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HOST PLANT RELATED PROPERTIES OF THE ANTENNAL OLFACTORY SYSTEM IN THE OAK FLEA WEEVIL, *RHYNCHAENUS QUERCUS*. ELECTROANTENNOGRAM STUDY

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Electroantennogram (EAG) recordings from the antennae of a highly specialized phytophagous species — the oak flea weevil, *Rhynchaenus quercus* L. — revealed two general properties of its antennal olfactory system: (1) a high and to some extent selective sensitivity to chemotaxonomically nonspecific compounds, namely the so-called "general green leaf volatiles" as well as to geraniol and linalool, (2) a lack of marked differences in the general excitation level caused by stimulation with vapours from leaves of both host plants and non-host plants. It was also observed, that the shapes of EAG's evoked by vapours from cut leaves and general green leaf volatiles differed from those elicited by corresponding concentrations of geraniol and linalool, where recovery was distinctly slower.

The results of EAG tests suggest that the general green leaf volatile complex is important in host-odour perception by the oak flea weevil. A possible function of these compounds in host-plant odour discrimination is discussed.

KEY WORDS: insect-plant relationships, host plant selection, oak flea weevil, *Rhynchaenus quercus*, olfaction, electroantennograms, plant volatiles, leaf vapours.

The oak flea weevil, *Rhynchaenus quercus*, is an example of a phytophagous insect whose behaviour is specially related to the particular features of its host plant. The larvae are leaf miners possessing morphological and behavioural adaptations for feeding on the parenchyma of oak leaves (Traghardh, 1910, Kleine, 1925). The fate of the larva seems to be completely dependent upon the ovipositing female, owing to the necessary precision of egg deposition into the main vein of an appropriate leaf. Field observations reveal that this species is an exclusive oak feeder, ovipositing only on developing leaves of various species of the genus *Quercus*. The weevils are active flyers, and specially in spring, when sexual activity and oviposition take place, they often change host trees, aggregating on the individuals of the appropriate phenological state before deposition (M. W. Kozlowski, unpubl.).

To be effective, such a mode of host plant selection may require the ability to distinguish, at a distance, certain features of host plant from non-host plants.

Preliminary behavioural tests, made by the first author, indicate that both sexes of the oak flea weevil are albe to descriminate the oak leaf smell from smell of the leaves from other plants such as beech, linden, apple-tree or larch. Therefore, it seems reasonable to examine the properties of the olfactory system in this species by making use of the electroantennogram (EAG) technique. This method, applied in the field of insect-plant relationships, may serve as a tool in the bioassay of plant-odour agents conveying relevant information to a particular insect species (Schoonhoven, 1974).

MATERIAL AND METHODS

The weevils were collected from oak trees in a wood near Warsaw late in August. After transporting to Wageningen, they were maintained in diapause (+3°). One hour before antennectomy the individuals were transferred to the experimental room, so that all experiments were performed on animals freshly recovered from diapause. Separate tests indicated that the antennae of individuals displaying reproductive activity did not show significant differences in responsiveness to the tested chemicals.

For EAG recordings the antenna was ampu-

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tated at the base of the flagellum. The recording electrode was a glass capillary (tip diam 20 μm) inserted into the club of the antenna via its middle segment. The indifferent electrode was a similar pipette slipped over the base of the antenna. Both recórding and indifferent electrodes were filled with insect saline and connected with the electronic unit as described by Visser (1979a). An experimental antenna was continuously bathed in a stream of charcoal-filtered and humidified air (80 cm/s). Test stimuli were applied via puffs of air (1ml) from a syringe connected with a pasteur pipette which contained a piece of filter paper (6 × 0.5 cm) impregnated with the test compound dissolved in paraffin oil (25 μl). The solvent itself did not elicit a measurable response. Concentrations of test compounds are referred to as dilutions volume/volume. The chemicals were ≥ 97% pure except for α-phellandrene and Δ^3-carene (95%), camphene (92%) and myrcene (90%).

