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ELECTROANTENNOGRAM RESPONSE OF *PLODIA INTERPUNCTELLA* TO ITS SEX PHEROMONE AND WING GLAND EXTRACTS[1]

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Y. S. Chow, M. S. Mayer and J. H. Tumlinson (1980) Electroantennogram Response of *Plodia interpunctella* to Its Sex Pheromone and Wing Gland Extracts. *Bull. Inst. Zool., Academia Sinica* **19**(1): 27-31. Electroantennograms (EAGs), an olfactory potentials to sex pheromone were recorded from the antennae of the male and the female Indian meal moth *Plodia interpunctella* (Hübner). Antennae of the male moth gave the strongest electroantennogram activities to 2 components of its sex pheromone, (*Z*, *E*)-9, 12-tetradecadienol acetate and (*Z*, *E*)-9, 12-tetradecadienol, and relatively strong to the related structure (*Z*)-9-tetradecenol acetate. Electroantennogram study obtained from the antennae of female moths indicated that the female also were sensitive to, but did not give a dosage-dependent response to these compounds. Extracts of wing glands from males elicited an EAG from female antennae above that of the control, but the EAG amplitude did not change with changes in concentration over a range of 5-150 male equivalents. The reason for this poor result to the linear response and concentration relationship is possible due to a low concentration of the constituent extracted from wing gland.

The sex pheromone of the female Indian meal moth, *Plodia interpunctella* (Hübner), was first identified as (*Z*, *E*)-9, 12-tetradecadienol acetate (ZETA) (Brady *et al.*, 1971, Kuwahara *et al.*, 1971) and later a secondary component, (*Z*, *E*)-9, 12-tetradecadienol (ZETOH) was also identified (Coffelt *et al.*, 1978). We wanted to compared in an EAG assay these 2 identified compounds with extracts of native glandular material. Also, because the EAG technique is commonly used as an aid in identifying components of the female glands (Roelofs 1976) we wanted to know if the female antenna would be useful in an assay for male produced pheromone. For this purpose we used native extracts of the pheromone produced by glands on the forewing of male Indian meal moths (Grant 1974). The bioassay for this pheromone is rather tedious, and we wished to ascertain if the EAG obtained from female antennae could be used to advantage in identifying the wing gland pheromone of males.

MATERIALS AND METODS

Insects were obtained from a laboratory colony reared on a mixed medium according to Silhacek and Miller (1972). Pre-sexed insects were kept in plastic boxes in different incubators

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until emergence. Forewings of male moths were clipped under a dissecting microscope into ether pentane (50:50 v/v). This solution was reduced to a known concentration under dry nitrogen. The method for extracting the female natural sex pheromone was modified only slightly from Sower and Coffelt (Sower *et al.*, 1973, Coffelt *et al.*, 1978). Synthetic pheromones were 99+% purity.

For electrophysiological tests the heads of males or females were excised from the throax and mounted in saline similar to the method devised by Roelofs (1976). Non-polarizing glass capillary Ag-AgCl electrodes filled with 3 M KCl solution were connected with the tip of an antenna from which the 3 distal flagellar segments had been removed. The indifferent electrode was inserted within the head near the base of the antenna.

The EAGs were obtained routinely and recorded permanently on an X-Y recorder. Cartridges were prepared on the day of use by pipetting a know amount of odorant in mineral oil onto a folded piece of 0.5 × 2.0 cm Whatman no. 1 filter paper. The paper was inserted into a Baketel adaptor (B-D, Co., Waltham, MA) and positioned 2-4 mm from the antenna. The stimulus was delivered for 1 sec in 1 ml of air.

