Ent. exp. & appl. **25** (1979) 86—97. *Ned. Entomol. Ver. Amsterdam*

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-hexenyl-acetate, 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.

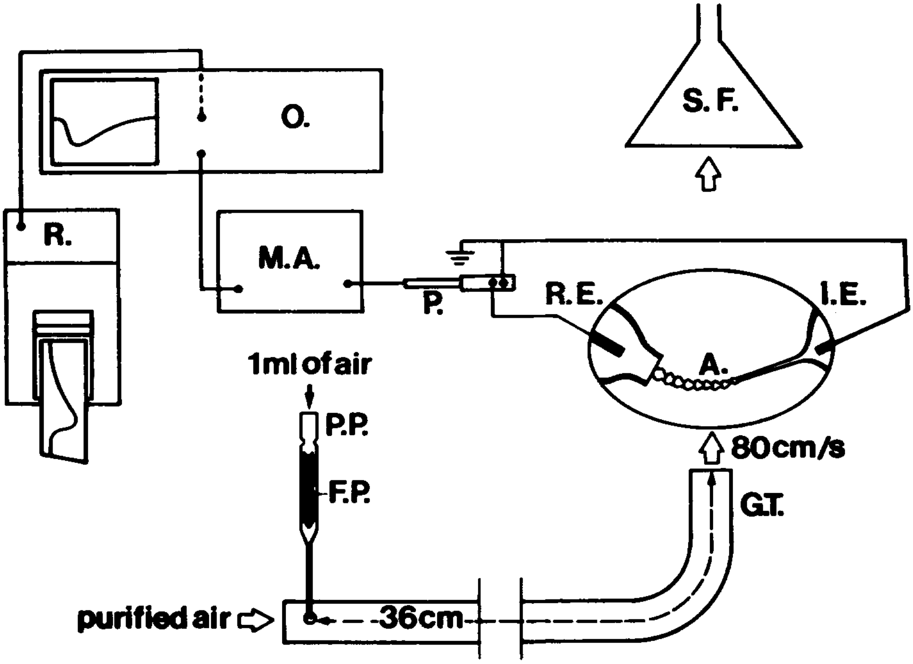

Fig. 1. EAG recording technique. A.: excised antenna; F. P.: filter paper; G. T.: glass tube; I.E.: indifferent electrode; M. A.: micro electrode amplifier; O.: oscilloscope; P.: input probe; P. P.: pasteur pipette; R.: recorder; R. E.: recording electrode; S. F.: suction-funnel. See text for further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

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 µl of each stock solution were pipetted on to a piece of filter paper (6 × 0.5 cm, S&S 589^2) 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} (1µl/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 $\geqslant$ 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

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

[1] c.i. = 95% confidence interval: standard error $\cdot t^{0.025}_{n-1}$

[2] concentration of the compound in paraffin oil $(^v/_v)$

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 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

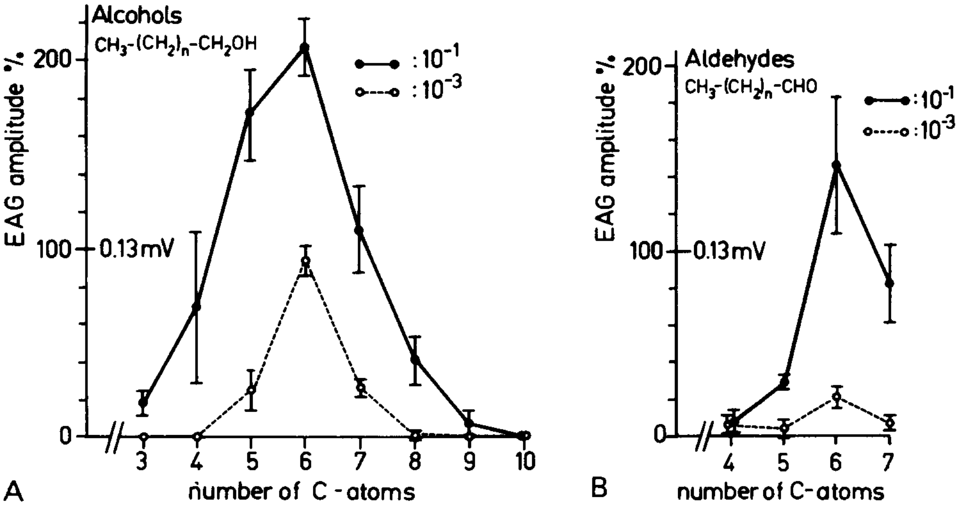

Fig. 2. Effectiveness of saturated alcohols (A) and aldehydes (B) in eliciting EAG responses of Colorado beetles, at two concentrations in paraffin oil (v/v). Vertical lines indicate 95% confidence intervals.

