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ELECTROANTENNOGRAM RESPONSES OF THE ARMYWORM (LEPIDOPTERA: NOCTUIDAE) AND CEREAL LEAF BEETLE (COLEOPTERA: CHRYSOMELIDAE) TO VOLATILE CHEMICALS OF SEEDLING OATS[1]

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ABSTRACT

Armyworm, *Pseudaletia unipuncta*, electroantennogram (EAG) responses to 10 volatile chemicals of seedling oats and three of injured green plants were significantly different from each other while cereal leaf beetle, *Oulema melanopus*, EAG responses were not significantly different. The EAG responses of both species did not vary significantly with respect to sex, age, or between the antennae of the same specimen. (E)-2-hexenol, a compound extracted from injured green plants, yielded the highest peak response for the armyworm while more cereal leaf beetle antennae responded to this chemical than any other chemical. Armyworm antennal life averaged 38 ± 20 min while those of the cereal leaf beetle averaged 6 ± 14 min.

All phytophagous insects must solve the problem of finding their specific hosts from among all of the other plants in the environment. Finding such a host involves a series of behavioral reactions toward plant cues that are both physical (color, shape, size, etc.) and chemical.

Although some information is available on the volatile chemicals of cereal grain (Maga 1978, Mikolajczak et al. 1983), little information was available on the volatile chemicals of cereal leaves until Buttery et al. (1982) reported on those of oats. These authors reported on the relative percentage of 26 chemicals isolated by either a Tenax trap or vacuum steam distillation. More information is available on the insect-host relationship to volatile cereal chemicals that emanate from the seeds (Nara et al. 1981, Seifelnasr et al. 1982) than the leaves.

We used the electroantennogram method (EAG) (Schneider 1957) to record the summated responses of antennal olfactory receptors to volatile chemicals. This method allows one to evaluate the tested chemicals based on the summation of receptor potentials of individual olfactory neurons in an antenna. Although a wide range of generator potentials may be recorded, it is impossible to determine an insect's behavior relative to these specific chemicals without additional bioassay tests.

Two insects that feed primarily on the leaves of gramineous plants are the cereal leaf beetle (CLB), *Oulema melanopus* (L.), and the armyworm (AW), *Pseudaletia unipuncta* (Haworth). The purpose of this study was to determine whether isolated volatile chemicals of oats and some general green plants would elicit different antennal responses (EAG) by the AW or CLB.

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MATERIALS AND METHODS

Test Insects. The CLB and AW were collected from cereals, native grasses, or corn, in southern Michigan from 1979 to 1982. The progeny of these species were reared on barley seedlings in the laboratory (26°C, LD 16:8 at 70 ± 10% RH), and the diet of late instar AW larvae was supplemented with a modified wheat germ diet (Vanderzant 1967). Adult AW were usually less than 5 days old and CLB varied from one to 330 days old when the antennal flagellum was amputated on both ends and inserted between the two glass electrodes. About five CLB and 10 AW antennae were tested for each chemical.

EAG Apparatus. The electrode tips were filled with an aqueous solution of NaCl (7.5 gm/l), $CaCl_2$ (0.21 gm/l), KCl (0.35 gm/l), and $NaHCO_3$ (0.20 gm/l). Ag-$AgCl_2$ wires in the glass electrodes were connected with the recording instruments: an input probe (Grass, H1P511), a differential amplifier (Tektronic, 5A22N), a time base amplifier (Tektronic, 5B10N), and a dual beam storage oscilliscope (Tektronic, 5103N). The air flow carrying the volatile chemicals over the amputated antennae was 1.5 m/sec for the CLB and 3.0 m/sec for the AW.

The oscilloscope trace was measured directly from the screen. The antennae, probes, and manipulator were located within a grounded Faraday cage made of ¼-in. mesh galvanized wire that reduced extraneous electromagnetic induction.

Test Chemicals. Test chemicals were obtained from reliable commercial sources (e.g., Aldrich Chemical Co.) or synthesized by established methods. Their identities were verified by spectral means (mass and infrared) and purity checked by capillary gas chromatography. Purity was better than 95% for all compounds. Those chemical compounds available (of those reported in oat leaves by Buttery et al. [1982]) were tested with the CLB and AW. One percent solutions of each chemical dissolved in hexane were placed in ampules which were then sealed. The chemical solutions were stored at −12°C until diluted to the desired dosage (100 ppm), and after dilution stored in a volumetric flask in a refrigerator (5.5°C) until used. Four volatile compounds not reported in Buttery et al. (1982) were also tested: FQ 44-118A (a mixture of oat leaf chemicals that was a vacuum steam volatile concentrate), (E)-2-hexenal, hexanal, and (E)-2-hexenol. The last three compounds are commonly found in green plant material, especially in plants that are cut, bruised, blended, chewed, or otherwise damaged (Visser et al. 1979).

