Effect of Vapor Repellents on the Electrical Response of Insect Antenna

The problem of finding suitable chemical repellents for protection of man, animals and commodities from insect bites and damages is receiving ever-increasing attention.

Behavioral response is the only criterion currently used for evaluating the efficiency of repellents. Studies concerning the physiological effect of repellents have so far been very limited. Recently, recordings have been made of the electrical response of single olfactory sense cells in mosquitoes stimulated by repellents[1]. In the present study, changes in the electroantennogram (EAG) response to amylacetate were recorded while exposing cockroach (female *Periplaneta americana*) antenna to vapors of various chemicals, including repellents. An attempt was made to relate the electrical response of the antenna to the behavioral reaction of the insect exposed to the same chemicals.

Materials and methods. Amylacetate was used as a standard stimulus for the cockroach because of its reproducible effect on the antennal electrical response as well as on the behavioral response, which is normally manifested by an immediate rapid movement. The following chemicals were tested in the vapor phase for their effect on the EAG and on the behavioral response to amylacetate: formaldehyde, propanal, butanal, potassium cyanide, ethylacetate, DEET (N,N-diethyl-*m*-toluamide), R-11 (2, 3, 4, 5-bis (2-butylene tetrahydrofurfural)), MGK-264 (N (2-ethylhexyl) bicylo-2, 2, 1-heptene-2, 3 dicarboxanamide) and glycerol.

Schematic drawing of the stimulation and the EAG recording process are given in Figure 1. The insect was tied with masking tape and fastened to a glass plate, ventral side down, leaving one free antenna. The latter was threaded perpendicularly, up to its mid-length, through 2 small holes in a glass tube of 9 mm ⌀, which was also fastened to the glass plate. The holes around the antenna were sealed off with parafin wax of a low melting point. Stimulation was accomplished by continously blowing air through 2 glass tubes of 7 mm ⌀ at a rate of 3 l/min per tube. The air in one tube was either directed through a flask (100 ml) containing the experimental chemical, or bypassed it; the air in the second tube was either directed through a similar flask filled with amylacetate or, again, bypassed it. The chemicals tested were in the liquid form; their exposed area in the flask was 27 cm². After passing the flasks the stream of air could be interchanged with the aid of a four-way solenoid valve. One exit of the valve was connected to the tube through which the cockroach antenna was threaded; a tube from the other exit bypassed the insect and joined the first tube forming a single tube which led the air-borne chemicals out of the room.

For EAG recordings essentially the same procedure as described by SCHNEIDER[2] and by BOECKH et al.[3] was followed. The indifferent electrode was inserted into one of the basic intersegmental membranes of the flagellum. The recording electrode was slipped over the tip of the antenna after cutting the 3 or 4 terminal segments. The electrical signals of the antenna were led through a cathode follower (NF 1, Bioelectric Instruments), displayed on an oscilloscope (502A, Tektronix) and photographed. The solenoid valve, camera shutter and the oscilloscope beam were triggered and controlled by a timer. Each test started with a continous flow of pure air over the antenna, and was interrupted, to serve as control for the system, by brief air puffs lasting 2.2 sec. Normally no response, or a very slight response, to such a change was recorded (Figure 2e). The flow of air was then continued with brief stimulations of amylacetate vapors; the height of the antennogram amplitude obtained served as control to subsequent responses of the antenna. The antenna was then exposed for 45 min to vapors of one of the experimental chemicals followed by at least 45 min of exposure to fresh air. The antennal response to brief stimulations of amylacetate was recorded at various intervals during the exposure and recovery periods. The effect of each chemical was tested on 3–4 cockroaches. Usually a fresh cockroach was used for each test.

The present method of stimulation, in which odorants are applied in a closed system, differs from the commonly used stimulation procedure described by BOECKH et al.[3] where odorants were blown on the antenna from a nearby open outlet tube. Thus, any variation due to a change in position of the outlet tube (Figure 2, a–d), which is sometimes necessary during tests, was avoided. On the other hand, EAG amplitude recorded by the present method was smaller because only a part of the antenna was stimulated (Figure 2f, as compared to a, b).