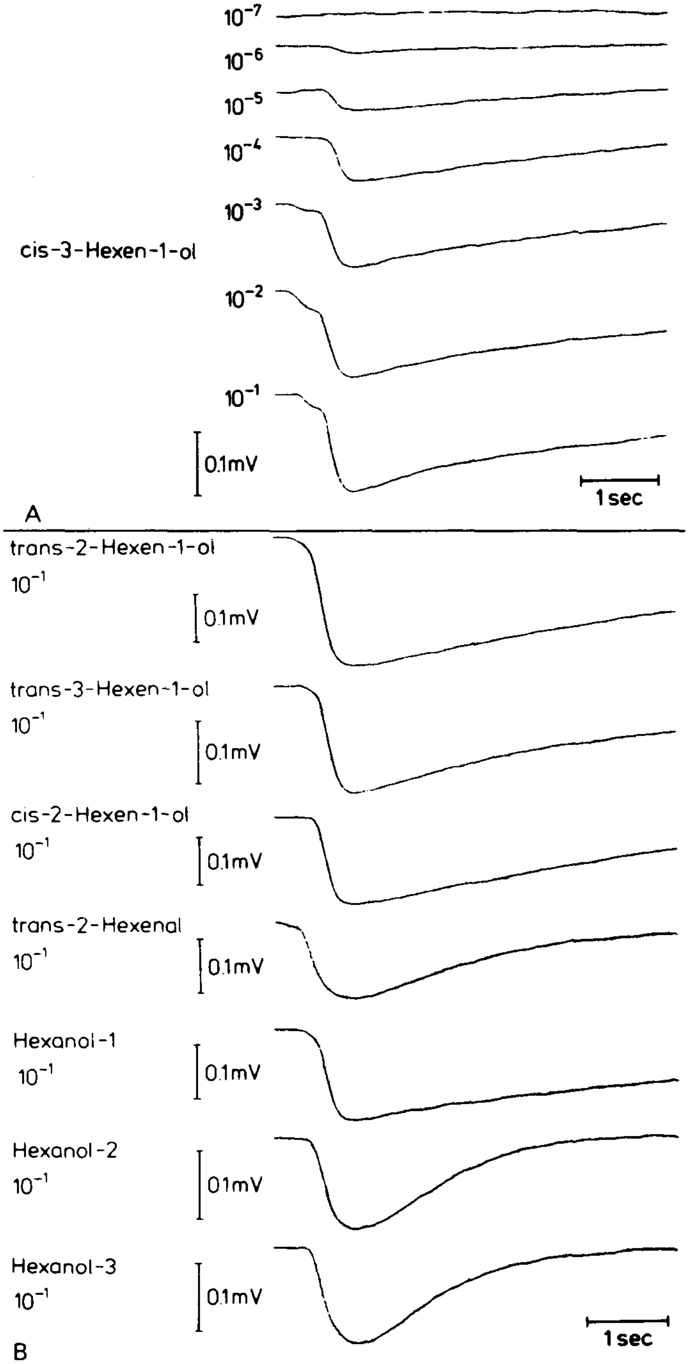

Fig. 3. EAG's of Colorado beetles responding to general green leaf volatiles and their isomers, dissolved in paraffin oil (v/v). A: responses to a range of concentrations of cis-3-hexen-1-ol; B: different shapes are observable.