Each chemical (10 μl) was pipetted onto a small filter paper disc. The disc was placed in the barrel of a 10-cm syringe and a 2-cm volume of air containing the volatile chemical was injected within 15 sec into the airstream passing over the amputated antenna. Tests were conducted every 5 min until there was no response or after 1 h of test time. The results of the traces include (a) the height of the initial response; (b) the duration of response time up to 1 h that each antenna responded (i.e., antennal life); and (c) comparisons between the antennae of each insect, of the sexes, and of varying age.

RESULTS AND DISCUSSION

There were no significant differences in EAG responses between sexes (61 pairs of AW and 43 pairs of CLB) or between antennae of the same insect (51 pairs of AW and 36 pairs of CLB) considering maximum EAG peak heights and durations of antennal life. Thus, the data for sexes are pooled. CLB antennal life averaged only 6.7 ± 14.1 min (SD, n = 103), whereas AW antennal life averaged 38.7 ± 20.1 min (SD, n = 144). EAG peak heights or duration of antennal life were unaffected by AW or CLB age.

AW antenna peak responses were significantly affected by different chemicals (ANOVA, $P < 0.05$, Table 1) but not those of the CLB (Table 2). In addition, 4% of the AW tested did not respond whereas 45% of the CLB were unresponsive.

The compound that elicited the greatest peak height for the armyworm insects was (E)-2-hexenol, a compound not reported from oats, but one that is present in many green plants when damaged. Tables 1 and 2 depict the average peak height in mV for AW and CLB, respectively, relative to total responses and positive response (this value excludes those antennae tested that did not respond).

Table 1. Armyworm EAG responses to volatile oat chemicals (100 ppm, 3.0 m/sec air speed).

Chemical	Positive response: No. of antennae	Positive response: EAG avg peak height in mV	Percent response
hexanal[a]	11	0.88 A[c]	85
(E)-2-hexenal[a]	14	1.71 AB	100
β-cyclocitral	14	1.85 AB	100
β-ionone	4	1.98 ABC	100
(Z)-3-hexenol	3	2.08 ABC	100
octanol	11	2.13 ABC	92
FQ 44-118 A[b]	12	2.57 BC	100
methyl salicylate	12	2.66 BC	100
geranylacetone	14	2.86 BC	100
nonanal	10	3.28 BC	100
1-octen-3-ol	12	3.47 C	80
(E)-β-ocimene	11	3.56 C	100
(E)-2-hexenol[a]	10	5.64 D	100

[a](E)-2-hexenal, hexanal, (E)-2-hexenol are compounds from green plants in general, and not reported in Buttery et al. (1982).
[b]FQ 44-118 A is the vacuum steam volatile concentrate mentioned on p. 791 in Buttery et al. (1982).
[c]Means in a column the same letter are not significantly different at the 0.05 level of probability by Student-Newman-Kuels test.

Table 2. Cereal leaf beetle EAG responses to volatile oat chemicals (100 ppm, 1.5 m/sec).

Chemical	Positive response: No. of antenna	Positive response: EAG avg peak height in mV	Percent response
nonanal	2	0.25	33
β-ionone	1	0.50[c]	25
FQ 44-118 A[a]	9	0.57	53
(E)-2-hexenal[b]	2	0.63	40
methyl salicylate	2	0.63	25
octanol	4	0.88	40
nonanol	8	1.14	80
hexanal[b]	6	1.31	86
(E)-2-hexenol[b]	5	1.40	100
β-cyclocitral	6	1.54	86
(Z)-3-hexenyl acetate	9	1.68	82
(E)-β-ocimene	1	4.00	20
1-octen-3-ol	1	4.00	25
geranylacetone	1	8.00	20

[a]FQ 44-118 A is the vacuum steam volatile concentrate mentioned on p. 791 in Buttery et al. (1982).
[b](E)-2-hexenal, hexanal, (E)-2-hexenol are compounds from green plants in general, and not reported in Buttery et al. (1982).
[c]No significant difference at 0.05 level.

These studies provide additional information on those volatile chemicals of seedling oats that the AW may perceive as odors. The chemicals that yielded the greatest peak heights (EAG) could be bioassayed to determine their attractiveness. Visser and Ave (1978) found that the composite volatile odor from potato plants elicited a positive anemotactic response of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, but that none of the individual volatile chemicals alone were attractive. Thus, it is very plausible that whole plant extracts or specific proportions of some of the volatiles would be much more attractive than the specific volatile chemicals tested.

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