mass spectra were recorded on the gas chromatograph (Varian 2700, fitted with a WCOT SP 2300 column)-mass spectrometer (Varian MAT CH4) -computersystem (Varian Spectrosystem 100) of the Central Institute for Nutrition and Food Research TNO.

Authentic samples were obtained from commercial sources: the hexenols (98-99%) from Roth, trans-2-hexenal (99%) from Koch-Light Lab., linalool (99%) and hexanol-1 (99%) from Fluka.

RESULTS AND DISCUSSION

The main components of the oil ($40\ \mu\text{l}/\text{kg}$) were identified by mixed glc analyses with authentic samples on Carbowax 20M and Apiezon L columns, and by comparison of their mass spectra with those of authentic samples (Figs. 4 and 5). The identity was further confirmed by analysis on the SP 2300 column. The mass spectra of cis-2- and trans-2-hexen-1-ol exhibited a high degree of similarity, as did cis-3- and trans-3-hexen-1-ol (Fig. 4). However, glc analyses of authentic samples showed consistent differences in their retention indices (Table 1) and allowed

VOLATILES OF POTATO FOLIAGE

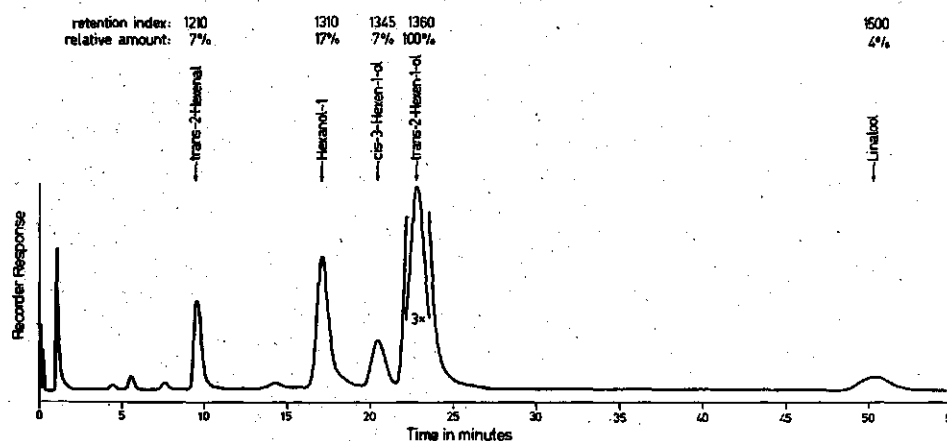


Fig. 3. Gas chromatogram of the potato plant oil: 0.1 μ l of oil injected on a stainless steel column, 3 m long and 4.4 mm I.D., filled with 10% Carbowax 20M on Chromosorb P-AW-DMCS, 60-80 mesh. Chromatogram run isothermally at 100°C, carrier gas nitrogen at 30 ml/min. Retention indices were calculated according to Kováts (1961).

discrimination between geometrical isomers. The composition of the oil is shown in Fig. 3, in decreasing order of magnitude: trans-2-hexen-1-ol, hexanol-1, cis-3-hexen-1-ol, trans-2-hexenal and linalool. At retention times > 55 min. only small quantities of compounds were detected. The most volatile components were lost in the final removal of the extractive solvents.

No evidence could be obtained that cis-2-hexen-1-ol, identified by Murray *et al.* (1972) as one of the components of *Solanum campylacanthum* oil, or trans-3-hexen-1-ol were present in the oil of potato plants. It is not clear to what extent the identification of cis-2-hexen-1-ol by Murray *et al.* (1972) had been based on mass spectral data only (see Table I).

TABLE I.

Retention indices^a

	present investigation 10% Carbowax 20M ^b		Murray <i>et al.</i> (1972) 8% Carbowax
	authentic samples	potato plant oil	<i>Solanum campylacanthum</i> oil
trans-2-Hexenal	1210	1210	1200
Hexanol-1	1310	1310	1312
trans-3-Hexen-1-ol	1320		
cis-3-Hexen-1-ol	1345	1345	1343
trans-2-Hexen-1-ol	1360	1360	
cis-2-Hexen-1-ol	1370		1360
Linalool	1500	1500	1500

^aRetention indices were calculated according to Kováts (1961)

^bSee legend of Fig. 3 for glc conditions

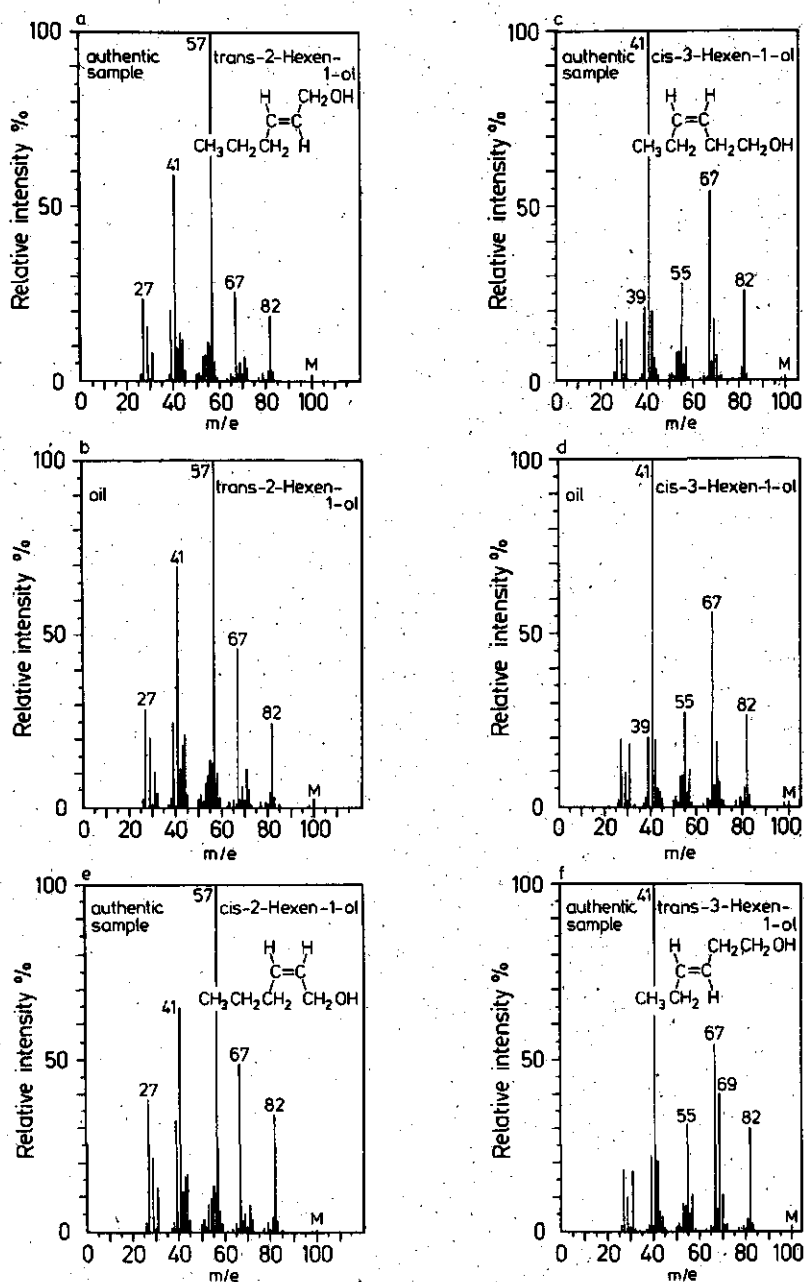


Fig. 4. Mass spectra of the main volatiles and of authentic samples.

VOLATILES OF POTATO FOLIAGE

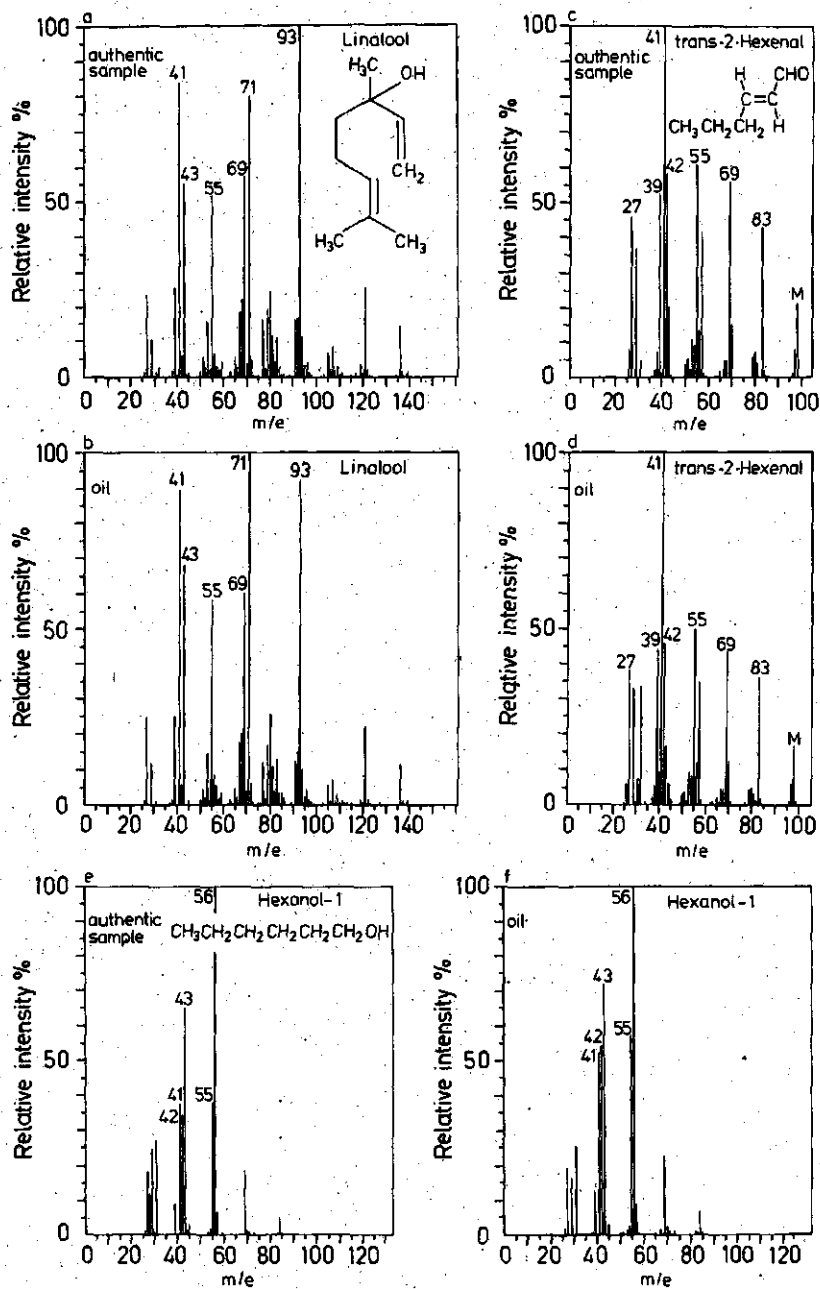


Fig. 5. Mass spectra of the main volatiles and of authentic samples.

TABLE II

Distributions of identical compounds in the oil of solanaceous plant species

<i>Capsicum annuum</i>	bell peppers: trans-2-Hexenal ^{1,21} , cis-3-Hexen-1-ol ^{1,21} , Linalool ^{1,21}
<i>Capsicum frutescens</i>	tobasco peppers: Hexanol ⁹ , cis-3-Hexen-1-ol ^{9,21}
<i>Solanum campylacanthum</i>	leaves ¹² : Hexanol -1, 2-Hexenal, cis-2-Hexen-1-ol, cis-3-Hexen-1-ol, Linalool
<i>Solanum lycopersicum</i>	tomatoes: Hexanol ^{2,19,21} , Hexanol-1 ^{4,5,7,11,13,14,17,22-24} , 2-Hexenal ^{7,15,16} , trans-2-Hexenal ^{2,6,11,14,18,21-24} , trans-2-Hexen-1-ol ^{19,22} , 3-Hexen-1-ol ²⁰ , cis-3-Hexen-1-ol ^{2,10,11,14,18,21-24} , Linalool ^{2,11,21,23}
<i>Solanum tuberosum</i>	potatoes: 2-Hexenal ⁸ , trans-2-Hexenal ²¹ , 2-Hexenol ¹⁵ leaves ²⁵ : Hexanol-1, trans-2-Hexenal, trans-2-Hexen-1-ol, cis-3-Hexen-1-ol, Linalool
<i>Nicotiana glauca</i>	flowers ³ : Hexanol-1, Linalool

¹Buttery *et al.*, 1969; ²Buttery *et al.*, 1971; ³Chang & Collins, 1972; ⁴Dalal *et al.*, 1967; ⁵Dalal *et al.*, 1968; ⁶Galliard & Matthew, 1977; ⁷Grosch, 1963; ⁸Grosch *et al.*, 1976; ⁹Haymon & Aurand, 1971; ¹⁰Johnson *et al.*, 1968; ¹¹Kazeniak & Hall, 1970; ¹²Murray *et al.*, 1972; ¹³Nelson & Hoff, 1969; ¹⁴Pyne & Wick, 1965; ¹⁵Ryder, 1966; ¹⁶Schormüller & Grosch, 1962; ¹⁷Schormüller & Grosch, 1964; ¹⁸Schormüller & Kochmann, 1969; ¹⁹Seck & Crouzet, 1973; ²⁰Shah *et al.*, 1969; ²¹Shankaranarayana *et al.*, 1975; ²²Sieso *et al.*, 1976; ²³Viani *et al.*, 1969; ²⁴Wick, 1965; ²⁵present investigation.

The main components of the potato plant oil are also present in other solanaceous plant species. They have been identified in *Solanum campylacanthum* leaves, potatoes, tomatoes, bell and tobasco peppers, and in the flowers of *Nicotiana glauca* (see Table II). These compounds are widely distributed in fresh foliage, vegetables and fruits (Gildemeister & Hoffmann, 1960, 1963; Van Straten, 1977). The well-known leaf aldehyde 2-hexenal and the leaf alcohols hexanol-1, 2-hexen-1-ol and 3-hexen-1-ol have been reported as volatile components of numerous plant species belonging to a variety of plant families (Table III).

The straight chain, saturated and unsaturated aldehydes and alcohols are formed by oxidative degradation of plant lipids, as illustrated in Fig. 6. Lipolytic acyl hydrolases liberate free fatty acids from the endogenous membrane lipids. The poly-unsaturated fatty acids, linoleic and linolenic acid are oxidized by the action of lipoxygenase to respectively hexenal and cis-3-hexenal. Alcoholdehydrogenase converts hexenal to hexanol-1, whereas cis-3-hexenal easily isomerizes to trans-2-hexenal and is converted to cis-3-hexen-1-ol. Trans-2-hexen-1-ol is formed

TABLE III

Distributions of the leaf aldehyde 2-hexenal and the leaf alcohols hexanol-1, 2-hexen-1-ol and 3-hexen-1-ol in various plant families

2-Hexenal	: <i>Polypodiaceae</i> ^a , <i>Lauraceae</i> ^a , <i>Piperaceae</i> ^b , <i>Saxifragaceae</i> ^b , <i>Rosaceae</i> ^{a,b} , <i>Mimosaceae</i> ^a , <i>Papilionaceae</i> ^{a,b} , <i>Theaceae</i> ^{a,b} , <i>Betulaceae</i> ^a , <i>Fagaceae</i> ^a , <i>Ulmaceae</i> ^a , <i>Polygonaceae</i> ^a , <i>Moraceae</i> ^a , <i>Cruciferae</i> ^{a,b} , <i>Myrtaceae</i> ^b , <i>Umbelliferae</i> ^{a,b} , <i>Caprifoliaceae</i> ^a , <i>Oleaceae</i> ^{a,b} , <i>Apocynaceae</i> ^a , <i>Rutaceae</i> ^{a,b} , <i>Aceraceae</i> ^a , <i>Hippocastanaceae</i> ^a , <i>Ericaceae</i> ^b , <i>Vitaceae</i> ^{a,b} , <i>Cucurbitaceae</i> ^b , <i>Solanaceae</i> ^b , <i>Labiatae</i> ^{a,b} , <i>Salicaceae</i> ^a , <i>Juglandaceae</i> ^a , <i>Musaceae</i> ^b
Hexanol-1	: <i>Lauraceae</i> ^a , <i>Saxifragaceae</i> ^b , <i>Rosaceae</i> ^{a,b} , <i>Papilionaceae</i> ^b , <i>Theaceae</i> ^{a,b} , <i>Violaceae</i> ^a , <i>Passifloraceae</i> ^b , <i>Caricaceae</i> ^b , <i>Cannabinaceae</i> ^b , <i>Cruciferae</i> ^b , <i>Myrtaceae</i> ^b , <i>Umbelliferae</i> ^b , <i>Oleaceae</i> ^b , <i>Rubiaceae</i> ^b , <i>Geraniaceae</i> ^a , <i>Rutaceae</i> ^{a,b} , <i>Ericaceae</i> ^b , <i>Vitaceae</i> ^b , <i>Cucurbitaceae</i> ^b , <i>Buettneriaceae</i> ^b , <i>Solanaceae</i> ^b , <i>Labiatae</i> ^{a,b} , <i>Musaceae</i> ^b , <i>Palmae</i> ^b
2-Hexen-1-ol	: <i>Saxifragaceae</i> ^b , <i>Rosaceae</i> ^{a,b} , <i>Papilionaceae</i> ^b , <i>Theaceae</i> ^b , <i>Cannabinaceae</i> ^b , <i>Umbelliferae</i> ^b , <i>Oleaceae</i> ^b , <i>Rutaceae</i> ^b , <i>Ericaceae</i> ^b , <i>Vitaceae</i> ^b , <i>Solanaceae</i> ^b , <i>Labiatae</i> ^b , <i>Musaceae</i> ^b
3-Hexen-1-ol	: <i>Piperaceae</i> ^b , <i>Saxifragaceae</i> ^b , <i>Rosaceae</i> ^{a,b} , <i>Mimosaceae</i> ^a , <i>Papilionaceae</i> ^{a,b} , <i>Theaceae</i> ^{a,b} , <i>Violaceae</i> ^a , <i>Passifloraceae</i> ^b , <i>Betulaceae</i> ^a , <i>Fagaceae</i> ^a , <i>Moraceae</i> ^a , <i>Cannabinaceae</i> ^b , <i>Cruciferae</i> ^{a,b} , <i>Myrtaceae</i> ^b , <i>Umbelliferae</i> ^b , <i>Oleaceae</i> ^b , <i>Geraniaceae</i> ^a , <i>Rutaceae</i> ^{a,b} , <i>Ericaceae</i> ^{a,b} , <i>Vitaceae</i> ^{a,b} , <i>Cucurbitaceae</i> ^b , <i>Solanaceae</i> ^b , <i>Labiatae</i> ^{a,b} , <i>Gramineae</i> ^a , <i>Musaceae</i> ^b

^aGildemeister & Hoffman (1960, 1963); ^bVan Straten (1977).

from trans-2-hexenal. Possibly trans-3-hexen-1-ol and cis-2-hexen-1-ol originate from isomerization during processing and storage of plant products. These biosyntheses are operative in several plant species: potatoes (Grosch *et al.*, 1976), tomatoes (Galliard & Mattew, 1977; Kazeniak & Hall, 1970; Sieso *et al.*, 1976), tea leaves (Hatanaka & Harada, 1973; Hatanaka *et al.*, 1976a), peas (Grosch, 1968, 1969), apples, other fruits and leaves (Drawert *et al.*, 1965, 1966), legumes like soybeans, and some cereal grains (Tappel, 1961), and are regarded as widely distributed.