RESULTS AND DISCUSSION

The antenna of both females and males responded with negative-going potential to all 3 materials tested, which is commonly interpreted to mean that primary receptor cells were stimulated by the chemicals tested. The absolute magnitude of the antennal responses of both sexes to ZETA, ZETOH, and the wing gland extracts declined, and responsiveness to 1 μg of pentyl acetate declined by about 1/2 within 14 min following surgery. The response of the female EAG was relatively insensitive to different concentrations of the synthetic chemicals tested, while the male antenna responded to various concentration of ZETA, ZETOH and (*Z*)-9-tetradecen-1-ol acetate. The true electroantennogram traces of female and male insects responded to different concentration of pheromones were shown in Fig. 1, and the replicate results were averaged and presented in Fig. 2. From these results, we knew that the female antenna could perceive its own sex pheromone, pheromone analogus and its male wing gland pheromone. Among the synthetic chemicals tested, female could detect Z-9, E-12-tetradecadienol more easily than the other two. Although most EAG traces of the sex pheromone response were higher than that of control, the dosage-response relationship was not regular. This finding was not consistent with that of *T. ni.* Light and Birch (1979) reported female antennae of *T. ni* elicited EAG similar to males but only 25% in amplitude. On the other hand, when male antenna was tested, a dosage-response curve was obtained (Fig. 2). Because of its best responsiveness, Z-9, E-12-tetradecadienyl acetate was confirmed to be the most important component in the sex pheromone of Indian meal moth. (Brady, *et al.*, 1971; Kuwahara *et al.*, 1971; and Coffelt, *et al.*, 1979). When using the *t*-test, we knew that the response curve of this component was significantly different from the control curve at 5% level. The standard error of the Z-9, E-12, tetradecadienyl acetate at concentration 10, 1, 0.1 μgs were 0.65, 0.89, and 0.99 mvs respectively. These data agree with what have been reported that higher concentration tested, the higher standard error shown in cabbage looper. (Light and Birch 1979). We did not test the various mixture of the 2 synthetic chemicals Z-9, E-12-tetradecadienyl acetate and their alcohol, but even in purified form, both chemicals elicited better response than that of the pheromone analogus Z-9, tetradecenyl acetate itself (Fig. 2). Z-9-tetradecenyl acetate also gave good concentration-dependent response to the male antenna. Even in the rice-storage house, it also presented good attraction to the male moth (Chow *et al.*, 1975).

Extracts from the female sex pheromone gland were compared directly with pure ZETA. These glands contain a mixture of the various compounds previously tested separately and each