The same stimulating procedure was used in observations of the motor response of the insect, but instead of stimulating the antenna, the odor-carrying air was blown over an intact cockroach kept in a glass tube of 36 mm ⌀,

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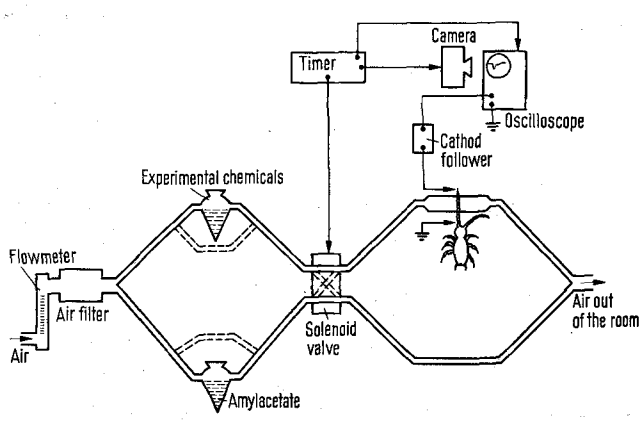

Fig. 1. Schematic drawing of stimulation and EAG recording procedure in the American cockroach.

Typical motor response[a] to amylacetate[b] of *P. americana* exposed to various chemicals

	Time from beginning of test (min)										
	Exposure period							Recovery period			
	0	1	5	10	20	30	45	50	55	65	90
Formaldehyde	++	++	+++	+++	+++	+++	+++	+++	+++	++	++
Propanal	++	0	0	0	0	0	0	0	0	0	0
Butanal[c]	++	0	0	0	0	+	+	++	++		
R-11	++	++	++	+	0	0	0	0	+	++	++
DEET	++	++	++	++	+	+	0	0	+	++	++
MGK-264	++	++	++	++	++	++	++	++	++	++	++
Glycerol	++	++	++	++	++	++	++	++	++	++	++

[a] 0 – No response; +, weak response, insect moves 1 or 2 steps; ++, normal response, incest moves up to 10 cm; +++, strong response, insect moves for a longer distance; response often continues after stimulus had stopped. [b] Stimulation with amylacetate lasted 2.2 sec. [c] Because of its toxicity, exposure to butanal lasted only 10 min. Beyond this time all values represent recovery period.

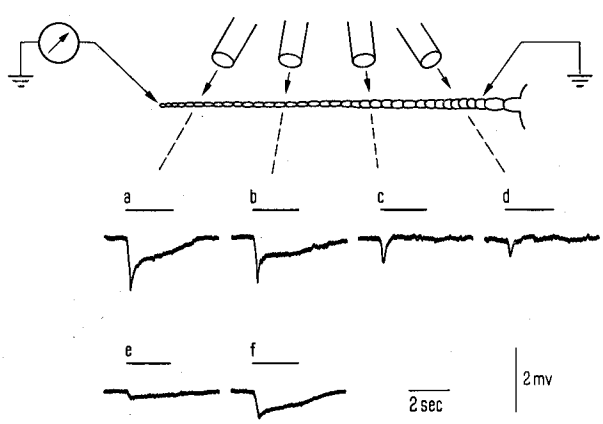

Fig. 2. EAG recordings from a single antenna of *P. americana*. a–d) outlet tube opens 10 mm in front of the antenna and puffs of amylacetate odor are directed on the recording electrode (a) and gradually (b, c) toward the ground electrode (d). e–f) closed system; stimulated portion of the antenna is confined in a tube through which a puff of clean air is given (e) followed by a puff of amylacetate odor (f).