Because enzyme inhibitors were not employed except for nitrogen in the present study, these products might be formed during the steam distillation of potato plant leaves and for that reason be regarded as biologically insignificant. However, vapour sampling of the air over potato plant leaves showed that these leaf alcohols and aldehydes are present (Visser & Schaefer, unpublished data).

These compounds are smelled by man as a grass-like odour. However, the ratio between the several products of this biosynthesis - the relative proportions of the different components - vary in and over different plant species. Within the same

plant species the proportions are modified seasonally (Hatanaka *et al.*, 1976b) as caused by the expressions and/or the shift in the expressions of the several enzymes involved, owing to plant ageing and injury (Buttery *et al.*, 1971; Kazeniak & Hall, 1970; Sayo & Takeo, 1975). Consequently, unbalanced mixtures containing overdoses of one or more components, give an "off-flavour" to the human sense, the hay-like odours of several food products like deteriorated beans and peas (Whitaker, 1972).

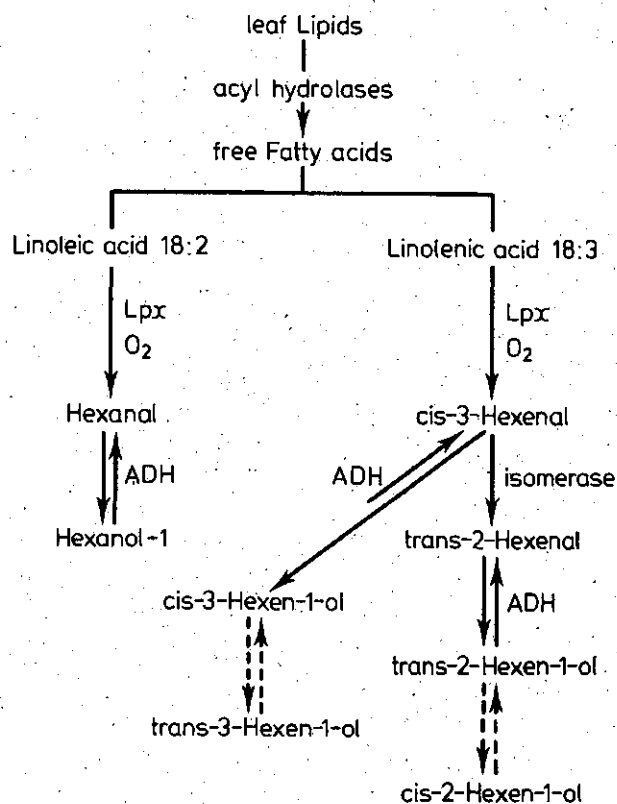


Fig. 6. The biosyntheses of the leaf aldehydes and alcohols, constituting a general green leaf volatile complex. Lpx: Lipoxygenase (Linoleate: oxygen oxidoreductase; E.C. 1.13.1.13); ADH: Alcoholdehydrogenase (Alcohol: NAD oxidoreductase; E.C. 1.1.1.1). According to: Drawert *et al.*, 1966; Galliard & Mattew, 1977; Grosch, 1968, 1969; Hatanaka & Harada, 1973; Hatanaka *et al.*, 1976a; Kazeniak & Hall, 1970; Sieso *et al.*, 1976; Wardale & Galliard, 1977.

In the same way these leaf aldehydes and alcohols constituting a general green leaf volatile complex, act in the olfactory orientation of the adult Colorado beetle. Electroantennogram recordings show the olfactory sensilla of the Colorado beetle to be mainly responsive to these type of compounds (Visser, 1979). In behaviour

tests none of these components applied singly, are attractive, whereas some of them, in minute quantities with potted potato plants, mask the attractive host plant vapours, that is the beetles do not react any longer with an odour-conditioned positive anemotaxis (Visser & Avé, 1978).

The natural potato plant odour, attractive to Colorado beetles, appears to be the result of complex interactions between these leaf components. Detailed information of this system, probably operative to a variety of phytophagous insects, will be presented in subsequent papers.

The first author owes the late Dr. C. Weurman a great debt of gratitude for his valuable advice, criticism and support. A part of the identification was carried out at the Central Institute for Nutrition and Food Research TNO, using their GLC-MS facilities, and the technical assistance of Mrs. C.A. Visscher and Mrs. G.F. La Vos is appreciated. We also thank Mr. J.H.C.T. van den Kerkhof for his assistance in the isolation procedure and Mr. P.M. Guerin for correcting the English text.

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CHAPTER 5

ELECTROANTENNOGRAM RESPONSES OF THE COLORADO BEETLE, *LEPTINOTARSA DECEMLINEATA*, TO PLANT VOLATILES

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Electroantennogram responses of Colorado beetles were recorded to 53 plant volatiles including isomers. The system of antennal olfactory receptors is selective, even at high doses several compounds cause fairly small responses. Diminishing the concentration to a moderate stimulus strength reduces the number of perceptible chemicals. Distinct electroantennogram responses are obtained to a group of closely related components, namely the general green leaf volatiles trans-2-hexen-1-ol, cis-3-hexen-1-ol, hexanol-1, trans-2-hexenal, hexanal and cis-3-hexenylacetate, and to isomers such as trans-3-hexen-1-ol and cis-2-hexen-1-ol. The threshold concentration of the most effective compound, trans-2-hexen-1-ol is 1.2×10^8 molecules per ml of air. The antennal olfactory receptors of the Colorado beetle are sensitively tuned to the perception of these general green leaf volatiles. As olfactory receptors of a number of phytophagous insects have been reported to respond to these components, this volatile complex probably plays a part in the host selection behaviour of various phytophagous insects.

In host selection behaviour, sensory systems of phytophagous insects face a wide diversity of information from the environment. The sensory systems can cope with this excess of information, excluding irrelevant parts, and in this way limiting the input to beneficial elements (Dethier, 1971). As in all insect chemoreceptors, the antennal olfactory receptors are primary neurons lacking synaptic connections until they enter the central nervous system (Kaissling, 1971). The selected input to the brain can be directly analysed by making use of electrophysiological techniques.

The electroantennogram method (EAG; Schneider, 1957) records the responses of antennal olfactory receptors to volatile compounds. In principle, this should segregate irrelevant chemicals from the volatiles which are potentially beneficial to the particular insect species. It reflects the summation of receptor potentials of the individual olfactory neurons in the antenna (Boeckh *et al.*, 1965), and thus indicates the sensitivity of the main olfactory system. The EAG has been a vital tool in the analyses of sex pheromones (see e.g. Roelofs & Comeau, 1971). However, this method has been scarcely employed as a bioassay of host plant volatiles (see Simpson, 1976).

Initial olfactory orientation directs the adult Colorado beetle *Leptinotarsa decemlineata* Say, towards solanaceous plant species (Visser & Nielsen, 1977). The

olfactory receptors responding to the potato plant odour are located in the terminal five segments of the Colorado beetle antenna (Schanz, 1953; De Wilde *et al.*, 1969; De Wilde, 1976). The chemical analysis of the potato plant odour (Visser *et al.*, 1979) was accompanied by the present study using the EAG responses of the Colorado beetle as a bioassay for a variety of plant volatiles.

MATERIAL AND METHODS

Newly-emerged Colorado beetles were obtained from the laboratory stock culture. Unless otherwise stated, three male and three female Colorado beetles were used (one antenna/ individual) for calculating the mean responses to the test chemicals.

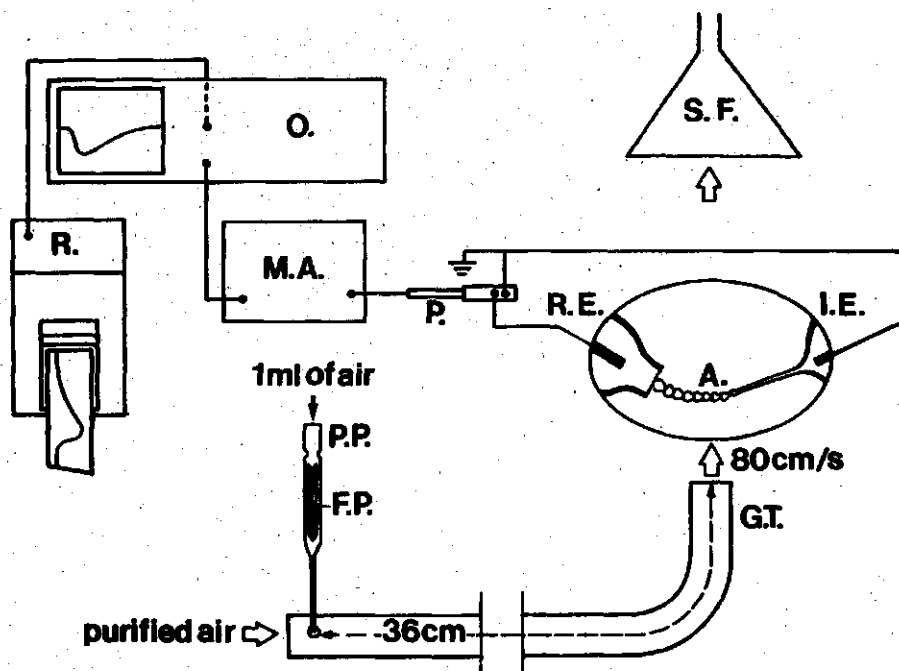


Fig. 1. EAG recording technique. A.: excised antenna; F. P.: filter paper; G. T.: glass tube; I.E.: indifferent electrode; M. A.: micro electrode amplifier; O.: oscilloscope; P.: input probe; P. P.: pasteur pipette; R.: recorder; R. E.: recording electrode; S. F.: suction-funnel. See text for further explanation.

The arrangement for the recording of an EAG of the Colorado beetle is illustrated in Fig. 1. The antenna was amputated at the base of the flagellum and the tip of the terminal segment was cut off. The excised antenna was fixed between two glass electrodes, the indifferent electrode inserted in the base and the recording electrode in contact with the cut tip. The glass electrodes had been filled with a solution containing glucose (354 mM), KCl (6.4 mM), KH_2PO_4 (20 mM), MgCl_2 (12 mM), CaCl_2 (1 mM), NaCl (12 mM) and KOH (9.6 mM). This solution (450 mosm, pH 6.5) resembles the haemolymph of the phytophagous moth

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Antheraea pernyi (Kaissling, pers. comm.). Ag-AgCl wires in the glass electrodes connected the preparation with the recording instruments: an input probe (Grass HIP16), a micro electrode AC/DC amplifier (Grass P16), a storage oscilloscope (Tektronix D11/5A20N/5B10N) and a hot-wire recorder (Astro-Med 102 F).

In order to prevent an early evaporation of the test chemicals, they were dissolved in paraffin oil (Merck Uvasol); 25 μ l of each stock solution were pipetted on to a piece of filter paper (6 \times 0.5 cm, S&S 589²) and placed in a pasteur pipette. The pasteur pipette was attached to a syringe and the pipette tip was inserted through a small hole in the glass tube, conducting a continuous air flow over the excised antenna. The syringe plunger was quickly depressed to pass 1 ml of air through the pipette into the air stream. The duration of this air puff (0.12 sec) was measured with a hot-wire anemometer. The concentrations of the test chemicals in paraffin oil refer to the concentrations of the original stock solutions (volume/volume).

Since the antennal responses diminished throughout an experiment, the responses (amplitudes) to the test compounds are expressed as a percentage of the EAG response to the standard, cis-3-hexen-1-ol 10^{-3} (1 μ l/ml of paraffin oil). This was achieved by stimulating the antenna alternately every 30 sec, with a test chemical or the standard. The mean of all recorded antennal depolarizations elicited by the standard is 0.13 mV.

The test chemicals were obtained from commercial sources (Fluka, Merck, Roth, Koch-Light Lab., Pfaltz & Bauer, K & K and Maschmeyer), and assayed using gas liquid chromatography. Most of them were \geq 97% purity, except for hexanol-3 (96%), Δ^3 -carene (95%) and α -pinene (95%).

RESULTS AND DISCUSSION

The test chemicals were arbitrarily selected, but based on their distribution in various plant families inclusive of solanaceous plant species, and their presence in potato plant essential oil (Visser *et al.*, 1979). The EAG responses of Colorado beetles to 43 chemicals at two concentrations, are listed in Table I. The observed EAG responses depend both on the sensitivity of the olfactory receptors and the concentration of the compound in the air flowing over them (see Fig. 3A). Relative comparisons can only be made between chemicals, since discrepancies in volatility were not corrected for. In spite of the limitations set to the interpretation of the results, it is concluded from the data presented in Table I that the antennal olfactory system is selective; a number of plant compounds do not stimulate, even at high concentrations. The solvent itself, paraffin oil, does not elicit measurable responses. Marked differences are not detected in the responses of male and female Colorado beetles.

It should be noted that in the experiments listed in Table I, one excised antenna responded to propanoic and butanoic acid by hyperpolarization, while the EAG responses to the standard consisted of a normal depolarization. Hyperpolarization of olfactory receptors stimulated with propanoic acid has been reported in

EAG RESPONSES OF COLORADO BEETLE

TABLE I

EAG responses of Colorado beetles to test compounds as a percentage of the response to *cis*-3-hexen-1-ol 10^{-3}

Compound	Mean \pm c.i. ¹ in %		Compound	Mean \pm c.i. ¹ in %	
	Concentration ²			Concentration ²	
	10^{-3}	10^{-1}		10^{-3}	10^{-1}
Propanoic acid	0	14 \pm 20.1	Butylacetate	1 \pm 3.0	70 \pm 11.3
Butanoic acid	0	13 \pm 17.4	Benzylacetate	7 \pm 0.9	76 \pm 2.8
Pentanoic acid	0	9 \pm 9.7	Benzaldehyde	5 \pm 3.1	74 \pm 21.8
Hexanoic acid	0	6 \pm 7.5	Salicylaldehyde	6 \pm 3.3	83 \pm 20.9
Heptanoic acid	0	1 \pm 3.4	Methylsalicylate	22 \pm 4.2	108 \pm 18.6
Octanoic acid	0	1 \pm 3.0	Eugenol	9 \pm 8.7	44 \pm 8.5
Linoleic acid	0	0			
Hexanal	21 \pm 6.2	146 \pm 36.5	Δ^3 -Carene	0	8 \pm 5.1
Hexanol-1	94 \pm 7.7	207 \pm 14.9	(+)-Limonene	0	15 \pm 8.3
Hexanol-2	17 \pm 9.3	157 \pm 25.2	α -Pinene	0	6 \pm 5.4
Hexanol-3	33 \pm 12.5	158 \pm 12.2	β -Ionone	0	3 \pm 4.9
trans-2-Hexenal	39 \pm 7.4	179 \pm 14.7	Geraniol	0	2 \pm 3.9
trans-2-Hexen-1-ol	146 \pm 13.1	274 \pm 48.9	Nerol	0	2 \pm 3.9
cis-2-Hexen-1-ol	75 \pm 9.9	199 \pm 19.4	Linalool	18 \pm 4.0	47 \pm 9.8
cis-3-Hexen-1-ol	100	247 \pm 41.8	Citronellol	4 \pm 3.5	33 \pm 12.6
trans-3-Hexen-1-ol	101 \pm 13.5	285 \pm 47.2	2-(methylthio)-Ethanol	2 \pm 2.7	38 \pm 7.1
cis-3-Hexenylacetate	69 \pm 18.7	195 \pm 44.0	3-(methylthio)-Propanal	4 \pm 3.6	71 \pm 7.9
2-Methylbutanol-1	0	68 \pm 22.7	2-Pentanone	6 \pm 7.5	47 \pm 28.7
3-Methylbutanol-1	1 \pm 3.0	78 \pm 18.6	6-methyl-5-Hepten-2-one	7 \pm 4.1	100 \pm 18.6
1-Penten-3-ol	6 \pm 6.6	70 \pm 11.6	2-Hendecanone	1 \pm 2.1	15 \pm 4.4
5-Hexen-1-ol	37 \pm 7.2	171 \pm 12.0	Pyridine	6 \pm 5.4	72 \pm 17.8
3-Hepten-1-ol	31 \pm 3.6	142 \pm 18.4			
1-Octen-3-ol	57 \pm 9.9	150 \pm 25.6	Paraffin oil		0

¹ c.i. = 95% confidence interval; standard error $t_{n-1}^{0.025}$ ² concentration of the compound in paraffin oil (%)

Necrophorus and *Thanotophilus* species (Kaissling, 1971). The acidity of these compounds possibly caused the artificial hyperpolarization.

Selectivity

Several chemicals in high doses (10^{-1}) commonly not met by insects, cause fairly small responses, i.e., the saturated fatty acids, Δ^3 -carene, (+)-limonene, α -pinene, β -ionone, geraniol, nerol and 2-hendecanone (Table I). Diminishing the concentration to a moderate stimulus strength (10^{-3}) reduces the number of perceptible compounds. At both concentrations, distinct EAG responses of Colorado beetles are obtained to a group of closely related components, namely the general green leaf volatiles trans-2-hexen-1-ol, cis-3-hexen-1-ol, hexanol-1,

trans-2-hexenal, hexanal and cis-3-hexenylacetate, and to isomers such as trans-3-hexen-1-ol and cis-2-hexen-1-ol. Besides these components, methyl-salicylate — an aromatic compound — causes definite responses, though relatively small compared with the responses to trans-2-hexen-1-ol.

It has been stated that acetaldehyde accounted for the phagostimulatory nature of the potato plant to the Colorado beetle (Hesse & Meier, 1950). From the failure to confirm their assumption (Ritter, 1967), it may be deduced that this volatile compound has been lost at the start of their experiments. The EAG response to 1 ml of a saturated vapour of acetaldehyde is 27%, a small response to a very high dose, throwing doubts upon its rôle in the olfactory orientation of the Colorado beetle. Since the EAG barely shows a response to geraniol (Table I), the reported behavioural responses of adult Colorado beetles to this chemical (Gottschalk, 1957) are questionable.