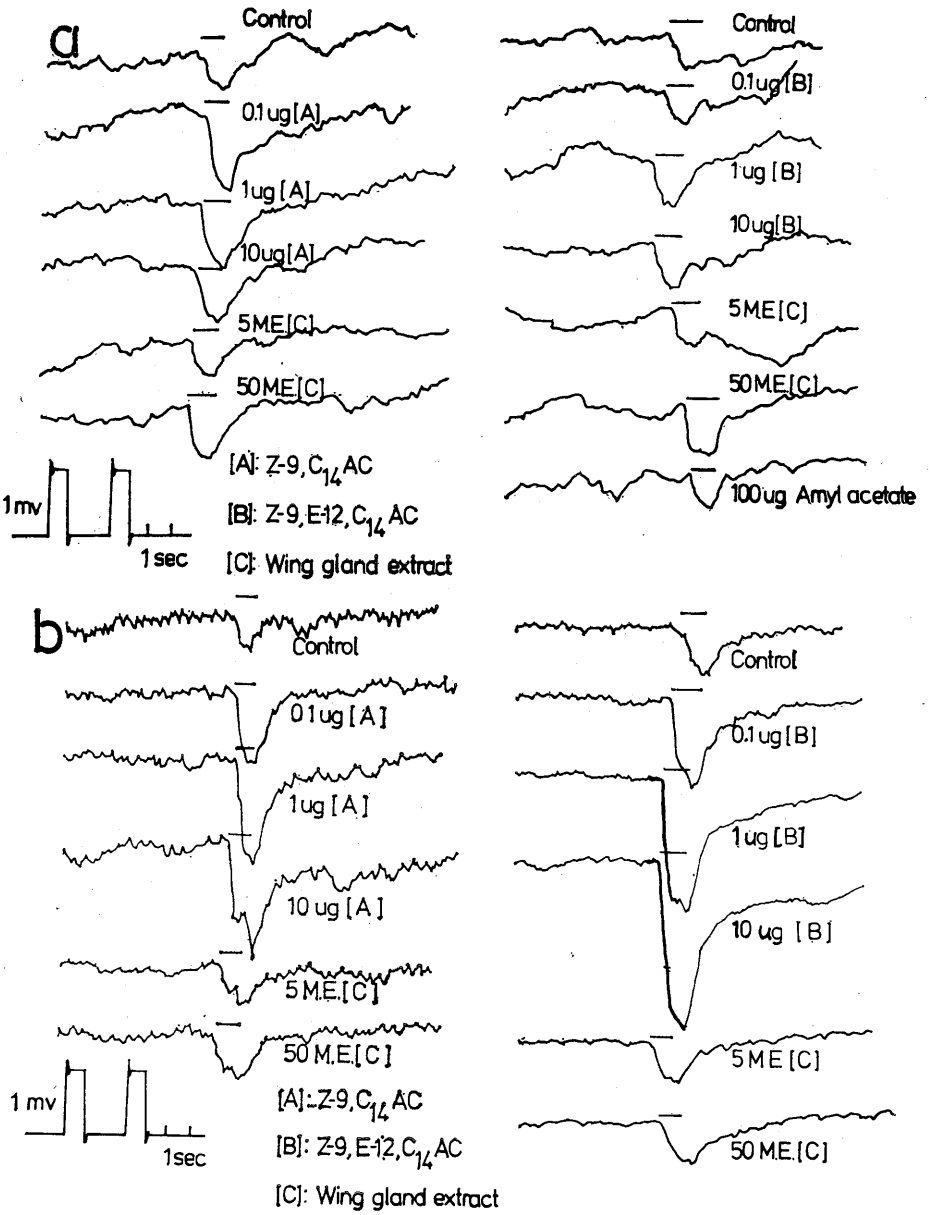

Fig. 1. Magnitude of EAG of Indian meal moth obtained by stimulation of its female sex pheromones, pheromone analogues and male wing gland extracts: (a). Female antenna (b) Male antenna. Black bars above each trace indicate the duration of air curent.

gland has been found by Coffelt *et al.* (1978) to contain about 2 μg each of readily-extractable ZETA and ZETOH. At a concentration equivalent to 0.3 female gland contents, the EAG elicited by the gland extract was almost equal to the EAG elicited by 10 μg ZETA. Thus the gland extract apparently was about 4000-5000 X more effective a stimulus of the EAG than

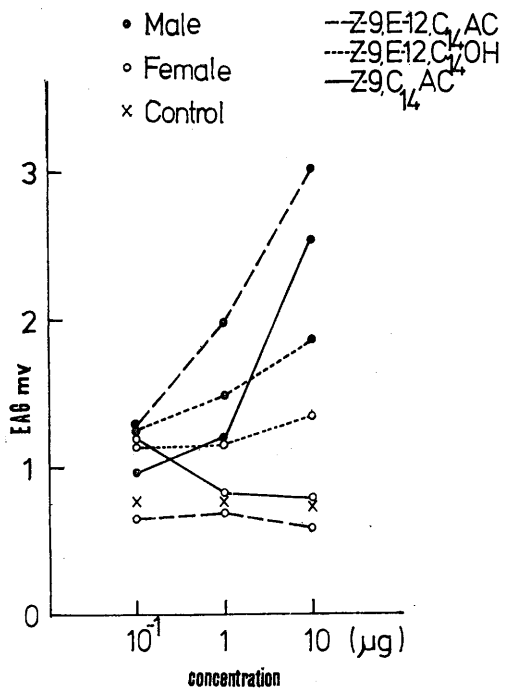

Fig. 2. Responses of Indian meal moth antennae to different concentrations of sex pheromones and other pheromone analogues (7 replicates).

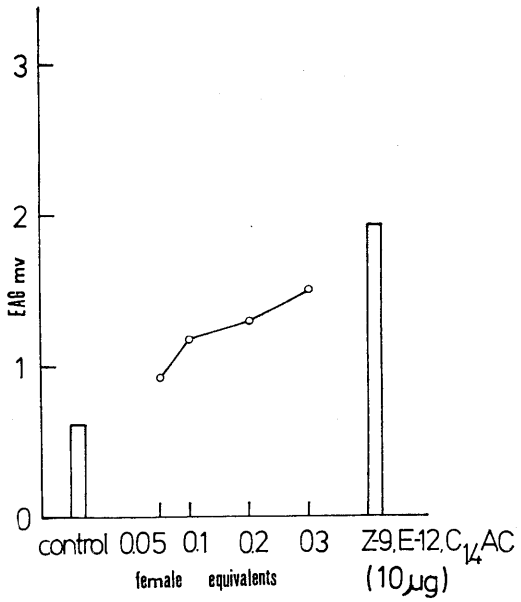

Fig. 3. Response of male antennae to pheromone gland extracts and ZETA. (3 replicates).