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 ($^{v}/_{v}$)[3]	Number of molecules/ml of air[4]
trans-2-Hexen-1-ol	10^{-6}	$1.2 \cdot 10^{8}$
trans-3-Hexen-1-ol[5]	10^{-6}	$2.3 \cdot 10^{8}$
cis-2-Hexen-1-ol[5]	10^{-5}	$1.8 \cdot 10^{9}$
cis-3-Hexen-1-ol	10^{-6}	$1.2 \cdot 10^{11}$
Hexanol-1	10^{-5}	$2.3 \cdot 10^{11}$
trans-2-Hexenal	$3.2 \cdot 10^{-5}$	$4.3 \cdot 10^{11}$

[3] see Fig. 4; [4] stimulating the antenna; [5] geometrical isomer

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

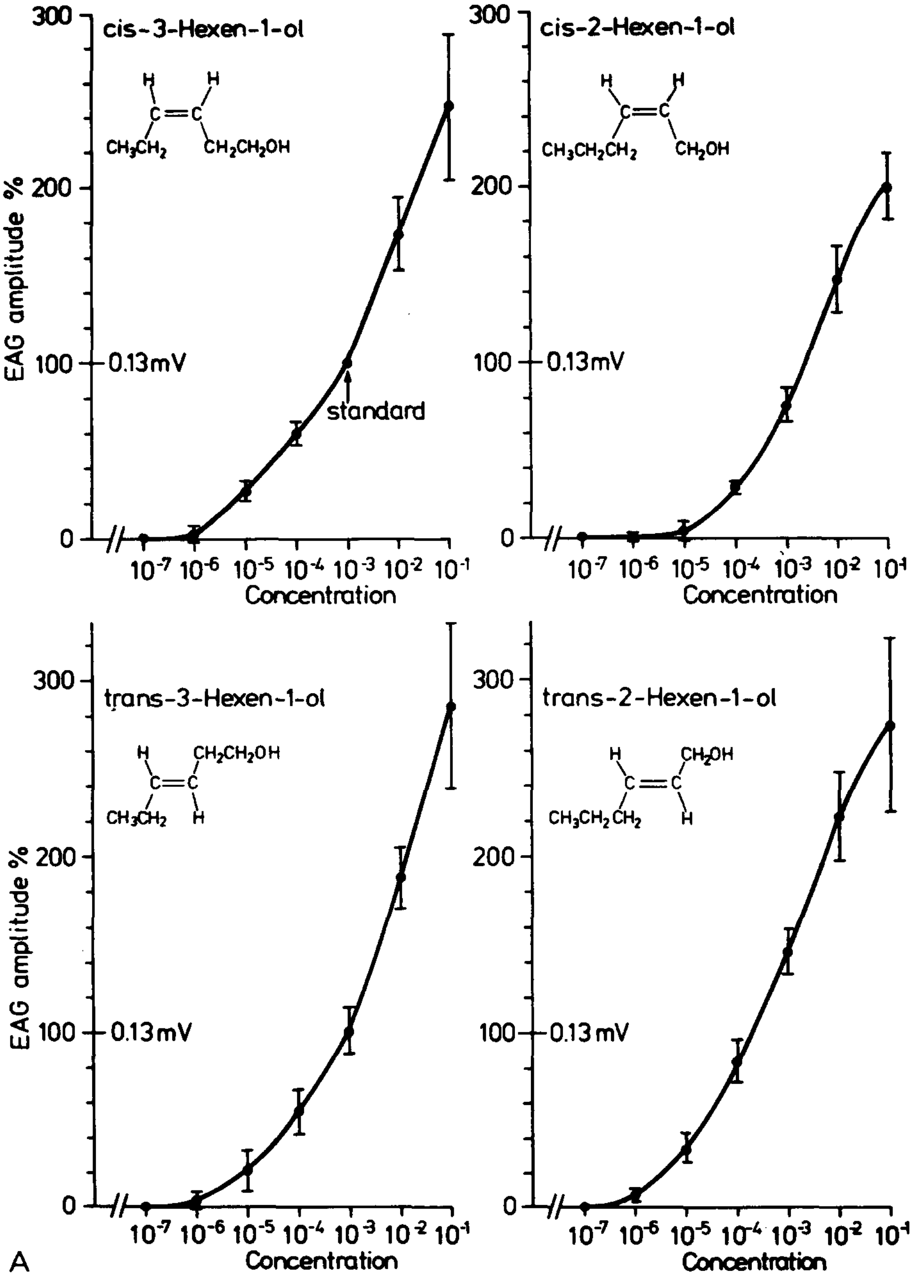

Fig. 4. Mean EAG responses of Colorado beetles to a range of concentrations of 8 test chemicals, dissolved in paraffin oil (V/v). Vertical liness indicate 95% confidence intervals.