Structure-activity relationship

The activity of the olfactory system is related to molecular structures. Stimulation with alcohols results in higher EAG responses than to aldehydes. The

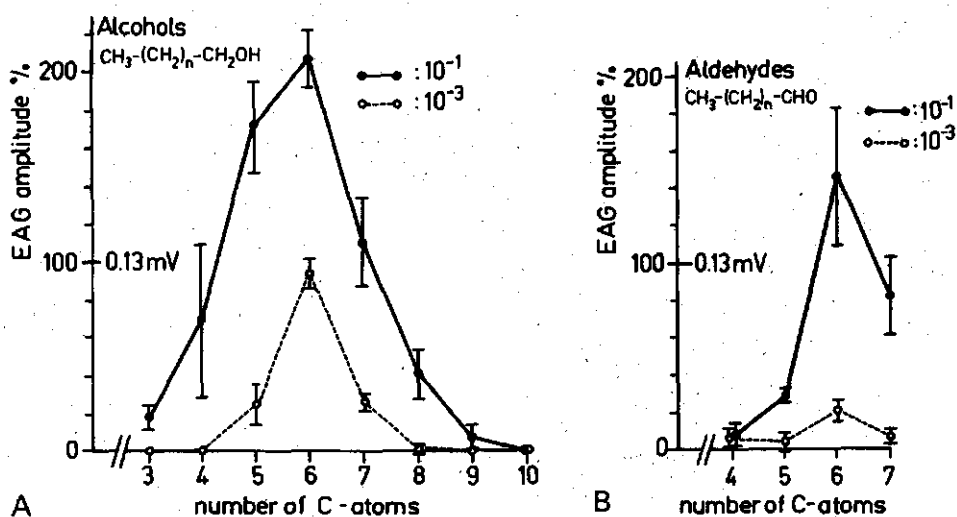


Fig. 2. Effectiveness of saturated alcohols (A) and aldehydes (B) in eliciting EAG responses of Colorado beetles, at two concentrations in paraffin oil (%). Vertical lines indicate 95% confidence intervals.

effectiveness of saturated alcohols and aldehydes appears to be optimal at a chain length of six carbon atoms (Fig. 2). Changing the position of the terminal hydroxyl-group to other carbon atoms, i.e. hexanol-2 and hexanol-3, reduces the responses. Except for cis-2-hexen-1-ol, the hexenols are more effective than the saturated alcohol hexanol-1. As the electroantennogram reflects the responses of many olfactory receptors, it is impossible to decide on one of the extreme interpretations. Whether there is one type of hexenol-receptor, responding in a

EAG RESPONSES OF COLORADO BEETLE

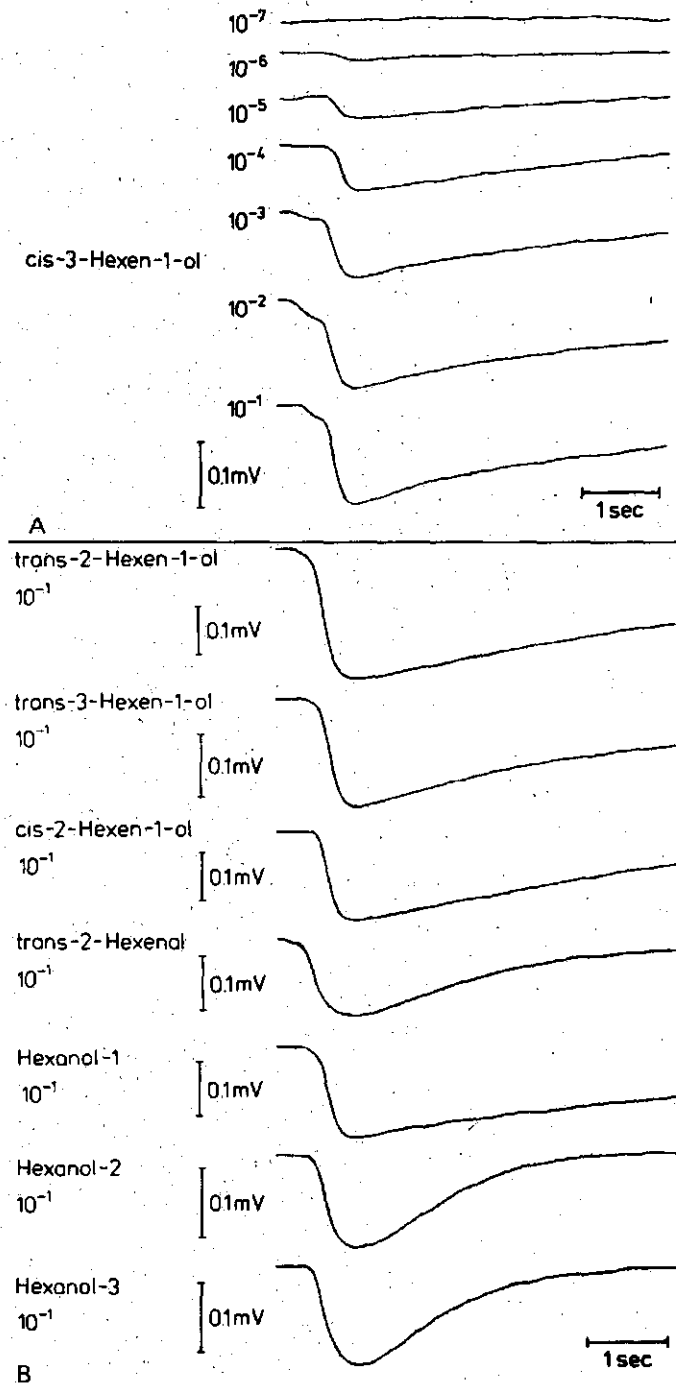


Fig. 3. EAG's of Colorado beetles responding to general green leaf volatiles and their isomers, dissolved in paraffin oil (%). A: responses to a range of concentrations of cis-3-hexen-1-ol; B: different shapes are observable.

differential way to closely related molecular structures (also to other unsaturated alcohols, e.g. the definite responses to 1-octen-3-ol), or several types of olfactory receptors, each responsive to a particular structure.

Recovery time

Representative EAG recordings to the general green leaf volatiles and some of their isomers, are shown in Fig. 3. It is observed that different EAG shapes occur, the responses to trans-2-hexenal, hexanol-2 and hexanol 3, recover faster than the responses to the other chemicals. Different shapes of the tracings are also detected in the responses to other chemicals listed in Table I. In general, EAG responses to components of sex pheromone blends are characterized by a slower return to the baseline than responses to other structurally related chemicals (Kaissling, 1974; Roelofs & Comeau, 1971). Roelofs & Comeau (1971) suggested that the shape corresponds to the affinity of the chemical for the receptor sites, and Kaissling (1974) proposed that the recovery is related to compound-specific velocities of an early inactivation. As the process of stimulus transduction in olfaction is still unknown, any interpretation will lack a real foundation.

Sensitivity

After the selectivity of the antennal olfactory system has been assessed, further experiments were conducted to determine the sensitivity of this system in the perception of the general green leaf volatiles and some of their isomers. The responses to various concentrations of these chemicals in paraffin oil are illustrated in Fig. 4. An example of the responses of one Colorado beetle antenna to a range of concentrations of cis-3-hexen-1-ol is presented in Fig. 3A. With the exception of trans-2-hexenal, hexanol-2 and hexanol-3, these components still stimulate the antenna at a dilution to 10 ppm in paraffin oil, however, these concentrations do not represent the actual stimulus strength. Therefore, the number of molecules per ml of air flowing over the antenna was determined using the flame ionisation detector of a gas chromatograph and calibration curves were

TABLE II

Threshold concentrations of the general green leaf volatiles and their geometrical isomers for EAG responses of the Colorado beetle

Compound	Concentration in paraffin-oil (% ³)	Number of molecules/ml of air ⁴
trans-2-Hexen-1-ol	10 ⁻⁶	1.2·10 ⁹
trans-3-Hexen-1-ol ³	10 ⁻⁶	2.3·10 ⁹
cis-2-Hexen-1-ol ³	10 ⁻⁵	1.8·10 ⁹
cis-3-Hexen-1-ol	10 ⁻⁶	1.2·10 ¹¹
Hexanol-1	10 ⁻⁵	2.3·10 ¹¹
trans-2-Hexenal	3.2·10 ⁻⁵	4.3·10 ¹¹

¹ see Fig. 4; ⁴ stimulating the antenna; ³ geometrical isomer

established (Ma & Visser, 1978). From these curves the actual threshold concentrations were calculated (Table II). The flame ionisation detector appears

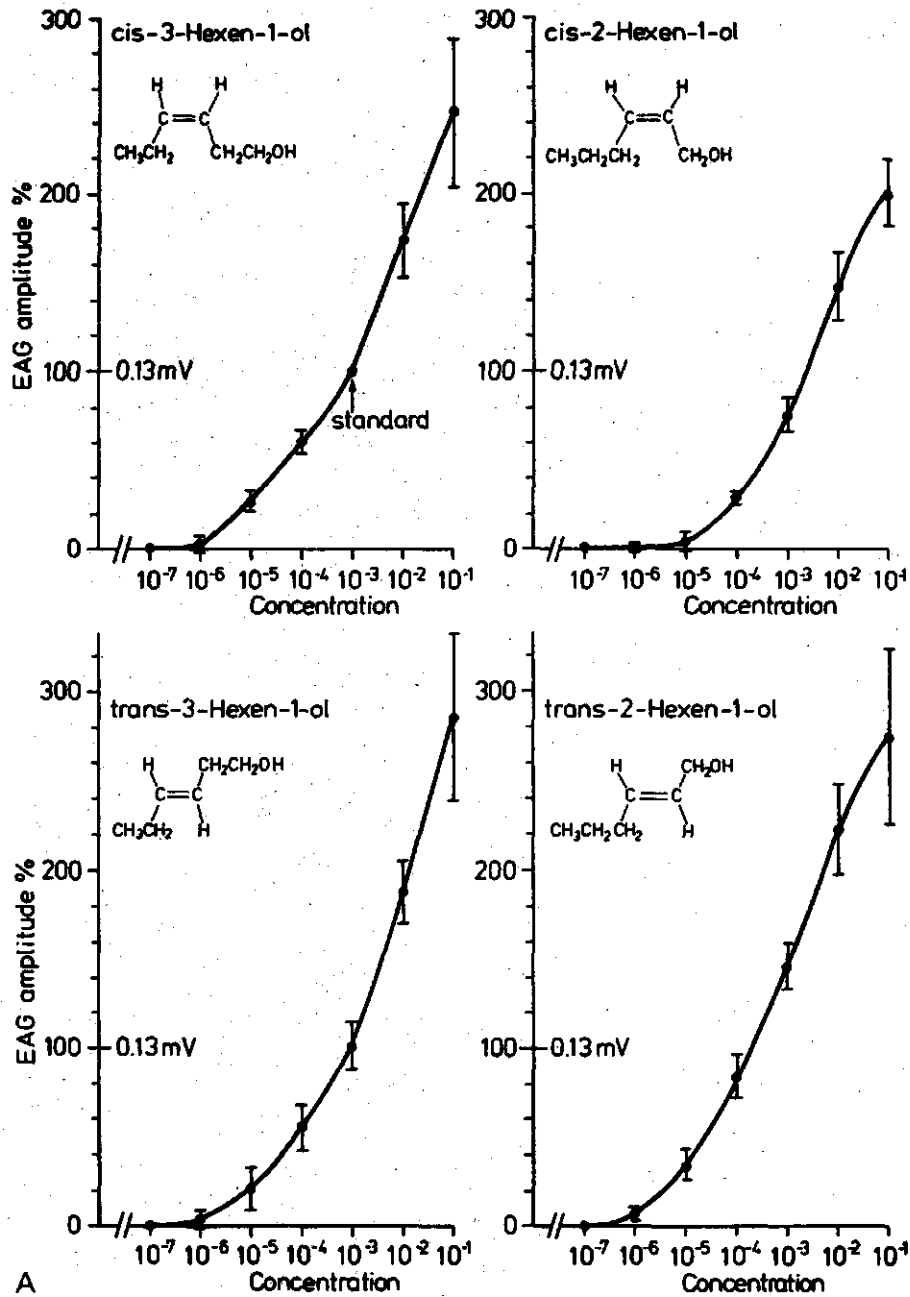
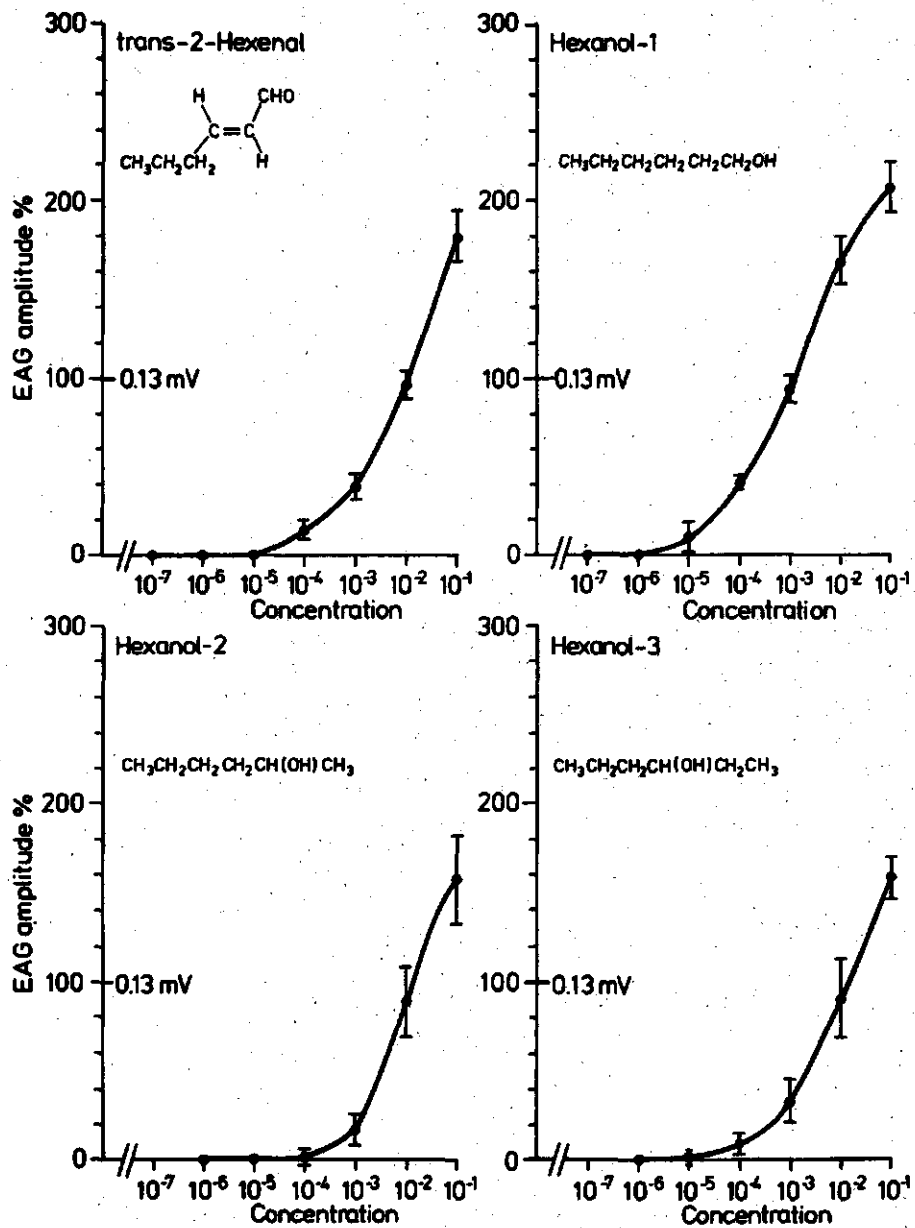


Fig. 4. Mean EAG responses of Colorado beetles to a range of concentrations of 8 test chemicals, dissolved in paraffin oil (v/v). Vertical lines indicate 95% confidence intervals.

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B

Fig. 4, continued.

to be less sensitive than the EAG, consequently the lower range of the calibration curves are obtained by extrapolation. Because of this, the data in Table II are estimated thresholds. Trans-2-hexen-1-ol (threshold at $1.2 \cdot 10^8$ molecules/ml of air) is the most effective chemical, followed by trans-3-hexen-1-ol (threshold at $2.3 \cdot 10^8$

molecules/ml of air). In comparison, at 760 mm Hg and 20°, 1 ml of air contains about 10^{19} molecules.

The responses of the sensilla coeloconica on the antennae of *Locusta migratoria* have been analysed by a group of German investigators (Boeckh *et al.*, 1965; Boeckh, 1967; Kafka, 1970; referred by Kaissling, 1971). These olfactory receptors responded to trans-2-hexenal, cis-3-hexenal, trans-2-hexen-1-ol, cis-3-hexen-1-ol, cis-2-hexen-1-ol and a few other geometrical isomers. Varying the chain lengths of the unsaturated aldehydes and alcohols showed optimal responses of these s. coeloconica to molecules of six carbon atoms (Kafka, 1970). This happens also in the EAG responses of the Colorado beetle (Fig. 2). Trans-2-hexenal was the most effective chemical for the s. coeloconica: thresholds at 10^8 (Boeckh, 1967), and $0.5 \cdot 10^8$ molecules per ml of air (Kafka, 1970), which is approximately of the same magnitude as the threshold of trans-2-hexen-1-ol for a response of the Colorado beetle. These thresholds do not reflect the lowest limit in the sensitivity of the olfactory system, as Kaissling (1971) observed marked differences in behavioural and electrophysiological thresholds of *Bombyx mori* to its sex pheromone bombykol. In addition, the sensilla basiconica on the antennae of *Locusta migratoria* has been studied (Boeckh, 1974). These were stimulated by both trans-2-hexenal and hexanol-1, the latter chemical did not affect the s. coeloconica (Boeckh *et al.*, 1965; Boeckh, 1967; Kafka, 1970). Unfortunately no behavioural responses of *Locusta migratoria* to these compounds or responses toward grass odour are known, since the observations of Kennedy and Moorhouse (1969) refer to *Schistocerca gregaria*.

General green leaf volatiles

The results of the present study lead to the conclusion that the olfactory system used by the Colorado beetle in olfactory orientation towards its host plant potato, is selective. It has a high sensitivity to general green leaf volatiles like trans-2-hexen-1-ol. The main components identified in the essential oil of potato leaves, are trans-2-hexen-1-ol, hexanol-1, cis-3-hexen-1-ol and trans-2-hexenal (Visser *et*

TABLE III

EAG responses of one Colorado beetle to leaf vapours of several plant species^a

Plant species	Response in %	Plant species	Response in %
<i>Solanum tuberosum</i>	95	<i>Rubus idaeus</i>	77
<i>Solanum luteum</i>	93	<i>Phaseolus vulgaris</i>	188
<i>Solanum nigrum</i>	76	<i>Brassica oleracea</i>	125
<i>Solanum lycopersicum</i>	137	<i>Daucus carota</i>	69
<i>Petunia hybrida</i>	121	<i>Chrysanthemum leucanthemum</i>	168
<i>Digitalis purpurea</i>	74	<i>Tropaeolum majus</i>	74
<i>Populus alba</i>	83		
<i>Convallaria majalis</i>	132		
<i>Holcus lanatus</i>	73		

^a 0.3 g of leaf in a pasteur pipette

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al., 1979). These volatiles are widely distributed in the leaves of numerous plant species. Stimulating the antenna of the Colorado beetle with leaf vapours of several plant species, causes distinct EAG responses (Table III). This is explained as a response to the general green leaf volatiles, however, other stimulating compounds cannot be excluded.