As illustrated in Fig. 1, the level of absolute response declined over the experimental period. Moreover, different preparations showed different rates of abatement. Since the ratios between responses to different stimuli, applied within small intervals, remained rather constant, EAG amplitudes are expressed relative to a standard, cis-3-hexen-1-ol 10^{-2}. The standard stimuli were applied from two separate cartridges and alternated with test stimuli. The antenna was stimulated at 30 s intervals. Values of responses were calculated as the percentage of the mean of the two adjacent standard response amplitudes. It is also seen in Fig. 1, that frequent application of the standard effected a slight loss of its original concentration. To reduce that effect, the test chemicals were applied in reverse order in each repetition. A test on a single antenna started 5 min after fixing it between the electrodes and lasted a maximum of 1hr unless responses to the standard decreased below 0.1 mV.

Each concentration of test compound was examined on 6 male and 6 female antennae. As the means of the relative EAG responses did not differ significantly between sexes, the results were pooled to give a total of 12 replicates.

Apart from responses to chemicals, EAG's evoked by leaf vapours of 6 tree species belonging to different genera: *Quercus* and *Fagus* (both Fagaceae), *Malus* (Rosaceae), *Populus* (Salicaceae), *Alnus* (Betulaceae) and *Larix* (Pinaceae), were recorded.

To obtain young leaves of similar size, branches from leafless trees were cut off and put in jars with a nutrient solution in a greenhouse. Branches were kept in the jars until leaves 1—2 cm long developed. A group of such leaves from a single bud was cut together with the associated bud scales, immediately before each test. This "one bud" unit was placed in a pasteur pipette from which the vapours were applied alternatively with the standard in the same way described above. Then the leaves were cut into small pieces and tested again. The leaf vapours were tested 6 times, using 3 male and 3 female antennae.

RESULTS AND DISCUSSION

Chemicals. To examine selectivity of the oak flea weevil antennal olfactory system, a number of volatile plant constituents was tested at two concentrations, *i.e.* 10^{-3} and 10^{-1}. As shown in Table I, for most of the chemicals, the response threshold appeared to be high and the lower concentration was either quite inactive or evoked fairly weak EAG's in only some of the tested antennae. Considering the small chance of an oak flea weevil encountering high concentrations of a single volatile in its habitat,

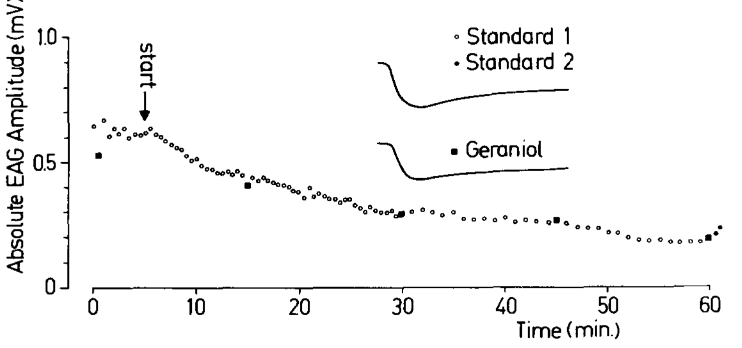

Fig. 1. Absolute EAG responsiveness throughout an experiment to the standard cis-3-hexen-1-ol 10^{-2} and a test stimulus, geraniol. Standard 2, freshly prepared. Explanation in text.

TABLE I

EAG responses of oak flee weevils to volatile plant constituents as a percentage of the response to cis-3-hexen-1-ol 10^{-2}