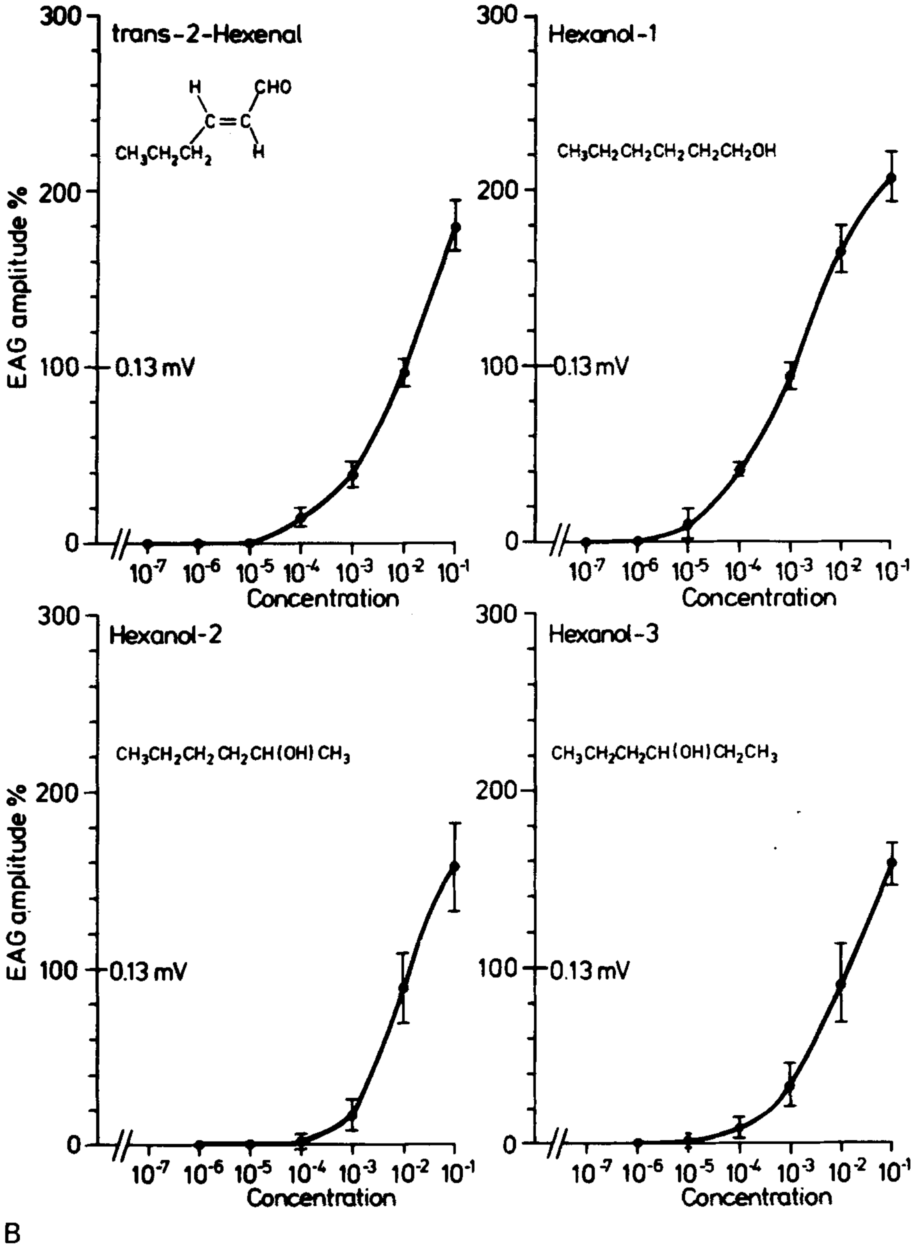

Fig. 4, continued.

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 [1]

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

[1] 0.3 g of leaf in a pasteur pipette

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, unpl.), *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).

I thank Mr. L. Koopman and Mr. G. Lubout for continuous supply of Colorado beetles; Dr. A. K. Minks for criticism and Mr. P. M. Guerin for correcting the English text.

RÉSUMÉ

ELECTROANTENNOGRAMMES DU DORYPHORE (LEPTINOTARSA DECEMLINEATA) *EN REPONSE A DES SUBSTANCES VOLATILES VEGETALES*

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-hexenyl-acetate, 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 x 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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Accepted for publication: October 12, 1978