This complex is perceptible to various phytophagous insects: responses of the sensilla basiconica on the antennae of silkworm larvae *Bombyx mori*, have been recorded to 3-hexen-1-ol and hexanol-1 (Morita & Yamashita, 1961). Also, EAG responses have been described of the cabbage rootfly *Erioischia brassicae*, to cis-3-hexen-1-ol and cis-3-hexenylacetate (Wallbank, 1972); of three noxious Lepidoptera species namely *Diatraea grandiosella*, *Manduca sexta* and *Heliothis virescens*, to trans-2-hexen-1-ol (Adler & Jacobson, 1972); of the shootborer *Hypsipyla grandella*, to 2-hexenal (Schoonhoven, 1974); and EAG responses of *Manduca sexta* to trans-2-hexenal, trans-2-hexen-1-ol and 3-hexen-1-ol (Schweitzer *et al.*, 1976). These general green leaf volatiles also elicit EAG responses of *Schistocerca gregaria* (Visser, unpubl.), *Pieris brassicae* (Schoonhoven, pers. comm.), *Yponomeuta* species (van der Pers, pers. comm.) and *Adoxophyes orana* (den Otter, pers. comm.). Considering the reported information, this complex of general green leaf volatiles probably plays a part in host selection behaviour of various phytophagous insects. Information concerning the particular rôle of this complex in the olfactory orientation of the Colorado beetle is presented by Visser & Avé (1978), and the present study is continued with a single unit analysis of odour quality coding (Ma & Visser, 1978).

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RÉSUMÉ

ELECTROANTENNOGRAMMES DU DORYPHORE (LEPTINOTARSA DECEMLINEATA) EN RÉPONSE À DES SUBSTANCES VOLATILES VÉGÉTALES

Les électroantennogrammes de *Leptinotarsa decemlineata*, en présence de 53 substances volatiles (y compris des isomères) ont été enregistrés. Le système des récepteurs olfactifs antennaires est sélectif, même à des doses élevées plusieurs composés ne provoquent que d'assez faibles réponses. La diminution de la concentration jusqu'à une puissance stimulante modérée réduit le nombre de substances perçues. Des électroantennogrammes différents ont été obtenus pour un groupe de composés très voisins, à savoir les substances volatiles de feuilles vertes: trans-2-hexen-1-ol, cis-3-hexen-1-ol, hexanol-1, trans-2-hexenal et cis-3-hexenylacetate, et aux isomères tels que trans-3-hexen-1-ol et cis-2-hexen-1-ol. La concentration seuil du composé le plus efficace, trans-2-hexen-1-ol, est de $1,2 \times 10^8$ molécules par ml d'air. Les récepteurs olfactifs antennaires du Doryphore sont sensoriellement ajustés à la perception de ces substances volatiles générales des feuilles vertes. Comme les récepteurs olfactifs de nombreux insectes phytophages ont été signalés comme répondant à ces composés, ce complexe volatile joue probablement un rôle dans le comportement de sélection de l'hôte par différents insectes phytophages.

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CHAPTER 6

GENERAL GREEN LEAF VOLATILES IN THE OLFACTORY ORIENTATION OF THE COLORADO BEETLE, *LEPTINOTARSA DECEMLINEATA*

BY

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The odour of fully grown potato plants elicits a positive anemotactic response in Colorado beetles, but none of the individual component volatiles alone is attractive. Certain component chemicals or isomers of them disrupt orientation to the host plant, probably by changing the relative proportion of the odour components. These general green leaf volatiles probably occur as important constituents of leaf odours attractive to various phytophagous insects. Such a complex represents "green odour" and confers different modalities on the leaf odours of diverse plant species.

Host selection behaviour of phytophagous insects is a catenary process, which is completed in the acceptance of a plant suitable for oviposition and/or feeding (Schoonhoven, 1968). Because of the spectacular selectivity of oligophagous insects in the final phase, selection behaviour has mainly been described in terms of "acceptance and rejection", resulting in "host plant recognition". Dual discrimination of both nutrients and secondary plant substances is thought to be crucial for the establishment of an insect on its host plant (see Beck, 1965). Acceptance of a plant is brought about at optimal concentrations of a feeding incitant — a "token stimulus" — of feeding stimulants and co-factors, provided that deterrents are absent.

The host plant specificity of the Colorado beetle, *Leptinotarsa decemlineata* Say, has been explained in this way (de Wilde, 1958; Hsiao, 1969). The isolation of a feeding incitant for this insect has been reported several times (Chauvin, 1945; Yamamoto & Fraenkel, 1960; Hsiao & Fraenkel, 1968). However, attempts to purify this "incitant" have failed and its chemical identity remains unsolved, throwing doubts upon its very existence (Ritter, 1967). Since the Colorado beetle feeds on solanaceous plant species lacking deterrents like demissine, tomatine and other related alkaloid-glycosides (Schreiber, 1958), the specificity of this oligophagous insect has been described in terms of rejection rather than acceptance (Jermy, 1961, 1966; Hsiao, 1974).

Since oligophagous insects searching for oviposition and/or feeding sites, are likely to meet suitable plants less frequently than their polyphagous counterparts, they would seem to need means of greater selectivity in approaching plants, as well as after arrival in the final stage of selection. This indicates that in spite of the selectivity in this final phase, host selection behaviour of oligophagous insects may

not be explained as merely acceptance and rejection. An initial olfactory orientation which is directed to a relevant part of the vegetation, would increase the effectiveness of host selection behaviour.

The initial olfactory orientation of the Colorado beetle is directed mainly towards solanaceous plant species, thus restricting the final part of the selection process (Visser & Nielsen, 1977). The main components in the essential oil of potato leaves are trans-2-hexen-1-ol, hexanol-1, cis-3-hexen-1-ol, trans-2-hexenal and linalool (Visser *et al.*, 1979). Electroantennogram recordings show that the olfactory system used by the Colorado beetle in olfactory orientation towards its host plant potato, is selective and has a high sensitivity to these generally distributed leaf volatiles (Visser, 1979).

The present study illustrates the effects of these chemicals and some of their isomers, on olfactory orientation of the Colorado beetle, and outlines their rôle in the context of our present knowledge.

MATERIAL AND METHODS

Newly-emerged Colorado beetles were obtained from the laboratory stock culture, and starved for 24 h prior to the experiments. The behavioural responses of individual female beetles to wind-borne volatiles were observed in a low-speed wind tunnel, described in detail by Visser (1976). This instrument (Fig. 1) is housed in a controlled environment room at 25° and 50% R.H. A centrifugal ventilator

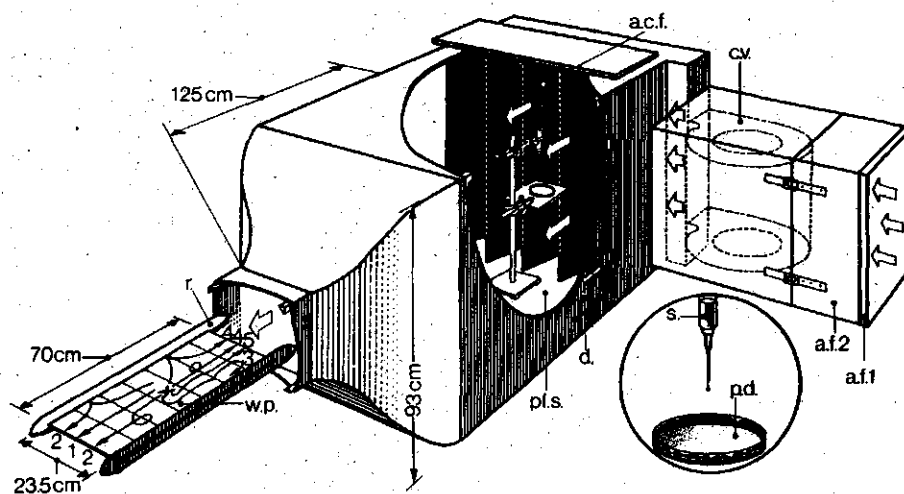


Fig. 1. Diagram of wind tunnel. a.c.f.: activated charcoal filter; a.f.1 — a.f.2: air filters; c.v.: centrifugal ventilator; d.: door; p.d.: petri dish; p.l.s.: plant section; r.: streamlined rails; s.: syringe; w.p.: walking plate. Outlines of insect test section not shown. A support holding syringe filled with a test chemical solution, stands in plant section. Inset illustrates the way test chemical is released into the wind. Arrows indicate direction of air flow. Beetles were released at the cross drawn on walking plate. 1—5: categories of response. See text for further explanation.

sucks air through two air filters, the air is purified by an activated charcoal filter and flows into the plant section. This dark section held the odour source: four pots of fully grown potato plants (cultivar *Eigenheimer*), and a support holding a syringe from which test chemicals could be injected into the air stream. The syringe was filled with pure paraffin oil (Merck Uvasol) in control treatments, or with a test chemical solution in paraffin oil in experimental situations. After the plunger had been removed, the solution dripped out of the syringe at a rate of 1.1 ml per hour into a petri dish, thus releasing volatiles continuously into the wind. In order to maintain the vapour concentration within certain limits in the course of an experiment, the petri dish was replaced every hour. The concentrations of the test chemicals in paraffin oil refer to the concentrations of the original solutions (volume/volume). The vapours were distributed over the total cross-sectional area of the wind tunnel by turbulence, which originated from the folded construction of the activated charcoal filter. This resulted in a uniform vapour concentration in the insect test section, downwind of the odour source (Visser, 1976). A glass walking plate was fixed with streamlined rails at half height in the test section, where the velocity of the main stream was set at 80 cm/sec.

In each experiment one beetle was placed in the centre of the walking plate. After it started walking, it was observed for 5 min., or until it reached one of the edges. So as to discriminate the behavioural responses, the observed tracks were divided into five categories, reported in detail by Visser & Nielsen (1977): 1 — straight to the downwind edge; 2 — indirectly to the downwind edge; 3 — reaching neither of the edges; 4 — indirectly to the upwind edge; 5 — straight to the upwind edge. In addition, the time needed to reach the upwind edge was noted. The same beetle was subjected to control and experimental situations. The resultant scores were analysed statistically by the Sign Test (Siegel, 1956) on the basis of the categories of responses called criterion A, and the periods required to reach the upwind edge called criterion B.

The test chemicals were obtained from commercial sources: the hexenols (98%—99%) from Roth, trans-2-hexenal (99%) from Koch-Light Lab., linalool (99%) from Fluka and the hexanols (96%—98%) from Merck.

RESULTS AND DISCUSSION

The individual responses of female Colorado beetles to wind, to wind-borne potato plant odour, and to wind supplied with the vapours of test chemicals, are shown in Table I. The potato plants strongly attract the beetles. The host plant odour elicits positive anemotactic responses in these insects; odour-conditioned positive anemotaxis is illustrated in the shift of the response categories from indifferent (3) to moving upwind (4 and 5); its significance is expressed in criterion A. Potato plant odour also increases the beetle's speed of locomotion or direct chemo-orthokinetic response; its significance is reflected in criterion B. As a consequence, the beetles reach the upwind edge faster in wind with potato plant odour than in an air flow devoid of vapours. The behavioural responses of

LEAF VOLATILES IN OLFACTORY ORIENTATION

TABLE I

Individual responses of female Colorado beetles to wind, to wind plus potato plant odour and to wind plus test chemicals¹

	Category of response					Compared with wind Criterion		Compared with potato Criterion	
	1	2	3	4	5	A	B	A	B
	wind	0	5	21	4	0			
potato	0	0	1	23	6	**	**		
cis-3-hexen-1-ol	0	4	19	7	0	ns	ns	**	**
wind	0	1	13	2	0				
potato	0	0	0	13	3	**	**		
trans-3-hexen-1-ol	0	3	12	1	0	ns	ns	**	**
cis-2-hexen-1-ol	1	4	7	4	0	ns	ns	**	**
wind	0	5	9	2	0				
potato	0	0	1	7	8	**	**		
trans-2-hexenal	0	3	10	3	0	ns	ns	**	**
wind	0	2	12	2	0				
potato	0	0	1	10	5	**	**		
trans-2-hexen-1-ol	0	5	10	1	0	ns	ns	**	**
linalool	0	5	9	2	0	ns	ns	**	**
wind	0	2	10	3	0				
potato	0	0	0	10	5	**	**		
hexanol-1	0	6	6	3	0	ns	ns	**	**
hexanol-2	0	7	5	3	0	ns	ns	**	**
hexanol-3	1	5	6	3	0	ns	ns	**	**

¹ Concentration of test chemical is 1 µl/ml of paraffin oil, 10⁻³.

ns: not significant at P > 0.01

** : significant at P < 0.001

Colorado beetles to the test chemicals are neutral, these individual volatiles being neither attractive nor repellent.

In spite of these "negative" results, further experiments were conducted in order to assess possible effects of these volatiles on the orientation response of Colorado beetles to potato plants.

Female beetles were tested successively with wind alone, with wind-borne potato plant odour, and with wind bearing both potato plant odour and the vapour of a test chemical (Fig. 2). In a control experiment Colorado beetles were tested once in wind and twice with wind-borne host plant odour, the interval between the first and second test with potato odour being 6 hr (Fig. 2a). It is observed that in the course of this experiment, the behavioural responses of Colorado beetles to the attractive potato plant odour do not change. Potato plants combined with trans-3-hexen-1-ol, cis-2-hexen-1-ol, trans-2-hexen-1-ol or trans-2-hexenal (Fig. 2c, d, f, h, j) are not

attractive, that is the beetles no longer react with an odour-conditioned positive anemotaxis (criterion A). Thus, a disruption of the olfactory orientation is attributed to the chemicals applied. The geometrical isomer *trans*-3-hexen-1-ol is the most effective chemical in this respect (at both 10^{-3} and 10^{-4} in paraffin oil). It is striking that direct orthokinetic responses of the beetles are not affected by these chemicals (criterion B). The beetles are more active than in a pure air flow, consequently several beetles reach the upwind edge of the walking plate. At the

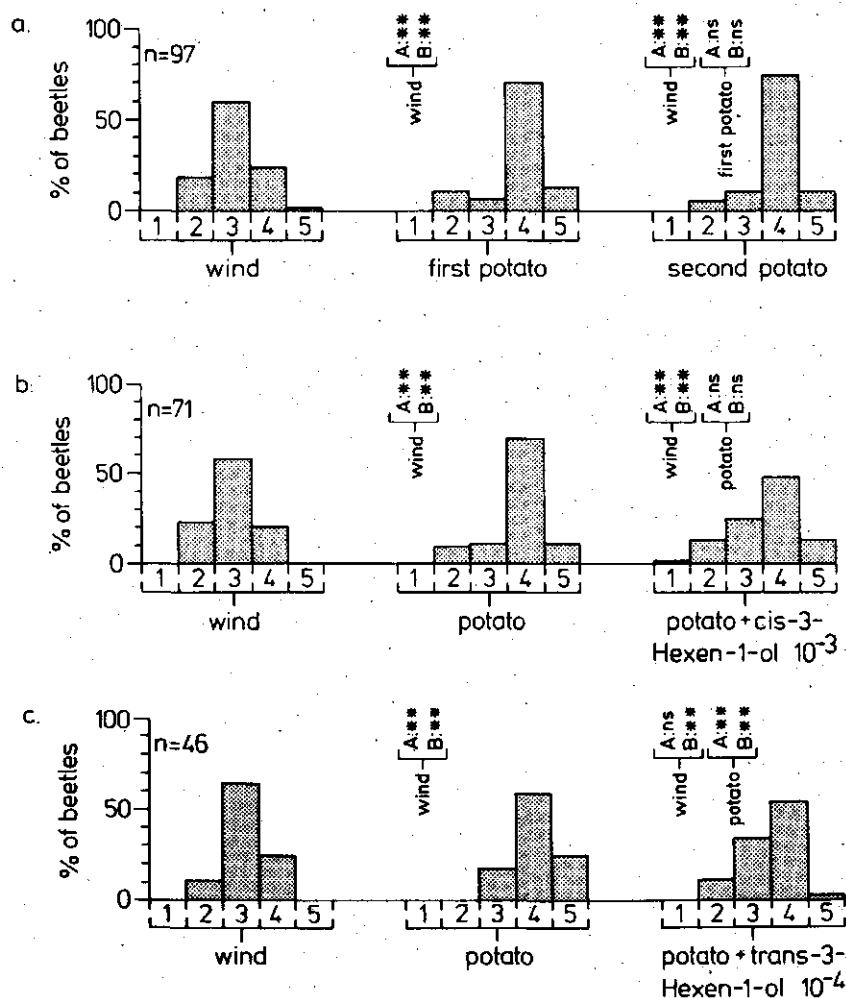


Fig. 2a-n. Individual responses of female Colorado beetles to wind, to wind plus potato plant odour, and to wind plus a combination of potato plant odour and a test chemical. Concentrations refer to test chemical solutions in paraffin oil (v/v). n, number of individuals tested. 1-5: categories of response. Response criteria A. and B. (see text) are compared with responses to wind and to potato. ns: not significant at $P > 0.01$; *: significant at $P < 0.01$; **: significant at $P < 0.001$.