Compound	Mean ± c.i.[3] Concentration[4] 10^{-3}	10^{-1}	Compound	Mean ± c.i.[3] Concentration[4] 10^{-3}	10^{-1}
1-Hexanol	47± 6	146±10	d-Limonene	0	18± 9
trans-2-Hexen-1-ol	65±11	304±75	Terpinolene	0	28± 7
cis-2-Hexen-1-ol	56± 5	240±59	α-Terpinene	0	25± 9
trans-3-Hexen-1-ol	34± 5	191±44	γ-Terpinene	0	6± 3
cis-3-Hexen-1-ol	49± 6	191±50	α-Phellandrene	1±1	78±14
cis-3-Hexenyl acetate	62±11	309±61	α-Terpineol	5±7	45±11
			Carvone	1±3	54±16
			Eugenol	0	1± 2
Hexanal	45± 8	130±14	trans-Methyl-iso-eugenol	0	2± 2
trans-2-Hexenal	48±14	193±43	p-Allyanisole	0	35± 8
trans-2-Nonenal	2± 2	82±17			
trans, trans-2,4-Decadienal	0	112±28	Δ^3-Carene	0	18± 8
			α-Pinene	0	7±10
n-Tridecane	0	1± 3	β-Pinene	0	3± 5
Myrcene	0	59±19	Camphene	0	3± 6
Linalool	48± 7	109±17	Bornylacetate	0	3± 4
Geraniol	58±12	109±18			
β-Ionone	0	8±10	γ-Bisabolene	0	10± 8
			β-Caryophyllene	0	19±13
2,4-Dimethylstyrene	0	6± 6			
Biphenyl	0	6± 6	Paraffin oil	0	

[3] c.i.: 95% confidence interval; n is 12.
[4] concentration of the compound in paraffin oil ($^v/_v$).

one may assume that these particular compounds are practically imperceptible by host-searching individuals. However, some may be present in the weevils' environment, e.g., terpenes associated with pine resin vapours (Smith, 1964) wherever oaks form mixed stands together with pines.

Against a background of clear-cut insensitivity to moderate concentrations of most of the tested chemicals, distinct EAG responses were obtained from 2 groups of related components: (1) the 6-carbon aliphatic alcohols, aldehydes and an acetate ester — the so-called "general green leaf volatiles" and their isomers (Visser *et al.*, 1979), (2) the acyclic monoterpene alcohols geraniol and linalool. All of these compounds evoked diminishing EAG's correlated with lowered test concentrations, but still caused repsonses at dilutions as low as 10^{-6} (Fig. 2). Volatiles delivered at the latter dilution reached the test antennae in concentrations of about 10^8-10^{11} molecules/ml air (Visser, 1979a). The perception of such low concentrations of a volatile is probably able to influence the behaviour of an oak flea weevil at some distance from a plant.

Both groups of the effective chemicals contain compounds which are rather widely distributed among plants. Geraniol and linalool are known constituents of floral volatiles, but they have also been found in essential oils from other plants organs (Van Straten, 1977, 1979), whereas complexes of general green leaf volatiles are ubiquitous in plant green tissues, being responsible for their characteristic "green odour" (Visser *et al.*, 1979).

High sensitivity to the perception of "green odour" by oak flea weevils was also reflected in the rank order of effectiveness of saturated alcohols and aldehydes. Compounds of six carbon atoms in a chain evoked the highest EAG responses, and increasing or decreasing the number of C-atoms resulted in marked abatement of EAG amplitudes (Fig. 3). Similar bell-shaped curves of effectiveness as a function of C-atoms can be obtained from EAG responses to the same range of saturated alcohols from other insects feeding on green plant tissue such