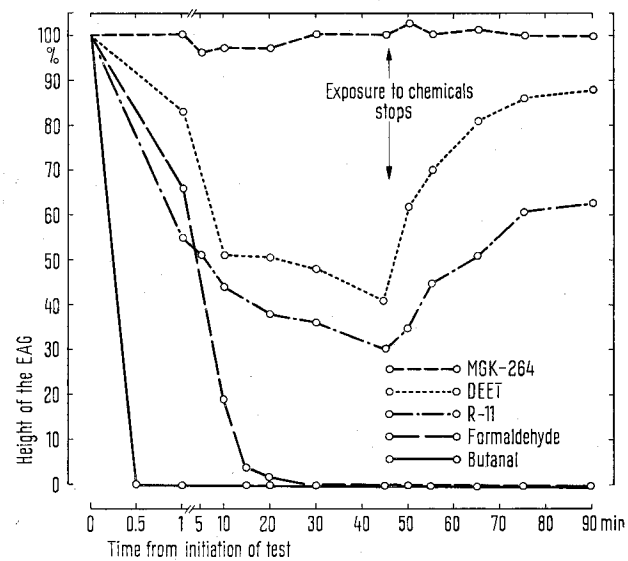

Fig. 3. Effect of continuous blowing of air-borne chemicals on the antennal response (EAG) to brief stimulation (2.2 sec) of amylacetate odor. Application of chemicals stops after 45 min.

20 cm long. A piece of paper placed mid-way along the tube furnished a suitable shaded area where the cockroach preferred to rest when unstimulated.

Results and discussion. The height of the EAG varied from one insect to the other (Figure 4, time 0), but the relative EAG response to the various chemicals was similar for all insects tested. The three aldehydes caused complete inhibition of the electrical response to amylacetate. The exposure time necessary to cause this effect was positively correlated with their molecular weights. Complete inhibition of the antennal response was reached within 30 sec, 5 min and 20 min for butanal, propanal and formaldehyde, respectively (Figures 3 and 4). Fresh air was blown for 3 h on the butanal-treated antenna and for 24 h on the propanal and formaldehyde-treated antenna, but no recovery was recorded. The effect of propanal and butanal on the behavioral response to amylacetate was similar to their electrophysiological effect; on the other hand, the behavioral effect of formaldehyde was entirely different and unexpected in its hypersensitizing influence (Table, Figures 3 and 4). One would expect formaldehyde, which easily binds to proteins[4], including apparently antennal protein[5], to be more efficient than the other aldehydes in blocking both the electrical and behavioral response.

Cyanide and ethylacetate were applied in a manner different from that described above. The cockroaches were placed in closed bottles containing cyanide and ethylacetate for 10 and 20 min, respectively; this treatment did not kill the insects. They were then mounted, exposed to pure air, and EAG response to amylacetate was checked at various time intervals during 24 h. No response was recorded during this period.

The complete and prolonged inhibitory effect of the aldehydes, cyanide and ethylacetate on the antennal response is apparently the result of toxicity to the chemoreceptive organs. Presumably, the adverse effect of these chemicals is correlated with repellency effect, which is a common feature of cockroach toxicants[6]. Yet, they are unsuitable for various reasons to be used as commercial repellents. On the other hand, DEET, a general purpose insect repellent[7] and R-11, one of the best commercial

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cockroach repellents[8], had a gradual and partial inhibitory effect on the antennal activity, with an apparent recovery after the repellent vapors were cut off; their behavioral effect was in accord with their electrophysiological effect (Figures 3 and 4, Table). MGK-264, the smell of which is stronger to the human nose than DEET and R-11, is non-repellent to cockroaches[8]. This material did not affect the EAG or the behavioral response of the insect (Figures 3 and 4, Table). Similar results were obtained with glycerol.

It is suggested that the partial blocking effect on EAG response, followed by a relatively fast recovery, is a typical feature of insect repellents. The practical implications of this suggestion require further investigations[9].