LEAF VOLATILES IN OLFACTORY ORIENTATION

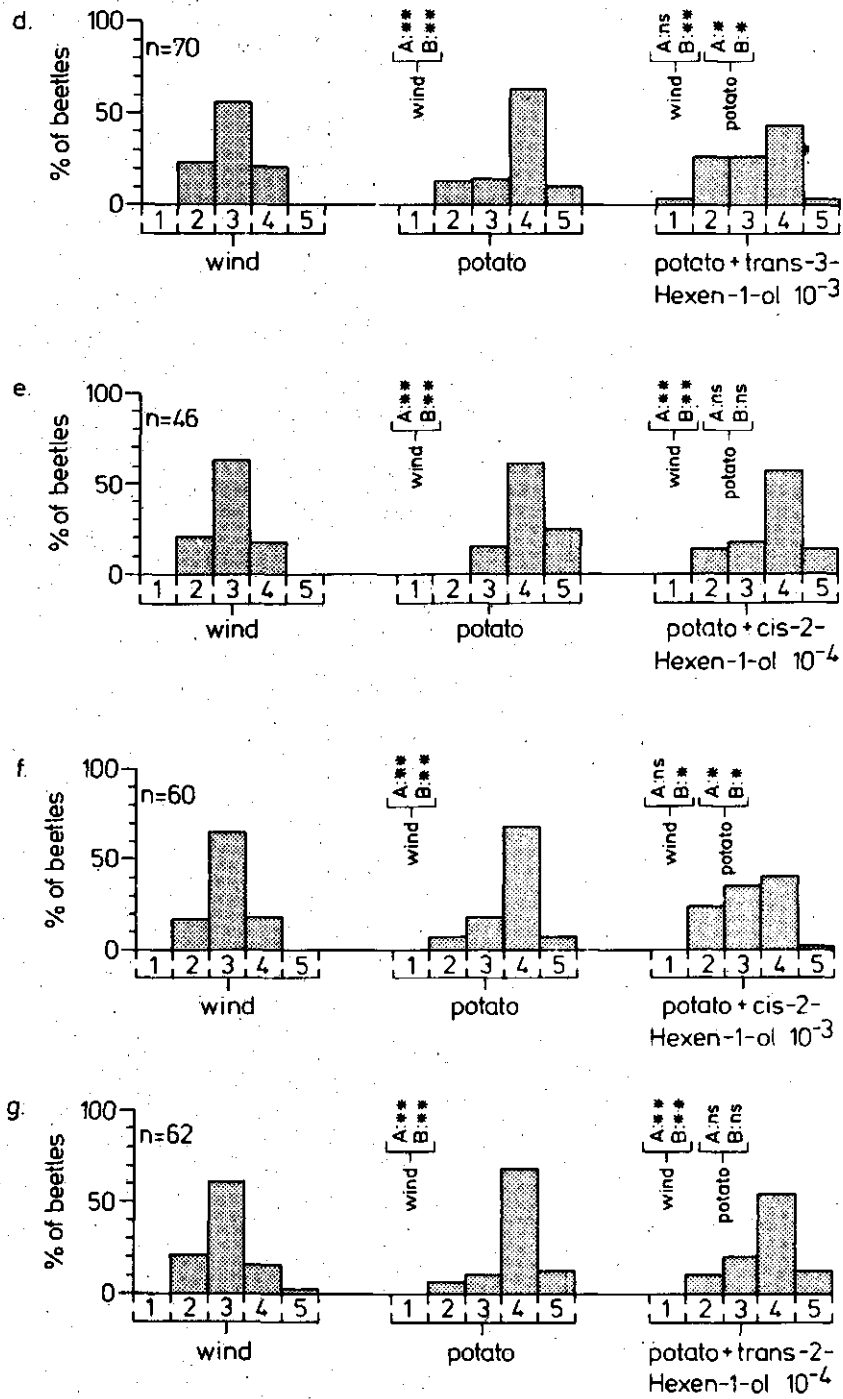


Fig. 2, Continued

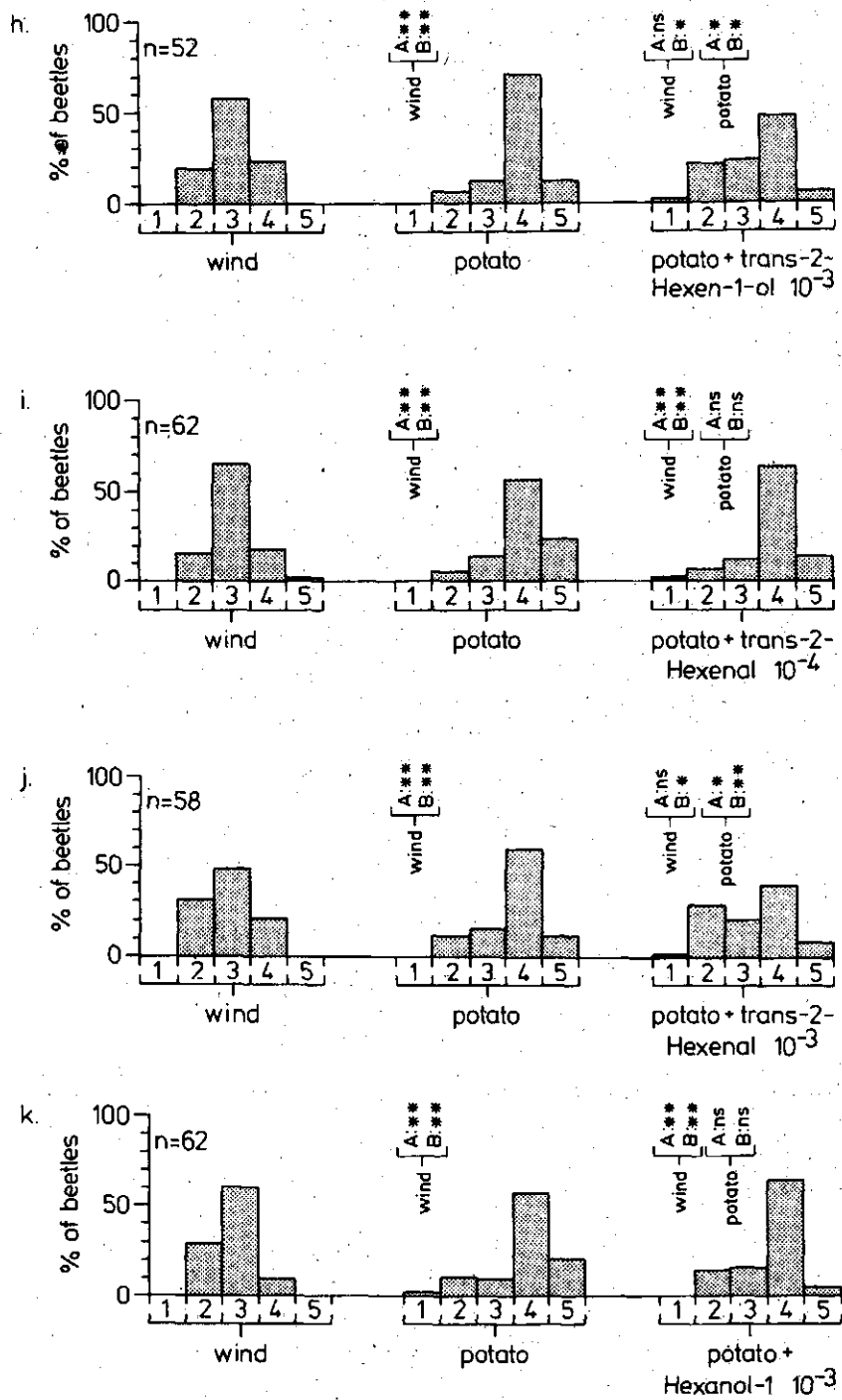


Fig. 2, Continued.

LEAF VOLATILES IN OLFACTORY ORIENTATION

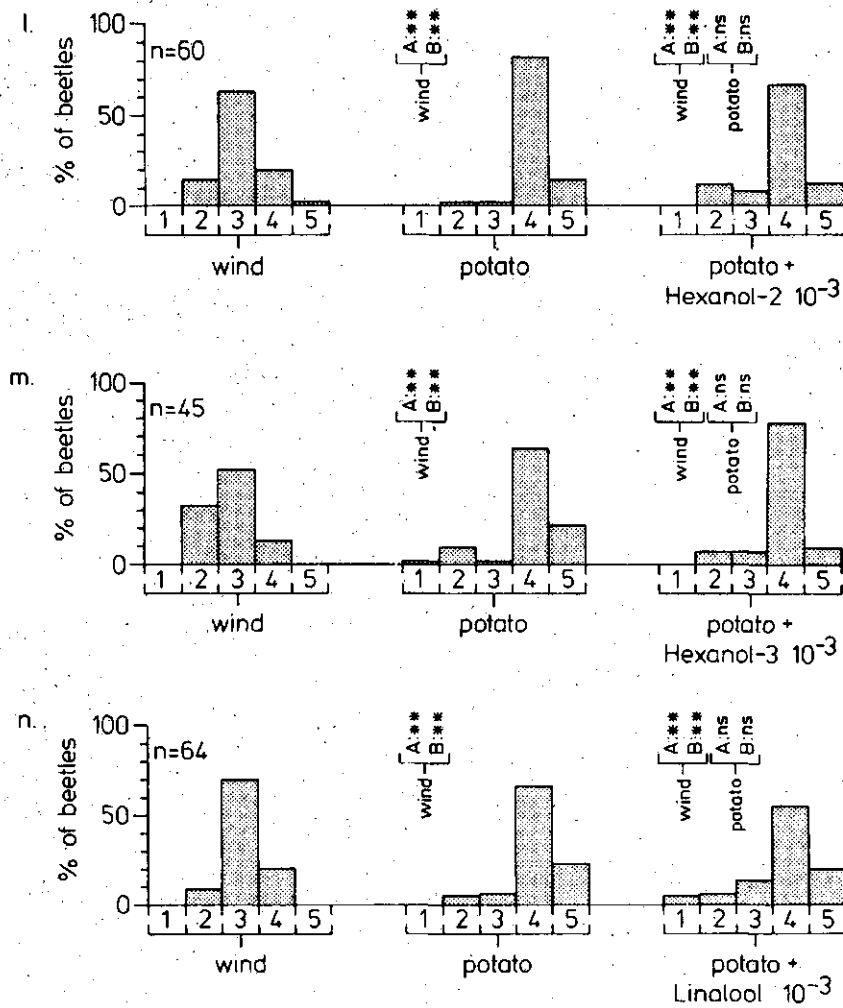


Fig. 2, Continued

concentration tested (10^{-3}), cis-3-hexen-1-ol, hexanol-1, hexanol-2, hexanol-3 and linalool (Fig. 2b, k, l, m, n) do not affect either of the responses of Colorado beetles to potato plant odour.

Duality of long-range olfactory orientation

In distant attraction, for the main part, odour-conditioned anemotaxis guides insects towards the odour source. The primary effect of attractive volatiles on insects, a chemo-orthokinesis, is also a requisite for this behaviour (see review by Kennedy, 1977). However, when chemo-orthokinesis is separated from the anemotactic responses its effectiveness in long-range olfactory orientation is

doubtful. Several volatiles "turn off" the odour-conditioned anemotaxis in Colorado beetles, but do not affect the orthokinetic responses, underlining the duality in long-range olfactory orientation. Thus, potato plant odour combined with some chemicals, is no longer effective for distant attraction.

Effective concentration

The geometrical isomer trans-3-hexen-1-ol interferes with the host plant odour at a concentration as low as 100 ppm in the syringe positioned upwind, and when released into the air flow this chemical is diluted, the resultant vapour concentration in the wind of the test section is at most 2.3×10^9 molecules per ml of air. As this concentration is near the threshold for an electroantennogram response of the Colorado beetle (2.3×10^8 molecules per ml of air at the same wind speed; Visser, 1979), the observed interference with potato plant odour is not caused by extreme stimulus strengths.

Masking of potato plant odour

The air over cut fully grown potato leaves, which is attractive to Colorado beetles (see e.g. Schanz, 1953; de Wilde *et al.*, 1969), differs from the essential oil (Visser *et al.*, 1979) in that it contains cis-3-hexen-1-ol (100%), cis-3-hexenylacetate (59%), trans-2-hexenal (37%) and trans-2-hexen-1-ol (16%), as identified by GLC-MS studies (Visser & Schaefer, unpubl.). The geometrical isomers trans-3-hexen-1-ol and cis-2-hexen-1-ol are not detected in either the essential oil or the headspace of cut potato leaves. The potato leaf volatiles trans-2-hexen-1-ol and trans-2-hexenal, and the geometrical isomers trans-3-hexen-1-ol and cis-2-hexen-1-ol, interfere with the attractive potato plant odour. As none of these chemicals applied singly elicits repellent responses in Colorado beetles, it is concluded that they mask the host plant odour. This masking is explained as a disturbance of the attractive complex by artificially changing the relative proportions of the components. The particular ratio of components, constituting an essential aspect of the attractive odour, is distorted by adding small quantities of its minor components, trans-2-hexen-1-ol and trans-2-hexenal. The geometrical isomers trans-3-hexen-1-ol and cis-2-hexen-1-ol may interfere with the perception of this complex. This has an obvious resemblance to the distortion of insect pheromone blends, as their ratios are changed or isomers added (Birch, 1974). A complex of volatiles rather than one single compound, is essential for olfactory orientation of the Colorado beetle towards its host plant.

General green leaf volatiles

The components involved in the attraction of Colorado beetles, also constitute a considerable proportion of the leaf vapours of numerous plant species (Visser *et al.*, 1979). They are formed by oxidative degradation of leaf lipids, and their biosyntheses are illustrated in Fig. 3. Owing to plant ageing and injury these volatiles are continuously released into the surrounding air. The observation that fully grown potato plants are more attractive to Colorado beetles than young plants

(Visser, 1976), may be related to the increase in the levels of these volatiles with age (Visser & Schaefer, unpubl.).

These general green leaf volatiles elicited behavioural responses in several phytophagous insects. Silkworm larvae, *Bombyx mori* are attracted by 3-hexen-1-ol and 2-hexenal (Watanabe, 1958). Adults of the vegetable weevil *Listroderes costirostris obliquus*, were attracted by 3-hexen-1-ol, and both 3-hexen-1-ol and hexanol-1 attracted the larvae (Matsumoto & Sugiyama, 1960). Trans-2-hexenal isolated from oak leaves, is an incitant for the "calling" behaviour of female

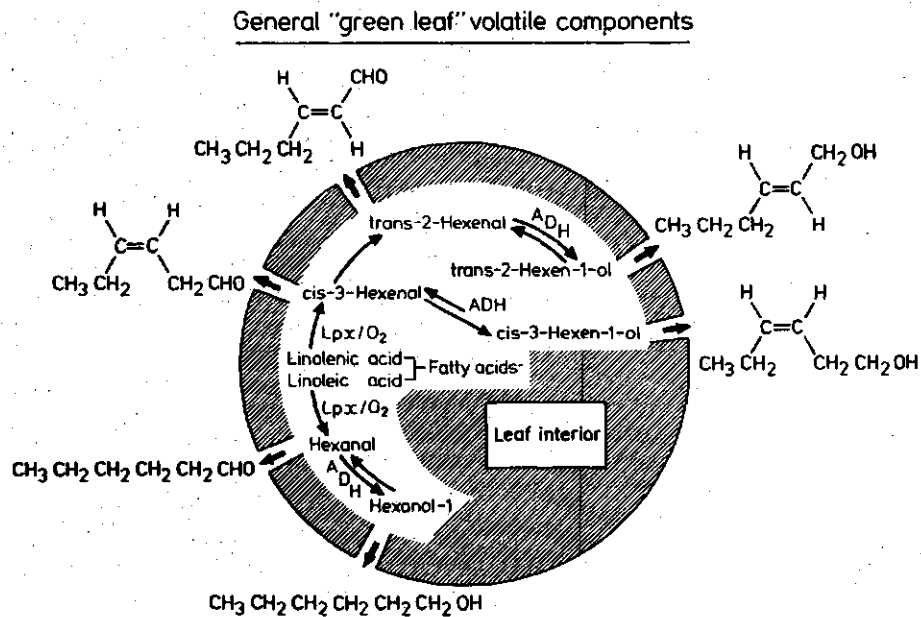


Fig. 3. Biosyntheses of the general green leaf volatiles (Visser *et al.*, 1979). ADH: Alcoholdehydrogenase; Lpx: Lipoxygenase.

polyphemus moths, *Antheraea polyphemus* (Riddiford, 1967). The phagostimulatory nature of *Solanum campylacanthum* for the larvae of *Epilachna fulvosignata* is attributed to a fraction of the essential oil, containing hexanol-1, cis-3-hexen-1-ol and cis-2-hexen-1-ol (Murray *et al.*, 1972), however data supporting the biological activity were not presented, and the identification of cis-2-hexen-1-ol is controversial (Visser *et al.*, 1978). The known behavioural responses to these components, and the more extended information concerning their perception by insects (see Visser, 1979), point to the conclusion that the complex of general green leaf volatiles represents "green odour" for various phytophagous insects.

Modality

The relative proportions of the components in the "green odour" complex vary

in and between different plant species, as influenced by the expression of the enzymes involved. The predominant vapour component of several cruciferous species is *cis*-3-hexenylacetate, while *cis*-3-hexen-1-ol and hexylacetate are minor constituents and *trans*-2-hexenal is absent (Wallbank, 1972; Wallbank & Wheatley, 1976). In this way the cruciferous "green odour" contrasts with the potato "green odour", and accordingly different modalities, or qualities, are conferred on the "green odours" of diverse plant species.

As a consequence, the "background odour" of other plant species in the field may prevent an initial olfactory orientation of the Colorado beetle towards susceptible plants by masking the attractive "green odour modality" peculiar to these susceptible plants. This interference should depend both on the density and variety of plants and the opposing natures of the several "green odours" involved. Information concerning the capability of the antennal olfactory system of the Colorado beetle to discriminate different modalities, is presented by Ma & Visser (1978).

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RÉSUMÉ

SUBSTANCES VOLATILES DES FEUILLES VERTES ET ORIENTATION OLFACTIVE CHEZ LE DORYPHORE, LEPTINOTARSA DECEMLINEATA

On a cherché à déterminer l'effet sur l'orientation olfactive de doryphores femelles des composants principaux identifiés dans l'huile des feuilles de pomme de terre, *trans*-2-hexen-1-ol, hexanol-1, *cis*-3-hexen-1-ol, *trans*-2-hexenal et linalool, et les isomères *trans*-3-hexen-1-ol, *cis*-2-hexen-1-ol, hexanol-2 et hexanol-3. Si des pieds de pomme de terre ayant atteint leur développement complet provoquent des réactions anémotactiques positives, causées par leur odeur, chez le doryphore, aucune de ces substances chimiques, appliquée isolément, n'est attrayante. Associés à des pieds de pomme de terre, les composés suivants masquent l'odeur attrayante de la plante-hôte: *trans*-2-hexen-1-ol, *trans*-2-hexenal, *trans*-3-hexen-1-ol et *cis*-2-hexen-1-ol. L'isomère géométrique *trans*-3-hexen-1-ol est à cet égard la substance la plus efficace. Les doryphores ne réagissent plus par une anémotaxie provoquée par l'odeur, mais ils sont pourtant activés. Le masquage de l'odeur de la plante-hôte s'explique par une perturbation du complexe attrayant par suite du changement des proportions relatives de ses composants. Ces substances volatiles générales des feuilles vertes constituent probablement une partie importante des odeurs de feuille qui attirent différents insectes phytophages et représentent "l'odeur verte".

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CHAPTER 7

SINGLE UNIT ANALYSIS OF ODOUR QUALITY CODING BY THE OLFACTORY ANTENNAL RECEPTOR SYSTEM OF THE COLORADO BEETLE

BY

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Peripheral events underlying plant odour quality discrimination in the Colorado beetle were investigated. Responses to saturated and unsaturated C-6 alcohols and aldehydes ("green leaf" volatiles) and to other plant compounds were recorded extracellularly. The olfactory receptors could respond by inhibition and/or excitation; inhibition was especially observed at the higher stimulus intensities. Variations in the response patterns were analyzed for a number of receptors constituting a representative sample, as indicated by rank correlation between mean spike responses and electroantennogram amplitudes for each odour stimulus. Application of cluster analysis showed that the olfactory receptors may be divided into two main groups, one reacting differentially to the complex of "green leaf" volatiles and their isomers, and another group showing a selective sensitivity to the aromatic compound methylsalicylate. Evidence is provided suggesting that individual olfactory sensilla can be innervated by more than one functional type of receptor unit. Responses to natural plant odours support the conclusion that plant odour quality is encoded by response patterning across a limited number of olfactory receptor types. The findings are discussed in relation to concepts of chemosensory coding and to the behaviour of the Colorado beetle.