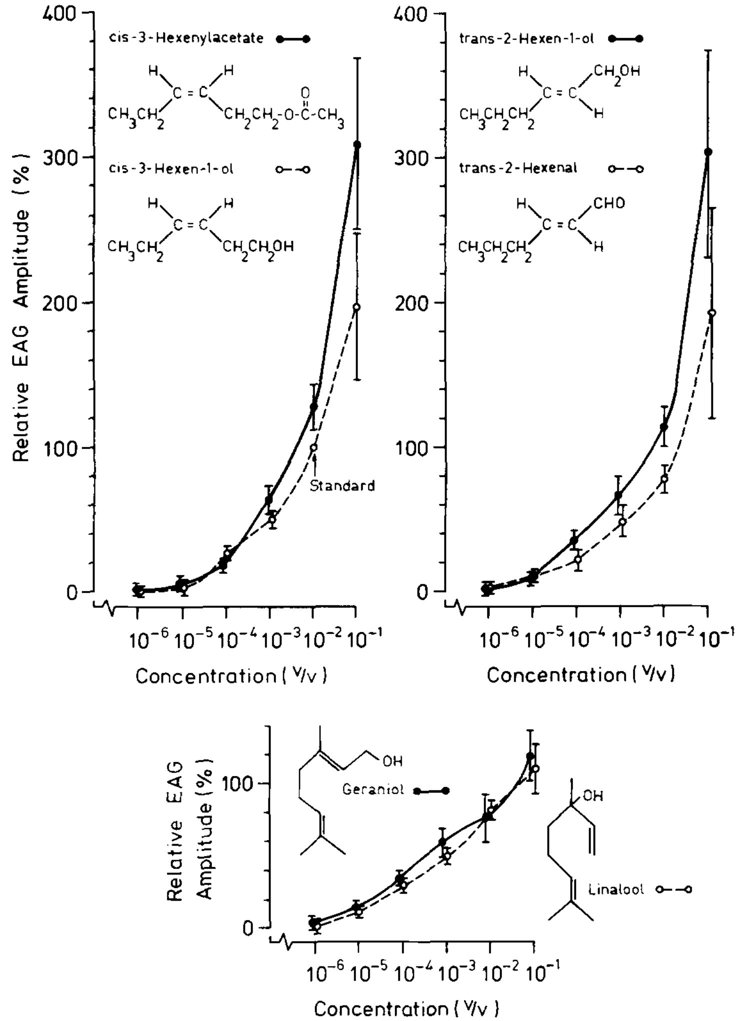

Fig. 2. Mean EAG responses of oak flea weevils to a range of concentrations of 6 plant volatiles, dissolved in paraffin oil. Vertical lines indicate 95% confidence intervals, n is 12.

as *Leptinotarsa decemlineata*, (Visser, 1979a), *Leptinotarsa haldemani*, *Piers brassicae*, *Sitobion avenae* and *Locusta migratoria*. Saturated alcohols with a chain length of six carbon atoms also appeared to be optimal in these cases (M. W. Kozlowski & J. H. Visser, unpubl.).

Plant vapours. The EAG responses to cut "one bud" leaves were generally several-fold higher than those to undamaged "one bud" leaves (Fig. 4). The mean values of EAG amplitudes elicited by vapours from cut "one bud" leaves of different plants are roughly similar to those evoked by general green leaf volatiles at dilution 10^{-2}. Also EAG responses to the undamaged leaves resembled the responses to these volatiles at dilution 10^{-4} (Figs. 2, 4). Although some differences between the mean values of the EAG responses to vapours from the leaves of each tree species can be observed, due to the relatively high variability, these differences are not significant (Fig. 4). This lack of different responsiveness to different leaf vapours indicates an equal ability of host and non-host leaf vapours to evoke general excitation of the antennal olfactory system in the oak flea weevil, and suggests the existence of a common effective factor or factors responsible for such unspecific EAG responses.

Leaf odour perception and general green leaf volatiles. As illustrated in Fig. 5, general green

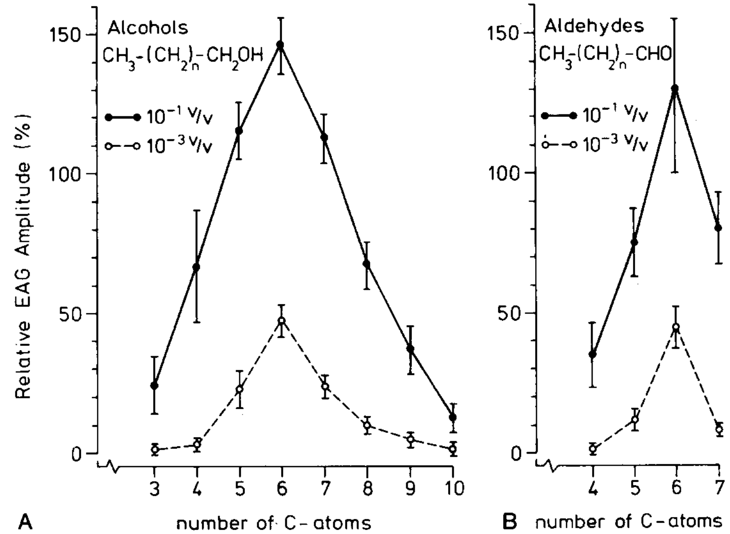

Fig. 3. Mean EAG responses of oak flea weevils to saturated alcohols (A) and aldehydes (B) at two concentrations in paraffin oil. Vertical lines indicate 95% confidence intervals, n is 12.