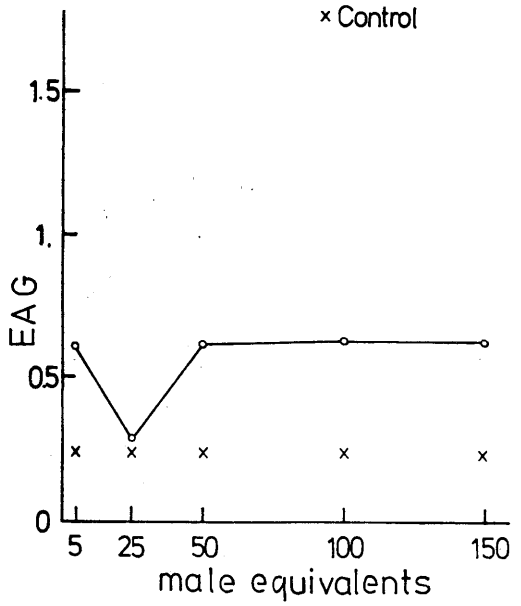

Fig. 4. EAG response of female antenna to different concentrations of male wing glands, 7 replicates.

ZETA alone (Fig. 3). This observation suggests the presence of other highly stimulatory compound(s) in the gland or other compound(s) which may synergize the EAG response.

Behavioral responses of the female have been observed to 2 male equivalents of wing gland extract (J. R. McLaughlin, personal communication). The EAG response of the female antenna was above the control level although it was insensitive to changes in concentration over a range of 5 to 150 male equivalent wing gland. Our results tested in higher concentration were presented in Fig. 4. One possible reason for this finding could be our poor evaporation procedures that some of the main components was lost during concentration process. Other possibility could be due to the smallness of the antenna and our poor recording techniques. Thus the EAG of females may be of use in studying the response of females to the male pheromone as well as an aid in its chemical identification.

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REFERENCES

1. BRADY, U. E., J. H. TUMLINSON, R. G. BROWNLEE and R. M. SILVERSTEIN, (1971) Sex stimulant and attractant in the Indian-meal moth and in the almond moth. *Science* **171**: 802-4.
2. CHOW, Y. S., L. C. YANG and M. Y. LEE (1975) Field evaluation of the synthetic sex pheromones of the almond moth and the Indian meal moth. *Plant Protection Bull.* **17**: 418-423.
3. COFFELT, J. A., L. L. SOWER and K. W. VICK (1978) Quantitative analysis of identified compounds in pheromone gland rinses of *Plodia interpunctella* and *Ephestia cautella* at different times of day. *Environ. Entomol.* **7**: 502-5.
4. GRANT, G. G. (1974) Male sex pheromone from the wing glands of the Indian meal moth, *Plodia interpunctella*. *Experientia* **30**: 917-8.
5. KUWAHARA, Y., C. KITAMURA, S. TAKAHANI, H. HARA, S. ISHII and H. FUMAKI (1971) Sex pheromone of the almond moth and the Indian meal moth: Cis-9, Trans-12-tetradecadienyl acetate. *Science* **171**: 801-2.
6. LIGHT, D. M. and M. C. BIRCH (1979) Electrophysiological basis for the behavioural response of male and female *Trichoplusia ni* to synthetic female pheromone. *J. Insect Physiol.*, **25**: 161-7.
7. ROELOFS, W. L. (1977) The scope and limitations of the electroantennogram technique in identifying pheromone components. *In*: Crop Protection Agents, Their Biological Evolution, N. R. McFarlane (ed.), pp. 147-165. Academic Press, New York.
8. SILHACEK, D. L. and G. L. MILLER (1972) Growth and development of the Indian meal moth, *Plodia interpunctella* (Lepidoptera: Phycitidae). *Ann. Entomol. Soc. Am.* **65**: 466-8.
9. SOWER, L. L., J. A. COFFELT and K. W. VICK (1972) Sex pheromone: A simple method of obtaining relatively pure material from females of five species of moths. *J. Econ. Entomol.* **66**: 1220-2.

印度穀蛾對性費洛蒙及其翅腺的觸角電析研究

周 延 鑫、M. S. MAYER、J. H. TUMLINSON

本文報導印度穀蛾 *Plodia interpunctella* (Hübner) 雌雄觸角對其性費洛蒙的嗅覺反應研究。其雄性觸角可以很强的感覺出牠的雌性性費洛蒙，(*Z*, *E*)-9, 12-tetradecadienol acetate 和 (*Z*, *E*)-9, 12-tetradecadienol，以及一種費洛蒙類似物質 (*Z*)-9-tetradecenol acetate。如用雌性的觸角研究時，則發現雌蛾可以認識這些費洛蒙物質，但是其觸角電壓圖的電位差和費洛蒙的濃度不成正比例增加而增加。如果用其雄性翅腺的抽出物試驗時，亦可得同樣情形。得到這種結果的原因，可能和翅腺的純物濃度太低，或觸角太小有關。