Many authors have concluded from behaviour studies that the adult Colorado beetle, *Leptinotarsa decemlineata* Say, possesses a sense of smell which allows it to distinguish between the odour of its solanaceous hosts and that of non-solanaceous plants (Visser & Nielsen, 1977). It has been suggested that the so-called "green leaf" volatiles, consisting of saturated and unsaturated C-6 alcohols and aldehydes, play a significant rôle in the process of host odour recognition in this insect (Visser & Avé, 1978). In fact, plant odour constituents eliciting strong electroantennogram responses (EAGs) largely comprise this group of volatile chemicals (Visser, 1979).

The present work focusses attention on the problem of odour quality coding, that is, the manner in which quantitative and qualitative differences in the composition of plant odours are detected. Input patterns analyzed for single antennal receptor cells (units) suggest that the Colorado beetle has a considerable sensory capacity of plant odour discrimination.

MATERIALS AND METHODS

Young female Colorado beetles reared on potato foliage under long-day

conditions were used throughout the experiments. Individuals with legs removed were attached dorsally to a cork base with a mixture of beeswax and colophonium. Extracellular recordings from olfactory receptors were made with electrolytically sharpened tungsten microelectrodes, using the method described by Boeckh (1962) with conventional apparatus for signal amplification and registration.

Air, purified through activated charcoal, was blown through two glass cartridges made of pasteur pipettes, one of which was loaded with a 6.0×0.5 cm strip of filter paper. The test chemicals were dissolved in paraffin oil (Merck, Uvasol) and pipetted in 25 μ l quantities on to the filter paper strip. The common outlet of both cartridges (1.5 mm internal diameter) was positioned by micromanipulators at a distance of 5-8 mm from the tip of the recording microelectrode. The delivery of an odour puff of one second duration at a rate of 1 ml/sec was alternated with a constant flow of purified air. The alternation of flow was controlled by electromagnetic valves operated through a timer which also provided a 50 Hz signal as a marker of stimulus application.

The number of molecules per ml air leaving the outlet was determined from direct injection into a gas chromatograph fitted with an open stainless steel tube. The time course of the stimulus is shown in Fig. 2 as the signal of the flame ionization detector to *cis*-3-hexen-1-ol. Calibration curves for a number of compounds are given in Fig. 1. For the sake of convenience the stimulus intensities are referred to as the dilution step (v/v) in paraffin oil of the chemical inside the odour cartridge.

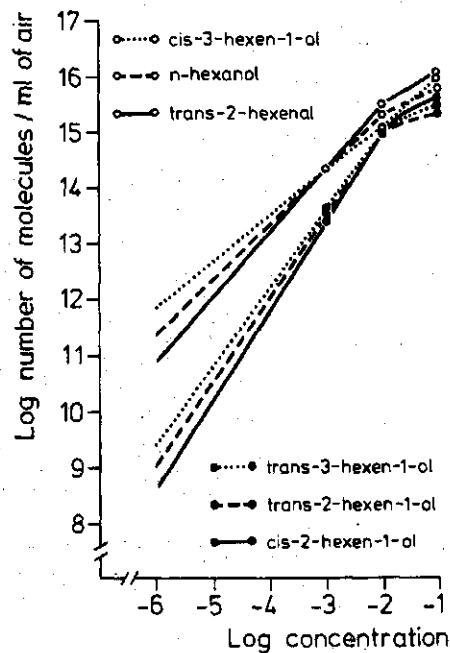


Fig. 1. Calibration of stimulus delivery system showing number of odour molecules per ml air for concentrations (v/v) of compounds in paraffin oil.

The chemicals selected for study had a known EAG activity (Visser, 1979), and include compounds belonging to the complex of "green leaf" volatiles, viz. cis-3-hexen-1-ol, trans-2-hexen-1-ol, n-hexanol, trans-2-hexenal, hexanal and cis-3-hexenyl acetate, and their isomers cis-2-hexen-1-ol, trans-3-hexen-1-ol, hexanol-2 and hexanol-3. The monoterpene alcohol linalool, the aromatic compound methylsalicylate and the monoterpene α -pinene were also studied. All chemicals were commercially obtained and checked for purity by gas liquid chromatography. Most compounds had a purity of greater than 97%, except for hexanol-3 (96%) and α -pinene (95%).

The response magnitude of a receptor unit was evaluated as the number of nerve impulses generated in an interval of 6 sec including the period of maximum spike density. A correction was made for the level of spontaneous discharge by deducting the number of nerve impulses over a similar 6-sec period preceding stimulus delivery. In experiments involving serial stimulation an interstimulus time of at least 2.5 min was imposed, while all trials were arranged in a random order.

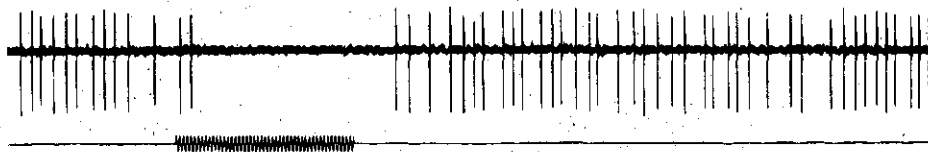
RESULTS

The olfactory receptor neurones associated with the sensilla basiconica on the antennae of the Colorado beetle displayed a spontaneous spike discharge which may vary considerably between individual receptors. The level of spontaneous activity appeared related to the type of receptor concerned. Thus, receptors which normally responded with an increase of neural activity showed constant spontaneous discharges which varied between 0.2 to about 5 impulses per sec (Fig. 2). Receptors responding with inhibition exclusively, and hence classified as inhibitory receptors, exhibited much higher levels of spontaneous activity of between 10 to 20 impulses per s. About 20% of a total number of approximately 100 receptors investigated were of the inhibitory type. They could be differentiated as receptors responding (1) to the C-6 alcohols only, and (2) to the aldehydes as well as the alcohols. These units had a response threshold of at least two log steps higher than the excitatory types of receptors. Typical complete cessation of spike activity such as shown in Fig. 2, was never recorded at stimulus intensities lower than 10^{-2} . Inhibition was occasionally observed to be followed by delayed excitation.

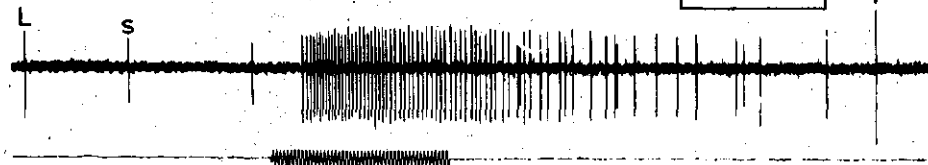
The inhibitory types of olfactory receptor are difficult to detect when the spontaneous level of activity is low, so that there may have been inhibition in those receptors that showed no discernable alteration in basal firing rate with any of the odour stimuli. This was the case for about half of the total number of recorded receptors. The significance of these non-responding receptors is not clear. Apart from the possibility of being hidden inhibitory receptors, it could be that none of the stimuli were of an adequate type. It is also possible, although less likely, that during the implantation of the microelectrode the functional properties of the receptor cell were affected.

In the following analysis attention has been concentrated on the excitatory type of olfactory receptor units. This type reacts to most of the odour stimuli by

Olfactory inhibition



Olfactory excitation

Stimulus: *cis*-3-hexen-1-ol [10^{-2}]

Stimulus duration: 1 sec

Fig. 2. Inhibitory and excitatory types of response of olfactory antennal receptor cells. Inset shows time course of stimulus as monitored by flame ionization detector (FID). Note firing of two receptor units ("L" and "s") in second trace; arrow points to coincidence of the two spike types. 50 Hz signal marks period of stimulus delivery.

excitation (Fig. 2), although instances of inhibition in the same unit may occur as well. The location of the recording sites was chosen randomly on the three terminal antennal segments for a sample of 25 units. Since each recorded unit was from a different beetle the group of receptors can be regarded as a truly random sample of the total receptor population present on these antennal segments. Virtually all the basiconic olfactory sensilla are concentrated on the five terminal segments (Schanz, 1953).

The stimulus intensity was set at a standard concentration of 10^{-2} in paraffin oil. The choice of this relatively high intensity was based on the consideration that at the higher intensities the stimulus-response curves are more reliably separated from each other. An example of stimulus-response relations for different odour stimuli is given in Fig. 3. The lowest threshold values measured for single receptor cells were at 10^{-6} concentration, determined for *cis*-3-hexen-1-ol and *trans*-2-hexen-1-ol. This would correspond to threshold values of approximately 10^{12} and 10^9 molecules per ml air, respectively (Fig. 1).

For each test chemical, the average response magnitude was calculated from three to four trials for the entire sample of olfactory receptors. The data were analyzed using a multivariate statistical method for sorting into groups with a high similarity among spectral characteristics (Blackith & Reymont, 1971). Fig. 4 shows

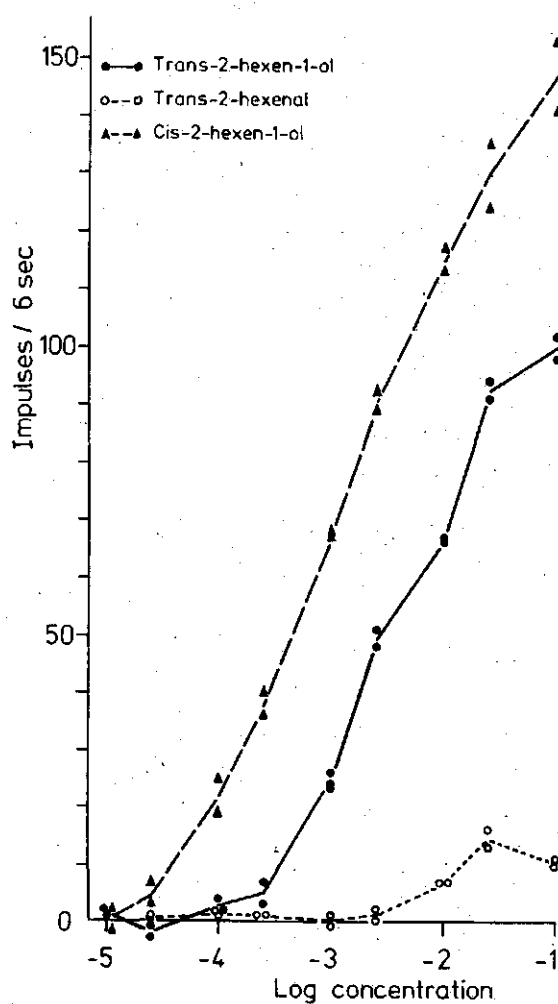


Fig. 3. Stimulus-response functions for three odour compounds for one single olfactory receptor unit stimulated at various intensities. Each point represents one trial.

the result of an agglomerative analysis, by way of a two-dimensional hierarchically branched figure, indicating a reasonable grouping when taking into account the degree of overlap of the various spectra. In Fig. 5, distinguishable groups have been visualized by presenting the average response spectrum per group. The grouping obtained can be considered as carrying a predictive value for the existence of separate receptor types, designated here as types A, B, C, D and E. To facilitate a comparison of the various types the response values in Fig. 5 have been computed as percentages of the highest value measured.

According to the respective response profiles, receptor type E is separated from the other four by having a selective sensitivity for the aromatic compound methylsalicylate. The other types are insensitive to this chemical, but show a

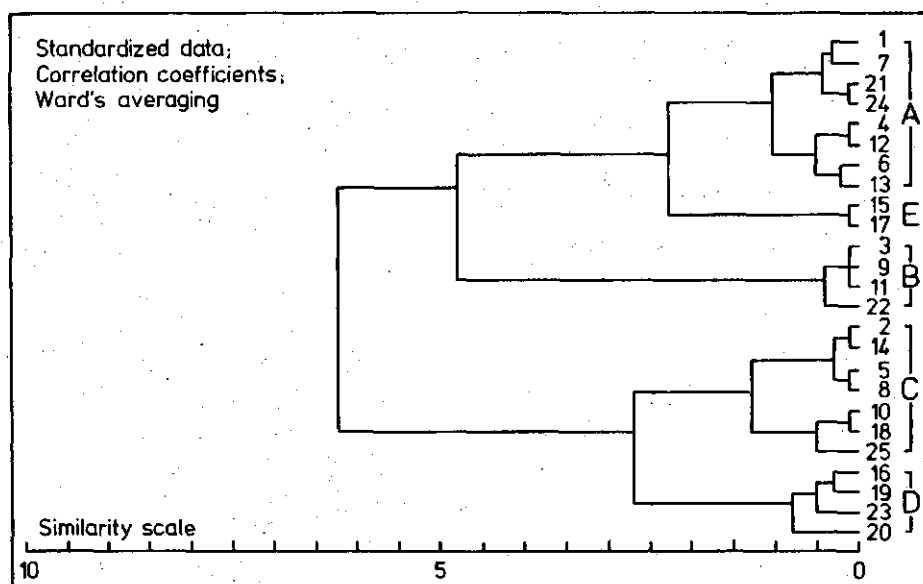


Fig. 4. Ward average cluster analysis based on correlation coefficients among 25 individual response spectra. Similarity scale indicates increasing similarity from left to right.

differential sensitivity to the array of "green leaf" volatiles and their isomers. Among these receptors, type A possesses the broadest spectrum by responding strongly to both the *cis* and *trans* isomers of 2-hexen-1-ol and 3-hexen-1-ol, as well as to *n*-hexanol. This type responds to the positional isomers *n*-hexanol, hexanol-2 and hexanol-3 in a characteristically decreasing order of magnitude; it does not show a significant sensitivity to the C-6 aldehydes. It is interesting to note that one of the above-mentioned inhibitory types of receptor forms in fact a mirror-image of the type A unit, except for the lower response threshold of the latter.

Receptor types B, C and D have more narrowly tuned reaction spectra; for example, both B and C have a low sensitivity to the *cis* form, but a high responsiveness to the *trans*-isomeric form of the unsaturated C-6 alcohols. On the other hand, type D shows a preferential sensitivity to *cis*-3-hexen-1-ol only. Another salient feature of the type D receptor is the time course of the response to *cis*-3-hexen-1-ol. After a one second stimulus at concentrations of 10^{-2} or higher these receptors continue firing almost tonically and require as long as 5-10 minutes before reaching baseline level. This slow decrement of the excited state occurred in spite of the constant flow of purified air over the preparation. In the other receptor types similar phasic-tonic discharges returned to resting activity much more rapidly within one or, at most, two minutes.

The reaction patterns further show that a sensitivity to the C-6 aldehydes *trans*-2-hexenal and hexanal is confined to receptor type C only. Sensitivity peaks for the saturated alcohols hexanol-2 and hexanol-3 are localized in type B, while a responsiveness to *cis*-3-hexenyl acetate is linked exclusively to receptor cell type D.

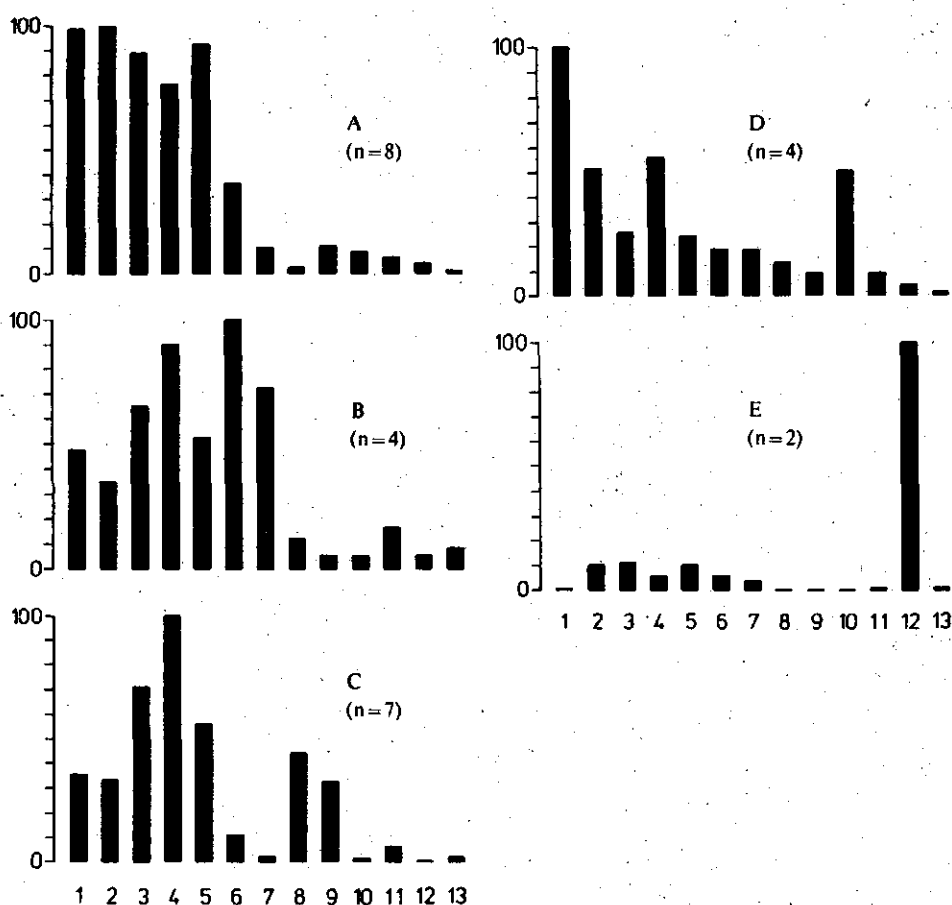


Fig. 5. Relative reaction spectra of receptor types A-E derived from grouping indicated in Fig. 4. The odour stimuli numbered: (1) *cis*-3-hexen-1-ol; (2) *cis*-2-hexen-1-ol; (3) *trans*-3-hexen-1-ol; (4) *trans*-2-hexen-1-ol; (5) *n*-hexanol; (6) hexanol-2; (7) hexanol-3; (8) *trans*-2-hexenal; (9) hexanal; (10) *cis*-3-hexenyl acetate; (11) linalool; (12) methylsalicylate; (13) α -pinene.

In order to estimate whether the sample studied was large enough to be considered as representative for the total receptor population on the antenna the average response values of the total sample to each odour stimulus were plotted against corresponding values of EAG responses. The reasoning behind this procedure is that the EAG represents the summated evoked receptor potentials from the majority of olfactory cells present in the antenna (Schneider, 1957; Boeckh *et al.*, 1965), while each receptor potential is related to the triggering of the impulse discharge of the afferent nerve (Boeckh *et al.*, 1965; Kaissling, 1971).