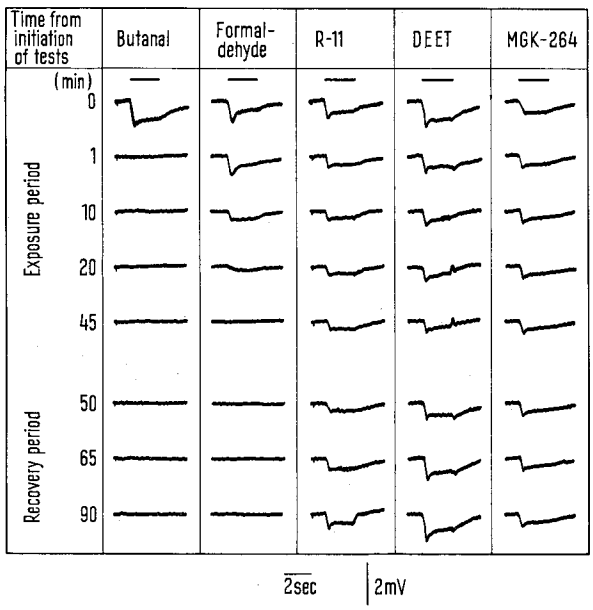

Fig. 4. EAG recordings from *P. americana* stimulated by amylacetate before (time 0), during (1–45 min) and after (50–90 min) exposure to various odorants. For each odor a different cockroach was used.

Zusammenfassung. Die elektrische Reaktion der Antennenrezeptoren von *Periplaneta americana* auf Reizung mit Amylacetat wird durch Formaldehyd, Propanal, Butanal, Cyanid und Aethylacetat vollständig gehemmt. Die Repellentien DEET und R-11 ergeben teilweise, Glyzerin und MGK-264 keine Hemmung. Antennogrammhemmung und Verhaltensreaktion ganzer Tiere sind korreliert, ausgenommen bei Formaldehyd, das sich im Verhaltenstest als hypersensibilisierend erweist.

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[9] This research has been financed in part by a grant made by the United States Department of Agriculture, Agriculture Research Service under P.L. 480.

Estrogens in 2-Chloroethylphosphonic Acid Induced Femaleness of *Cucurbita pepo* L.

Flower sex expression is subjected to genetic, environmental and chemical controls and may be regulated by endogenous levels of growth substances. Treatment with gibberellins usually increases the male tendency of cucumber and other plants[1,2] while treatment with auxins[3], growth retardants[4] or 2-chloroethylphosphonic acid (CEPHA)[5] induces femaleness. The influence of steroidal hormones on flower sex expression in plants was also stated. Löve and Löve[6] have found that these substances could produce male or female flower on *Melandrium dioecum*, if either androgens or estrogens are applied to the stems before flowering. Estrogens also increased the number of female flowers in *Ecballium elaterium* (L.) A. Rich[7].

The present investigation was undertaken to test whether the induction of femaleness by CEPHA, a substitute for ethylene, is connected with simultaneously occurring effect of this compound on endogenous estrogens content in pumpkin plants.

Material and methods. Monoecious plants of *Cucurbita pepo* L., cv. Weiser Bush were grown in clay pots during spring and summer (March 10 till July 13, 1971) in the greenhouse at maximum and minimum temperatures of 26 and 17 °C, respectively. CEPHA (AmChem formulation 68–250) was applied as aqueous foliar spray in 2 treatments, each at concentration of 200 ppm. Sprays were carried out to run-off at both 4th and 7th leaf stages when the plants were 42 and 49 days old, respectively, and the critical true leaves were 1 cm in diameter. Control plants were sprayed with distilled water. Number of male and female flowers produced by each plant was recorded, the observations being made every second day. First opened male flower appeared on May 14 and female one on May 26 on control plants, while on the CEPHAL-treated plants the first opened female flowers – on May 22; no male flowers appeared on treated plants until June 18.

Material for estrogens determination was taken at time indicated in the Table. Whole plants deprived of the roots, flowers and infructescences were used. Frozen material was homogenized with hot methanol and the homogenate was filtered. The filter cake was being extracted in a Soxhlet apparatus with benzene-methanol mixture (3:1) for 6 h. The methods of extraction, fractionation and rechromatography procedure were the same as described previously[8]. For the quantitative determination of the

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