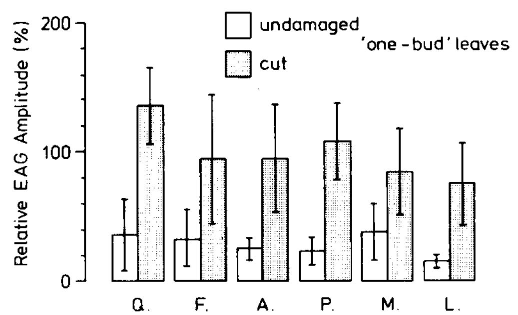

Fig. 4. Mean EAG responses of oak flea weevils to leaf vapours from different tree species. Q.: *Quercus* sp.; F.: *Fagus* sp.; A.: *Alnus* sp.; P.: *Populus* sp.; M.: *Malus* sp.; L.: *Larix* sp.. Vertical lines indicate 95% confidence intervals, n is 6.

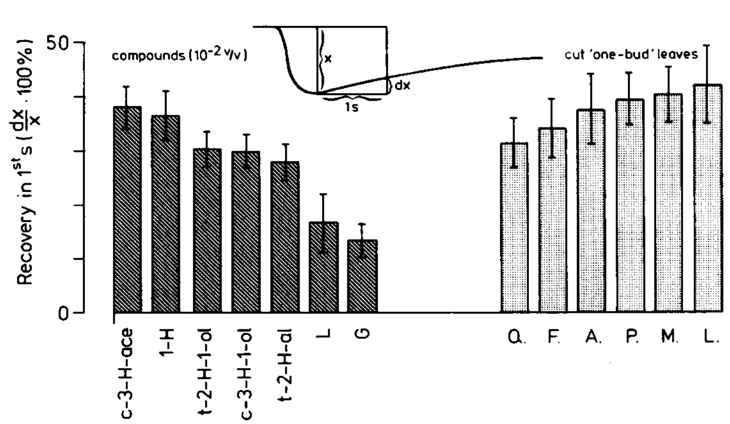

Fig. 5. Mean recoveries of EAG's in first second after maximum depolarization. Stimuli-plant volatiles, c-3-H-ace: cis-3-hexenyl acetate; 1-H: 1-hexanol; t-2-H-1-ol: trans-2-hexen-1-ol; c-3-H-1-ol: cis-3-hexen-1-ol; t-2-H-al: trans-2-hexenal; L: linalool; G: geraniol; or leaf vapours, Q: *Quercus* sp.; F.: *Fagus* sp.; A.: *Alnus* sp.; P.: *Populus* sp.; M.: *Malus* sp.; L.: *Larix* sp.. Vertical lines indicate 95% confidence intervals. Calculated from 5 recordings of similar absolute amplitudes.

leaf volatiles and leaf vapours caused EAG's with relatively fast recovery compared with 2 other effective chemicals: geraniol and linalool. The differences in the EAG shapes between these two groups of volatiles suggest that eliciting the responses to leaf vapours is largely independent of geraniol or linalool.

Based on EAG recordings alone, which correspond to the summated activity of a number of single receptors, it is impossible to conclude precisely to what extent the convergence between the responses to general green leaf volatiles and to leaf vapours can be attributed to the presence of these volatiles in leaf odour blends. The more so, as the natural vapours of plants are commonly complex mixtures of various volatiles (e.g., more than 100 volatile compounds have been isolated from buds and leaves of the cotton plant, *Gossypium* sp.; Hedin *et al.*, 1976).