As shown in Fig. 6 a reasonable degree of correlation between the single unit responses and the corresponding EAG's was observed with no exception for all of the thirteen odour stimuli investigated. The correlation computed as Spearman's coefficient of rank correlation was determined at $r_s = 0.9135$. It should be noted that the stimulus intensities shown in the ordinate of Fig. 6 should in fact be ten

times lower. This is because in the EAG measurements the odour stimulus was injected into a glass tube carrying a continuous air stream rather than applied directly onto the antenna as in the single unit study. As a result the intensity of the odour stimulus in the EAG study can be calculated as being diluted ten times in the air passing over the antenna. In the left half of Fig. 6 the stimulus intensity indicated for the EAG data therefore is a hundred times lower than the concentration shown in the abscissa. Even so, the rank correlation remained highly significant ($r_s = 0.8242$, $P < 0.01$). Consequently, the assumption that the sample of receptor units investigated is reasonably representative for the total antennal receptor population seems justified.

Depending on the depth of penetration of the sensillum base by the recording microelectrode the nervous activity from several neighbouring receptor cells can be picked up. Such multiple unit recordings can be analyzed from amplitude discrimination of the nerve impulses and offers the considerable advantage of permitting a direct comparison of receptor units without having to consider experimental recording conditions as a source of variability. In order to exclude any risk of confusion of the origin of the nerve impulses only those recordings were considered which allowed a clear distinction between the activity of not more than

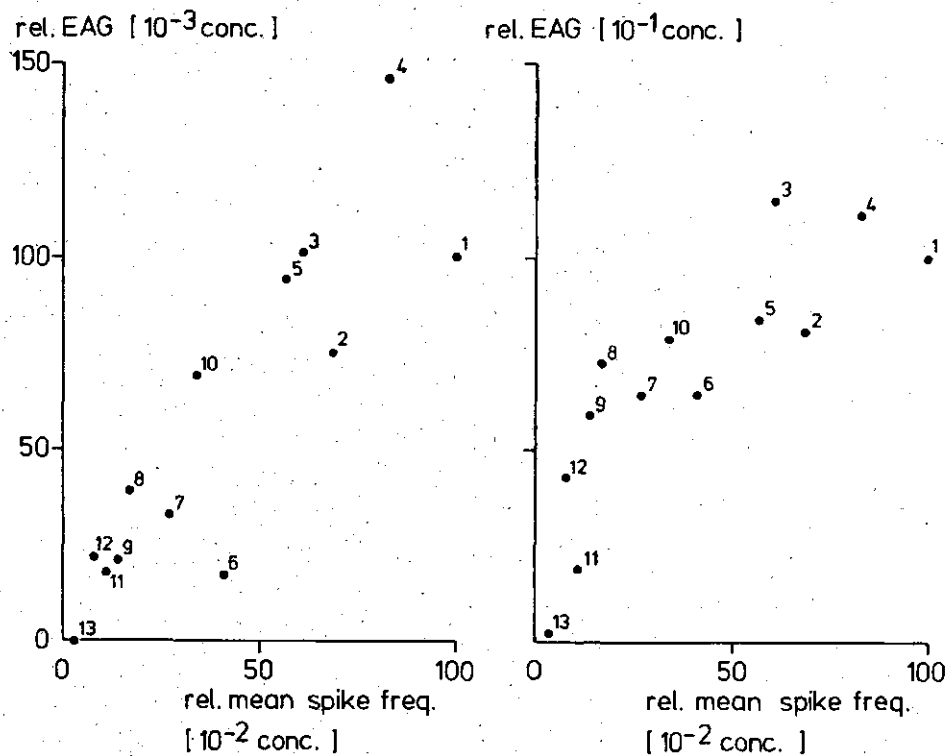


Fig. 6. Scatter diagram for average total evoked activity of nerve impulses and electroantennogram response as determined for series of odour stimuli listed in Fig. 5.

two units. In such recordings the unit firing with the largest spike was designated as the "L" cell, while its counterpart was named the "s" cell (Fig. 2).

In Fig. 7 the results of two of such simultaneous recordings, each obtained from a

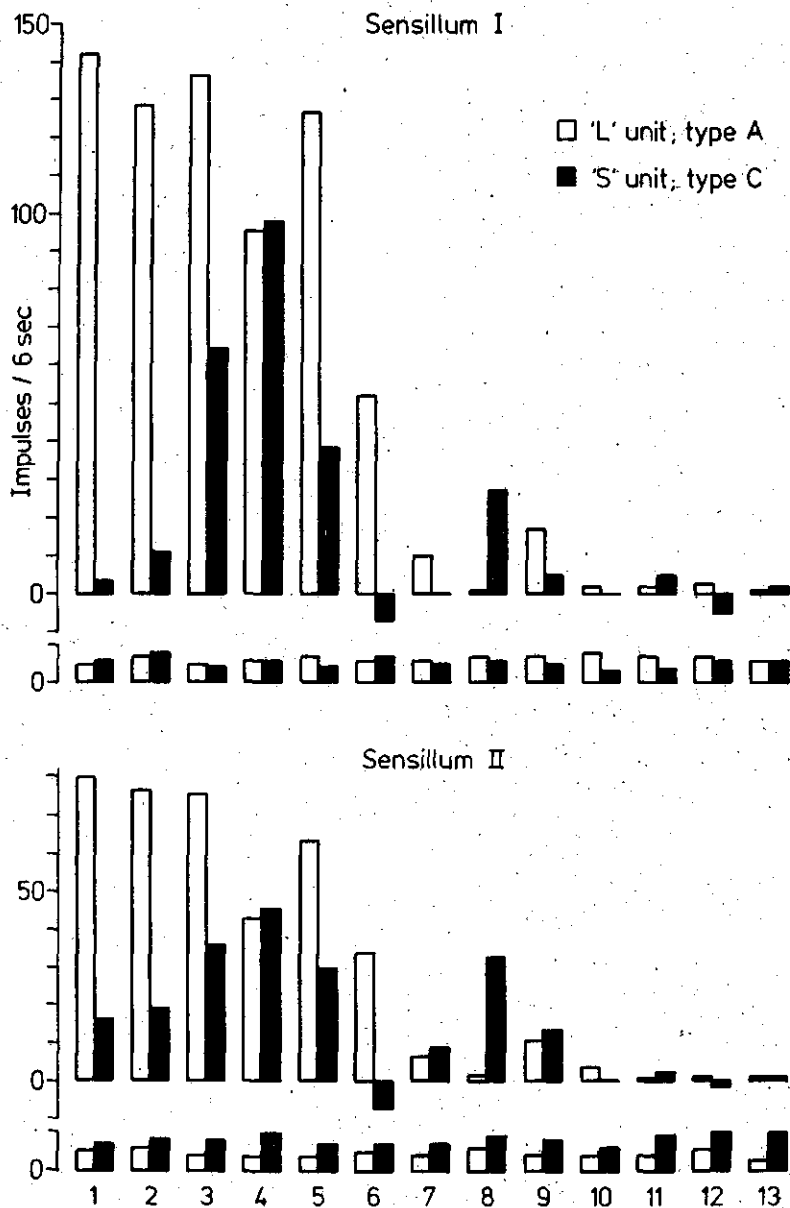


Fig. 7. Reaction spectra of two pairs of simultaneously recorded receptor units. Numbering of the odour stimuli same as in Fig. 5. Level of spontaneous neural activity preceding stimulus delivery is indicated under each set of spectra.

different beetle, are compared. The sensilla concerned were located on opposite sides of the annular groove of the terminal segment of the antenna. Although the general level of excitability appears considerably higher for the first than for the second sensillum, the response profiles of the paired units nonetheless remain qualitatively similar. In both cases one unit of each pair conformed to type A of the classification shown in Fig. 5 whereas the other corresponded to type C. A most significant feature, however, is the fact that between the two sensilla more or less similar ratios are observed for the "L" and "s" receptor responses.

It seems reasonable to conclude that a single sensillum can be innervated by receptor cells belonging to different types. Several combinations seem possible. Thus, the simultaneous neural activity was recorded from two cells which according to their reaction pattern, were identified as a type D unit showing a high sensitivity to *cis*-3-hexen-1-ol, and a type E unit with a specific sensitivity to methylsalicylate. In fact the latter receptor was inhibited by stimuli other than this aromatic compound. This specific pair of receptors was investigated for its responses to natural plant odours. To this end, equal pieces of fresh leaves of seven different plant species were crushed onto the test filter paper strip placed inside the odour cartridge of the stimulus delivery system, and the responses of the receptors to the emitted odour recorded immediately afterwards. The plant species tested included Solanaceae as well as non-Solanaceae (Fig. 8). The results show that each leaf odour evokes a certain ratio of activity between the two types of olfactory receptor units. The response ratios elicited by the three *Solanum* species were distinctly different from the proportions found for the other plants. Interestingly, only the odour emanating from crushed tomato leaves elicited a high neural activity in the type E unit. Since this type was specifically responsive to methylsalicylate it seems possible that the receptor had reacted to a significant above-threshold occurrence of this or some closely related chemical in the leaf odour of tomato plants (cf. van Straten, 1977). Thus, even with the combination of only two receptor types, a certain level of discriminatory capacity would be achieved. This pair of units would, for example, be able to discriminate the odour of crushed leaves of tomato from that of other Solanaceae, or, distinguish between the odour of Solanaceae and that of certain non-Solanaceae.

DISCUSSION

Plant odours encountered by insects in nature provide a complex of olfactory information, the perception of which involves an array of peripheral factors. These have been summarized by Dethier (1977) as: the number and kinds of specifically different receptors, band width of each receptor, character of each tuning curve, absolute threshold and rate of adaptation, and synergism and inhibition. Because of possible interactions between stimuli at the peripheral level the analysis of chemosensory input becomes even more complicated. Any analysis using pure compounds therefore only provides a simplified picture of the actual situation. Dethier (1977) rightly stated that such an approach "may provide an exaggerated picture in the sense that they overemphasize the rôle of labeled lines in (gustatory)

perception or a diminished one in the sense that by implication they oversimplify complexities of peripheral and central integration". However, because of the extreme complexities involved in the perception of natural odours or tastes, it may be profitable first to analyze the perceptual capacity of the sensory system using the combination of single receptors and pure compounds. Once the basic properties have been established, one may then endeavour to modulate the picture with increasingly complex stimulus conditions. Such an approach to the analysis of chemosensory coding seems particularly suitable for insects, where it may often be possible to relate responses from single receptor cells to quantifiable, graded behaviour output, as has been demonstrated, for example, for the gustatory perception in caterpillars (Ma, 1972).

The present work concerns such a pilot study of the perceptual coding of plant odours at the primary receptor level in the Colorado beetle. Peripheral effects of lateral interactions and mixed stimuli have not yet been examined, so that the picture presented obviously must be a simplified one. The antennal olfactory receptor system offers the considerable advantage of a prior selection of relevant chemical stimuli on the basis of electroantennogram responses. In the Colorado

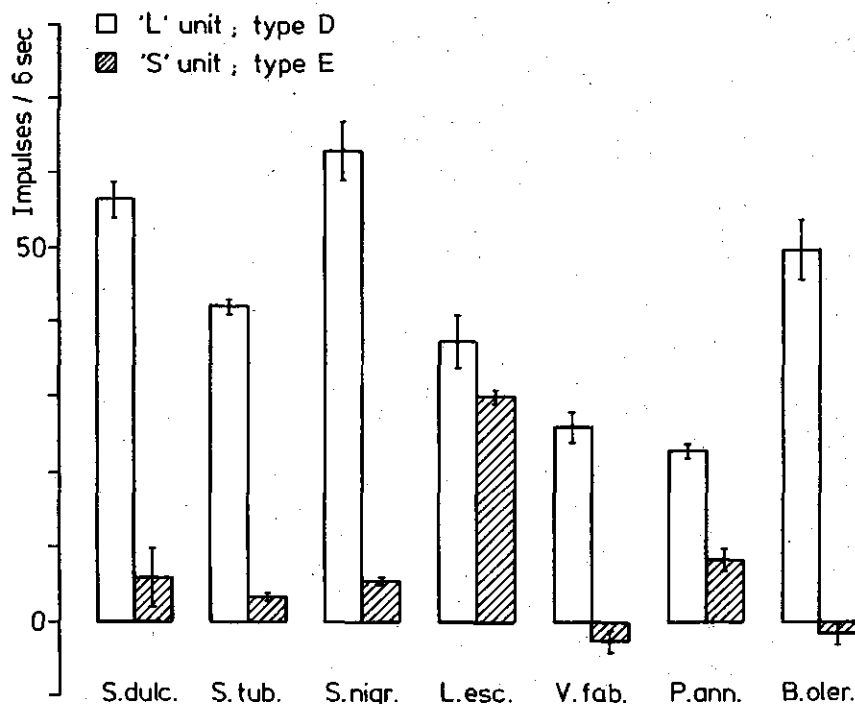


Fig. 8. Response magnitudes of two simultaneously recorded receptor units when stimulated with odour of following plant spp: *Solanum dulcamara* (S. dulc.); *S. tuberosum* (S. tub.); *S. nigrum* (S. nigr.); *Lycopersicon esculentum* (L. esc.); *Vicia faba* (V. fab.); *Poa annua* (P. ann.); and *Brassica oleracea* (B. oler.). Spontaneous discharge level 1-3 imp./s in "L" unit and 5-9 imp./s in "s" unit. Mean values from two trials per plant species.

beetle such studies so far indicate that the antennal receptor system has a strong filtering effect, with effective plant volatiles being largely restricted to "green leaf" volatiles (Visser, 1979). The results of the present study show that the Colorado beetle has a well-developed sensory capacity of quality discrimination of odour complexes that include "green leaf" volatiles, and possibly other chemicals as well.

Olfactory receptors responding to "green leaf" volatiles have also been described in *Locusta antennae* (Boeckh, 1967; 1974), and their properties worked out in great detail by Kafka (1970). Large differences have been noted between the individual receptor cells in *Locusta* with respect to the rank order of effective compounds, to the general sensitivity, and to the shape and the general steepness of the stimulus-response curves (Kafka, 1970, quoted in Kaissling, 1971). It would be interesting to investigate whether these differences can be clarified by the existence of "green odour" receptor types, with differential sensitivity spectra, similar to those which have been demonstrated in the present study for the Colorado beetle.

The range of chemical volatiles studied does not necessarily include all active constituents of the total complex of plant volatiles which may be perceived by the Colorado beetle. In fact, many recorded receptor neurones showed no significant change of resting activity to any of the odour stimuli applied. The question whether these receptors are specifically tuned to as yet unidentified components of the host plant odour has to await detailed chemical analysis of the odour composition. Insofar as the presently proposed system is concerned, it remains possible that with an extended sample additional receptor types may be found. However, the results of the rank correlation study (Fig. 6) suggest that a representative section of the total receptor population has been covered. The rank correlation is of special importance with regard to weakly EAG-active odour stimuli. Specific olfactory units that are relatively scarcely represented in the total receptor population obviously have a smaller chance of being selected in a limited random sample. Thus, the type E receptors constitute only 8% of the sample studied compared with 32% of type A (Fig. 5). Therefore, with regard to the total information coding, variations in the quantitative representation of the various receptor types have to be taken into account as well.

A basic and concrete example for understanding the mechanism of odour quality coding is given by the results shown in Figs. 7 and 8. These results, if extrapolated to the numerous olfactory receptor cells in the antenna, illustrate how each plant odour evokes a definite response pattern across the various receptor types, some of which are excited, others remaining indifferent, and still others inhibited. Thus, only a definite response patterning may convey the message for attractiveness of a plant odour. This idea is supported by behaviour observations which show that even a slight increase of the concentration of one "green leaf" volatile can reduce the attractiveness of the host plant odour for the Colorado beetle (Visser & Avé, 1978).

Odours from some non-host plants may evoke a similar response patterning to those emanating from host plants. Since the "green leaf" volatiles are common in nature the chance that the odour composition of a non-host plant resembles the

attractive odour of the host plant cannot be regarded as negligible. Moreover, comparable response patterns in one and the same set of receptors may be produced by different stimuli at different intensities. Therefore, it is not surprising that, apart from the solanaceous plants, some non-solanaceous species are also able to attract the Colorado beetle, as has been shown in wind-tunnel tests (de Wilde *et al.*, 1969; de Wilde, 1976; Visser & Nielsen, 1977). Thus, the idea of the total pattern or "Gestalt", as the significant parameter, as expounded by Dethier (1974) and reviewed by Städler (1976) for insect chemoperception, applies to olfaction in the Colorado beetle as well. It will be interesting to investigate the manner in which the primary input converges onto the higher order neurones in the deutocerebrum, as has been studied recently in other insects (*e.g.* Boeckh, 1974). This would yield information on whether the across-fibre patterning concept would actually extend to the level of the central nervous system. The results of the present work emphasize the potential of graded input patterning as a sensory basis for host odour discrimination in the Colorado beetle.

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RÉSUMÉ

ANALYSE CELLULAIRE DU CODAGE DE LA QUALITÉ DES ODEURS PAR LE SYSTÈME DE RÉ-
CEPTEURS ANTENNAIRES OLFRACTIFS CHEZ LE DORYPHORE

On a étudié la capacité de distinguer la qualité des odeurs végétales chez le doryphore. On a enregistré extracellulairement les réactions de cellules de récepteur olfactif séparées à des C-6 alcools et des aldéhydes saturés et insaturés (substances volatiles de la "feuille verte") et à d'autres composés végétaux. Les récepteurs olfactifs pouvaient réagir par inhibition et/ou par excitation; on a particulièrement observé l'inhibition aux intensités élevées de stimulus. Les variations des types de réactions ont été analysées pour un certain nombre de récepteurs, qui constituent un échantillon représentatif, sur la base de la corrélation de rang entre les réactions de pointe moyennes et les amplitudes de l'électroantennogramme pour chaque odeur-stimulus. L'application d'une analyse en grappe a montré que les récepteurs olfactifs peuvent être divisés en deux groupes principaux, un groupe qui réagit de façon différentielle au complexe des substances volatiles de la "feuille verte" et de leurs isomères et un autre groupe qui a une sensibilité sélective à l'égard du composé aromatique méthylsalicylate. Le premier groupe a été subdivisé en différents types de récepteurs avec des réactions qui se chevauchent. Il semble que les sensilles olfactives individuelles peuvent être innervées par plus d'un type fonctionnel de cellule réceptrice. Les réactions aux odeurs végétales naturelles enregistrées appuient la conclusion que la qualité d'une odeur végétale est codée par un modèle de réaction pour un nombre limité de types de récepteurs olfactifs. On discute les résultats par rapport aux concepts du codage chimiosensoriel et par rapport au comportement du doryphore.

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CHAPTER 8 SYNOPSIS

**LEAF ODOUR PERCEPTION AND OLFACTORY
ORIENTATION IN THE COLORADO BEETLE**

BY

J. H. VISSER

The insect's perception of plant odours differs essentially from their discrimination of nonvolatile plant constituents, as phytophagous insects may already perceive the odour at some distance from the plant. Hence, it can be assumed that insects make use of plant vapours by being attracted to host plant odours.