In spite of these limitations, certain characteristics seem to emphasize a possible rôle of general green leaf volatiles in the host plant odour perception by the oak flea weevil:

1. The similar EAG responses to host and non-host leaf vapours indicate that host-odour differentiation is not a matter of presence or absence of a "sign stimulus" associated with the host plant vapours, but requires the olfactory system to discriminate among the qualities of plant volatile complexes. Hence, the appropriate behavioural responses to host and non-host odour must include the concerted action of various olfactory receptors (Kaissling, 1971; Visser, 1979b).

2. One of the main functional features of insect chemosensory systems is filtering capacity, which restricts the range of perceptible compounds to elements yielding relevant information for a particular insect species (Dethier, 1971). This common characteristic can be related to high and perhaps selective sensitivity to general green leaf volatiles shown by the antennal olfactory system of the oak flea weevil.

3. Although relatively little information concerning the chemistry of oak leaf volatiles is available (Karrer, 1976, reports C_2, C_4—C_6 straight-chain aldehydes and trans-2-hexenal), it seems that leaf vapours of these plants do not contain marked amounts of highly specific (in the sence of chemotaxonomy) components. Characteristically a volatile "oak factor" which evoked calling behaviour in the polyphemous moth *Antheraea polyphemus*, has been identified as trans-2-hexenal, a general green leaf volatile (Riddiford, 1969).

4. Despite the common occurrence of general green leaf volatiles, these compounds seem to play a substantial rôle in plant selection in insects which demonstrate various degrees of host-plant specialization (Visser, 1979a; Visser & Avé, 1978; M. W. Kozlowski & J. H. Visser, unpubl. EAG's).

5. Similarities in high sensitivity to general green leaf volatiles can be observed between the oak flea weevil and 2 other distinctly oligophagous insects — the Colorado beetle (Visser, 1979a) and the carrot fly, *Psila rosae* (Guerin & Visser, 1980). Each of these species showed certain traits in the character of their EAG responses to particular general green leaf volatiles. These differences can be correlated with species-specific plant odour quality coding, since the make-up of general green leaf volatiles varies, qualitatively and quantitatively, with respect to plant species and its phenological state (Visser *et al.*, 1979).

6. Such a complex alone or with other, more specific volatiles, may theoretically provide an insect with relatively rich information via differential effects on numbers of antennal receptors.

This capacity of an antennal receptor system was demonstrated in a single unit analysis of a receptor population on the antennae of Colorado beetles (Ma & Visser, 1978; Visser, 1979b). Since the antennal olfactory system of the oak flea weevil is likewise selectively tuned to reception of general green leaf volatiles, one may assume that plant-odour quality coding in this insect can be based on analogous principles.

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ZUSAMMENFASSUNG

Wirtspflanzenabhängige Eigenschaften des Geruchsystems der Fühler des Eichenblattflohs, Rhynchaenus quercus. *Elektroantennographische Studie*

Elektroantennographische Aufzeichnungen an Fühlern einer hochspezialisierten phytophagen Art, des Eichenblattflohs *Rhynchaenus quercus* L. zeigten zwei allgemeine Eigenschaften des Geruchsystems der Antennen:

Erstens ist eine hohe und teilweise selektive Empfindlichkeit auf chemotaxonomisch nicht spezifische

Verbindungen, besonders auf die "allgemeinen Blattgründämpfe" wie auch auf Geraniol und Linalool vorhanden.

Zweitens fehlen ausgeprägte Unterschiede im allgemeinen Erregungsniveau hervorgerufen durch flüchtige Stoffe von Wirtspflanzen einerseits und Nichtwirtspflanzen andererseits. Es wurde auch beobachtet, dass die Form der EAGs hervorgerufen durch zerschnittene Blätter oder „allgemeine Blattgründämpfe" verschieden war von denjenigen verursacht durch Geraniol und Linalool, wo die Erholung eindeutig langsamer war.

Die Resultate der EAG deuten darauf hin, dass der Komplex der "allgemeinen Blattgründämpfe" wichtig ist bei der Wahrnehmung der Wirtspflanzengerüche durch *Rhynchaenus quercus*. Eine mögliche Funktion dieser Verbindungen bei der Erkennung der Wirtspflanzen wird diskutiert.

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