Olfactory attraction has been demonstrated in the Colorado beetle *Leptinotarsa decemlineata* Say, at an early stage (McIndoo, 1926). In a Y-tube olfactometer adult Colorado beetles preferred the branch containing potato plant odour. These findings have been confirmed by Schanz (1953), and additionally she attributed the reception of odour to the sensilla basiconica, covering the five terminal segments of the antennae. Jermy (1958) reported several contradictory results, and doubted of the significance of plant odours in host plant localization by the Colorado beetle. His interpretation virtually underlines the inadequacy of the concept of olfactory attraction, inasmuch as attraction refers solely to close-range orientation responses (see Kennedy, 1978). In the experiments of de Wilde *et al.* (1969), long-range olfactory orientation in the Colorado beetle appeared to involve the vital element of positive anemotaxis. This observation initiated the present analysis of olfactory orientation in the Colorado beetle.

Besides the behavioural responses of the Colorado beetle, both the composition of the potato leaf odour and the beetle's olfactory reception of leaf odour constituents have been studied (Visser, 1976; Visser & Nielsen, 1977; Ma & Visser, 1978; Visser & Avé, 1978; Visser, 1979; Visser *et al.*, 1979). Although the composition of the total "essence" still awaits elucidation, the main components of potato leaf essential oil were identified and turned out to be the general green leaf volatiles. The significance of the general green leaf volatiles in various leaf odours, stimulates to discussing the indispensable relation between olfactory orientation and leaf odour perception, and to deduce some functional principles from their interdependency.

Olfactory orientation

The beetle adapts its motor patterns to the stimulus situation, which results in

getting closer to the odour source. In outdoor conditions plant odours are continuously dispersed in the atmosphere, and at a long distance downwind from the source the odour concentration is extremely small. When coming into the vicinity of plants, the odour concentration increases and a steep odour gradient is present.

Newly-emerged female Colorado beetles (starved for 24 h) were subjected to these different stimulus situations. In the first type of experiments, the behavioural responses of individual beetles to a low concentration of potato plant odour were observed in a wind tunnel (see for methods: Visser & Nielsen, 1977). These orientation responses, which bear relevance to long-range olfactory orientation, are illustrated in Fig. 1. Potato plant odour elicits positive anemotactic responses in the beetles, as shown in the shift of the response categories from indifferent to moving upwind. The odour moreover increases the beetles' speed of locomotion, since they reach the upwind or downwind edge quicker than in the pure air flow (Fig. 1 lower half).

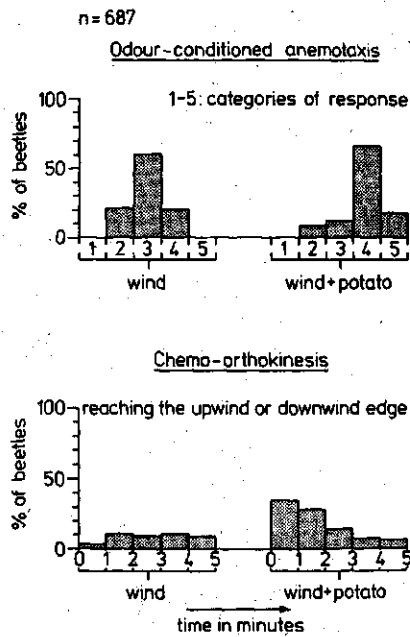


Fig. 1. Long-range orientation responses of female Colorado beetles to wind and to wind plus potato plant odour. n number of individuals tested. In upper half, tracks divided into five categories: 1 and 2 - straight and indirectly downwind; 3 - indifferent; 4 and 5 - indirectly and straight upwind. In lower half, time periods required to reach upwind or downwind edge.

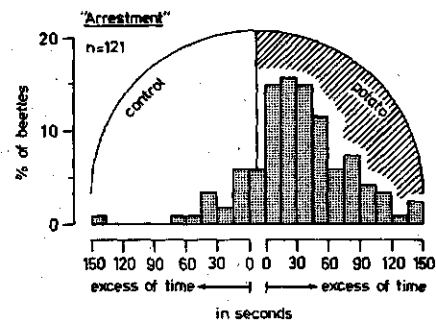


Fig. 2. Close-range "arrestment" of female Colorado beetles in screen tests. n number of individuals tested. Excess of time beetles spent on either control or potato leaf odour half in a 5 minutes' period.

When walking the beetles meet with a change of odour concentration in the second type of experiments, i.e. screen tests (methods after Bongers, 1970; with slight modifications). The bottom of a glass cylinder (11 cm in diameter) had been partitioned in two halves, one half was supplied with potato leaves and the control half with green paper. In each experiment, one beetle was placed onto a screen covering control and test side, and was allowed to walk about for 5 minutes. The position of the beetle was noted every 15 seconds, and the olfactory response was evaluated from the excess of time spent on either control or potato leaf odour half (Fig. 2). In this close-range situation the beetles stay longer on the potato half, as they are "arrested" by the odour. The orientation responses underlying "arrestment" in the Colorado beetle are obscure, and might involve the interplay of both chemokineses and -taxes.

Leaf odour components

Although chemical analysis in itself might reveal the identities of leaf volatiles, it does not discern which odour constituents are involved in olfactory orientation. For that purpose, the responses of the beetle's olfactory receptors want closer examination. The overall response of the olfactory receptors was determined by using the electroantennogram method (Visser, 1979). The olfactory receptor system of the Colorado beetle appears to be finely tuned to the unsaturated and saturated C-6 alcohols and aldehydes, like *trans*-2-hexen-1-ol, *cis*-3-hexen-1-ol, 1-hexanol and *trans*-2-hexenal. These volatiles are the main components of the potato leaf essential oil (Visser *et al.*, 1979).

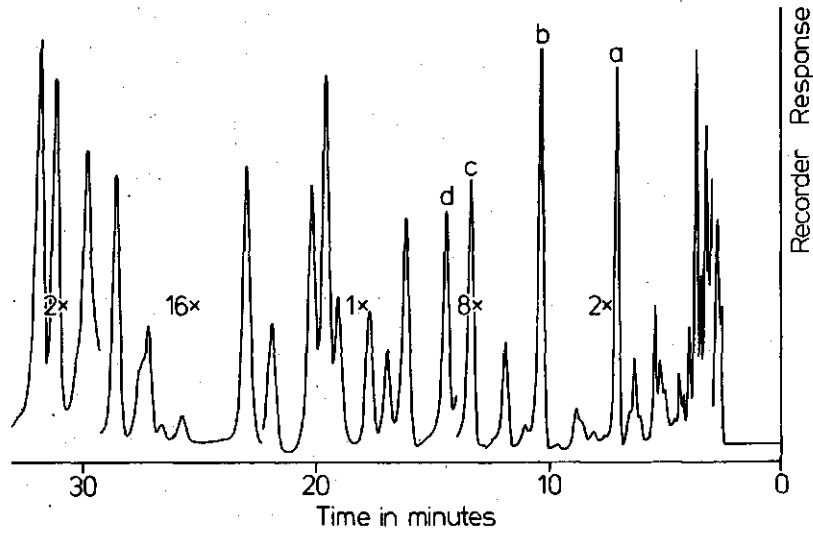
Some components present in the leaf essential oil could be irrelevant to the Colorado beetle for its orientation responses. Since the essential oil does not necessarily represent the composition of the odour emanating from the leaves, the airborne odour of cut potato leaves was analysed. In 20 minutes, 30 litres of air were sucked through a sample flask (volume: 5 litre) containing cut potato leaves, and the airborne components were trapped onto carbon (1-2 mg, see Grob & Zürcher, 1976). The carbon trap was extracted with CS₂, and the extract was subjected to gas chromatography (Fig. 3). Components were identified by using the gas chromatograph - mass spectrometer - computersystem of the Central Institute for Nutrition and Food Research TNO.

In the air space above leaves of fully grown potato plants, the following general green leaf volatiles were detected: *cis*-3-hexen-1-ol, *cis*-3-hexenylacetate, *trans*-2-hexenal and *trans*-2-hexen-1-ol. The leaf odour differs to some extent from the essential oil. Besides, sesquiterpenes are obviously present at retention times 23-29 minutes (Fig. 3 upper half). The identities of these sesquiterpenes were not worked out in detail, and their function, if any, in the olfactory orientation of the Colorado beetle is still obscure.

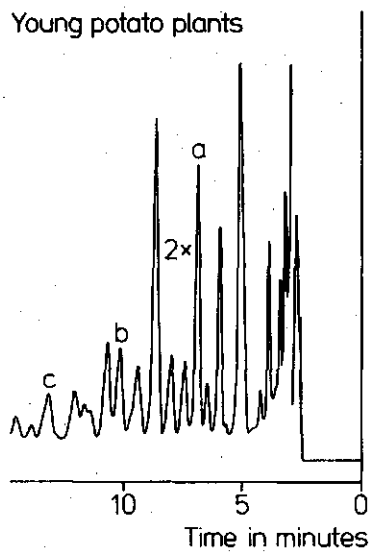
In the air surrounding leaves of young potato plants, the concentrations of the general green leaf volatiles are lower than in the leaf vapours of fully grown potato plants, and what is more, their concentration ratios differ markedly (Fig. 3

LEAF ODOUR PERCEPTION

Air over cut fully grown potato plants



Young potato plants



Fully grown potato plants

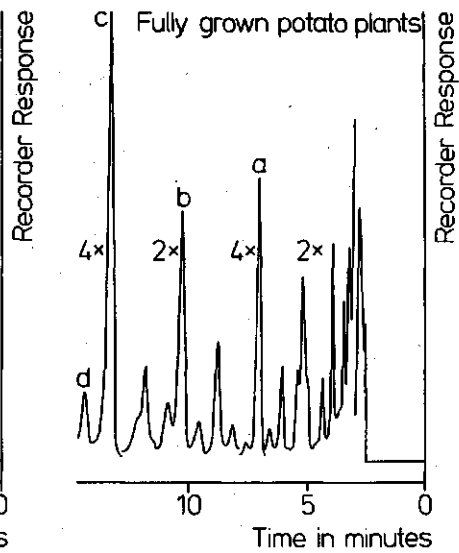


Fig. 3. Components trapped from the air over cut potato leaves. Carbon traps extracted with 60 μ l of CS_2 , 1 μ l used for GC (detector: FID). GC conditions: WCOT Carbowax 20 M column, 50 m long; temperature programmed 70-150 $^{\circ}C$. a: *trans*-2-hexenal; b: *cis*-3-hexenylacetate; c: *cis*-3-hexen-1-ol; d: *trans*-2-hexen-1-ol. In upper half, air sample from 930 g of leaves. In lower half, air sample from 280 g of leaves of either young (30 cm high) or fully grown plants (80 cm high).

lower half). The observation that young plants are less effective than fully grown plants in eliciting a positive anemotactic response in Colorado beetles (Visser, 1976), is attributed to a suboptimal composition of the former potato leaf odours.

Leaf odour perception and olfactory orientation

In long-range olfactory orientation the Colorado beetle discriminates between solanaceous and nonsolanaceous plant species. The airborne odours of solanaceous plants elicit a positive anemotactic response in Colorado beetles, whereas in general the vapours of nonsolanaceous species do not affect the beetles' motor patterns (Visser & Nielsen, 1977). When the concentration ratios of the general green leaf volatiles in the potato leaf odour are changed artificially, the release of the upwind locomotory response is prevented (Visser & Avé, 1978), which suggests that the Colorado beetle perceives the composition of the green odour complex and is able to discriminate the ratios as encountered in the potato leaf odour from the different ratios as found in the vapours of nonsolanaceous plants.

The responses of the single olfactory receptor cells to the general green leaf volatiles and their isomers have been analysed (Ma & Visser, 1978). For the present purpose, these data are reduced to the elements acting in long-range olfactory orientation of the Colorado beetle towards potato plants. The results from receptors responding with inhibition are discarded, as from their levels of spontaneous activity, the responses are suspected to represent artefacts. Moreover, the response thresholds of these receptors make their significance doubtful. The general green leaf volatiles of the potato leaf odour are considered as the adequate stimuli for a number of olfactory receptor cells in the Colorado beetle antenna. The relative intensities in the receptors responding with an increase of their neural activity to the known volatile constituents of potato leaf odour, are shown in Fig. 4.

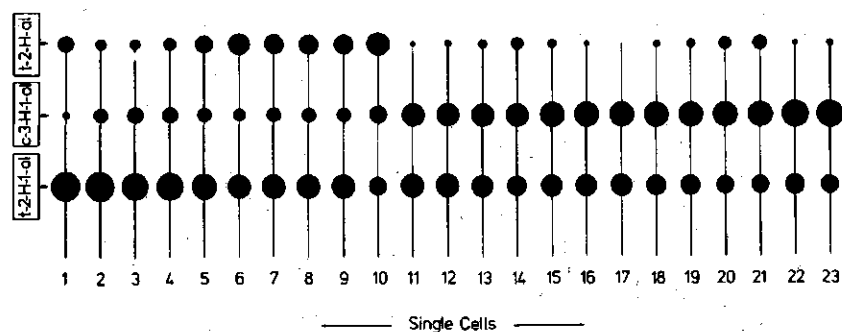


Fig. 4. Intensities in 23 olfactory receptor cells responding to trans-2-hexen-1-ol (t-2-H-ol), cis-3-hexen-1-ol (c-3-H-ol) and trans-2-hexenal (t-2-H-al), visualized in the areas of circles. Data derived from Ma & Visser (1978).

Since not all single cells were tested for their responses to cis-3-hexenylacetate, this compound is not discussed here.

The array of olfactory receptor cells reacts differentially to the component stimuli of potato leaf odour, which is manifest by the continuum of their response spectra (see Fig. 4). On increasing the proportion of trans-2-hexen-1-ol or trans-2-hexenal in the potato leaf odour, the beetle's upwind locomotory response is "turned off" (Visser & Avé, 1978). The observed interference with potato leaf odour is caused at concentrations near or even below the thresholds for an electroantennogram response. At these extremely low concentrations the level of spontaneous activity in one single olfactory receptor cell is hardly changed. Therefore, it is concluded that in long-range olfactory orientation the perception of leaf odour involves a concerted change of neural activity in numbers of olfactory receptors. An increase of the proportion of trans-2-hexen-1-ol or trans-2-hexenal, heightens the neural activity in lines 1-11 more than in lines 12-23 (see Fig. 4). This differential increment alters the contrast in the across-fibre pattern and, at the level of the central nervous system, modulates the beetle's orientation responses.

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ABSTRACT

Long-range olfactory orientation of the adult Colorado beetle was studied in a low-speed wind tunnel. The odour of fully grown potato plants elicits an upwind locomotory response in Colorado beetles (odour-conditioned positive anemotaxis), and increases the beetles' speed of locomotion (direct chemo-orthokinesis). The initial olfactory orientation of the Colorado beetle during its search for food, is mainly directed towards solanaceous plant species, thus restricting the final part of host plant selection.

Chemical analysis of potato leaf essential oil revealed the complex of general green leaf volatiles. In the potato leaf odour this complex is composed of *cis*-3-hexen-1-ol, *cis*-3-hexenylacetate, *trans*-2-hexenal and *trans*-2-hexen-1-ol. The olfactory antennal receptor system of the Colorado beetle is sensitively tuned to the reception of these volatiles, and the single olfactory receptors respond differentially to these component stimuli of potato leaf odour. When their particular ratio in the potato leaf odour has been changed, the beetles no longer react with an odour-conditioned positive anemotaxis. The complex of volatiles, rather than one single compound, is essential for long-range olfactory orientation of the Colorado beetle towards its host plant. The general green leaf volatiles probably occur as important constituents of leaf odours attractive to various phytophagous insects.

SAMENVATTING

Het onderzoek beschreven in dit proefschrift, heeft betrekking op de eerste fase in de waardplantselectie door de Coloradokever, namelijk de olfactorische oriëntatie. Tevens werden de geursamenstelling van aardappelloof en de reukzin van de Coloradokever, de elementen die ten grondslag liggen aan het oriëntatiegedrag, bestudeerd.

De gedragsexperimenten werden uitgevoerd in een windtunnel. De olfactorische oriëntatie van insecten over grotere afstand, vormt de basis van het windtunnel ontwerp. Aangezien insecten de geurbron benaderen door windopwaarts te bewegen, is waarneming van zowel de geur als de windrichting noodzakelijk (hoofdstuk 2). Naast de geur van de aardappelplant, de waardplant van de Coloradokever, zijn ook de geuren van andere Nachtschaden voor Coloradokevers attractief. De kevers gaan sneller lopen (chemo-orthokineses), en bewegen windopwaarts (geur-gekonditioneerde positieve anemotaxis.) Planten behorende tot andere families zijn over het algemeen niet in staat deze gedragsreacties in Coloradokevers op te wekken. De initiële olfactorische oriëntatie van de Coloradokever is gericht op een relevant deel van de vegetatie, waarin zich geschikte planten bevinden. Reeds op grotere afstand is de kever in staat ongeschikte planten van mogelijk geschikte planten te onderscheiden (hoofdstuk 3).

Vervolgens werd de chemische analyse van de geur van aardappelloof ondernomen (hoofdstuk 4). Daarnaast werd m.b.v. electrofysiologische methoden, het electroantennogram, vastgesteld welke vluchtige verbindingen Coloradokevers kunnen waarnemen (hoofdstuk 5). Het bleek dat de reukzin van de Coloradokever selectief functioneert, en juist gevoelig is afgestemd op de waarneming van een complex van bladalcoholen en -aldehyden, die door de chemische analyse uit aardappelloof waren geïsoleerd en geïdentificeerd. De voor Coloradokevers attractieve geur van aardappelloof is een mengsel, waarin dit complex bestaande uit cis-3-hexeen-1-ol, cis-3-hexenylacetaat, trans-2-hexenal en trans-2-hexeen-1-ol, een bijzondere positie inneemt (hoofdstuk 6). De attractieve werking van aardappelloof wordt verstoord door artificiële veranderingen in de onderlinge mengverhouding van genoemde bladcomponenten. De kevers bewegen niet meer windopwaarts, maar hun verhoogde loopsnelheid blijft gehandhaafd. Aangezien het effect van alleen deze chemo-orthokineses in het bereiken van de waardplant over grotere afstand verwaarloosbaar is, wordt geconcludeerd dat de kevers niet meer in staat zijn aardappelloof te lokaliseren, m.a.w. de attractieve geur is gemaskeerd. Deze verstoring zou tevens betekenen dat de olfactorische receptoren in staat zijn de samenstelling van dit complex van structureel gelijkende verbindingen, te onderkennen. Uit electrofysiologisch onderzoek aan de afzonderlijke olfactorische sensillen op de Coloradokever antenne, "single-unit" afleidingen, bleek dat zij inderdaad voor de kwaliteit van dit complex kunnen coderen (hoofdstuk 7).

Het beschreven complex komt in elk plantenblad voor. Het is aannemelijk dat veel fytofage insecten deze verbindingen kunnen waarnemen. Waarschijnlijk betreft het een algemeen principe in de olfactorische oriëntatie van fytofage insecten, dat

als volgt is te omschrijven (hoofdstuk 6):

- het complex bevat de "geur-informatie groen".
- de onderlinge mengverhouding van de componenten is min of meer plantensoort of -familie specifiek, zodat verschillende plantensoorten zich onderscheiden door verschillende kwaliteiten van deze "groene geur".