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EFFECT OF (E)-11-HEXADECEN-1-OL THE MINOR COMPONENT OF THE SEX PHEROMONE GLAND ON (E)-11-HEXADECENYL ACETATE THE SEX PHEROMONE OF THE BRINJAL PEST *LEUCINODES ORBONALIS* GUENEE IN AN ELECTROANTENNOGRAM ASSAY.

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Abstract: Before commencing field application, the attractiveness of (E)-11-hexadecenyl acetate (HDA), the female sex pheromone of the brinjal pest, *Leucinodes orbonalis* was evaluated in comparison to a series of mixtures of (E)-11-HDA containing the minor component, (E)-11-hexadecen-1-ol, found in the sex pheromone gland of the female *L. orbonalis*. The electroantennogram response of the isolated male antenna of *L. orbonalis* was recorded using five concentrations (range 0.02 μg-200 μg) of each of (E)-11-HDA and a mixture of (E)-11-HDA: (E)-11-hexadecen-1-ol, (90%: 10%, w/w). The results indicated an increased antennal response upto a concentration of 20 μg at the source and a decrease thereafter for both series. The responses obtained for the series of the mixture, however, were slightly higher (range 60.3% - 100%) at all concentrations than those of the pure (E)-11-HDA (range 50.5% - 92.8%) though the results were not significantly different statistically. The pure alcohol, (E)-11-hexadecen-1-ol, in contrast elicited poor responses at 20 μg and 200 μg with EAG values of 44.3% and 38.8% respectively which are comparable to that of the control (38.0%). The responses of the pure alcohol were significantly different ($P < 0.01$, t-test) from those of the corresponding mixtures of (E)-11 HDA: (E)-11 hexadecen-1-ol and pure (E)-11- HDA ruling out the possibility that the alcohol is itself a pheromone.

Key words: Electroantennogram assay, (E)-11-Hexadecenol, (E)-11-Hexadecenyl acetate, *L. orbonalis*, Sex pheromone.

INTRODUCTION

Leucinodes orbonalis[1] Guenee (Lepidoptera: Pyralidae: Pyraustinae) is a serious economic pest that damages the shoot and pod of the brinjal plant, *(Solanum melongena)* retarding growth and producing fruits that are unsuitable for consumption.[2] This pest occurs worldwide but serious damage to crops have been reported only in China,[3] India and Sri Lanka.[2] The only known method of control currently available involves the continuous spraying of insecticides during the growth of the fruit.[2,4] This method is effective for egg and first instar larval stages on leaves. Insecticides become ineffective once the larvae enter the fruit, since insecticides do not penetrate the thick skin of the fruit. However, spraying can be made more effective, if it is timed to coincide with those periods of the insect's life cycle during which it is exposed to the environment *viz.* eggs and the first instar larvae on young leaves, pupae on leaves and fruit, and adult moths. Spraying the eggs and first instar larvae would appear more practical than the destruction of pupae which are protected by a thick pupal case. Careful monitoring of the life cycle of the pest is

therefore necessary for successful spraying. For this purpose, a pheromone baited trap[5] will be helpful since it will indicate the mating period of the pest. With a knowledge of the time involved between mating and egg laying it will then be possible to predict the best period for spraying aimed at eggs and first instar larvae on leaves. In our previous work, the female sex pheromone of *L. orbonalis* was identified as (E)-11- hexadecenyl acetate (HDA).[6] Subsequent field studies using a synthetic sample of (E)-11-HDA in baited traps, with doses upto 1 mg, were not promising.[4] In comparison, traps containing virgin females caught males successfully. Based on the above experiments it was thought that the trace amounts of (E)-11-hexadecen-1-ol, probably the biosynthetic precursor of the acetate found in the sex pheromone gland of the female moth during calling time might be an important part of the pheromone system. The results of investigations into this possibility are reported here.

Electroantennography (EAG), originally described by Schnieder[7] in 1957, is a simple but powerful method for detecting and assessing biological activity of pheromones and plant derived attractants (Fig. 1). When EAG is used in combination with gas liquid chromatography (GC-EAG),[8] it leads to a unique, and effective way of identifying active component/s in a mixture. The present paper reports the results of testing the effect of (E)-11-hexadecen-1-ol on the pheromone of *L. orbonalis*, evaluated on the isolated antenna of male *L. orbonalis*, using the EAG technique.

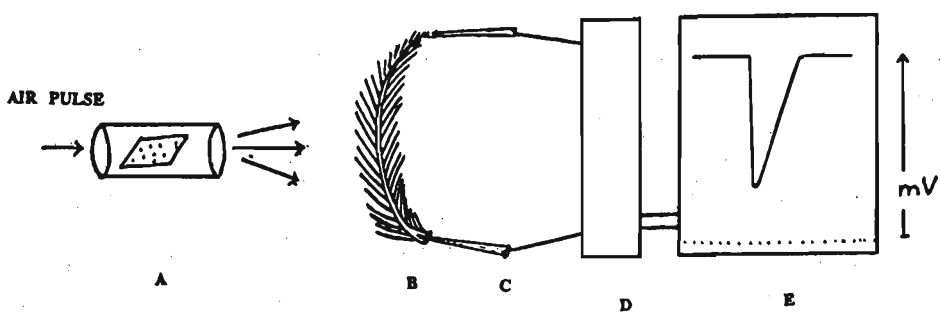

Figure 1: Electroantennogram (EAG), A. Stimulus source, B. Antenna, C. Microelectrodes, D. Amplifier, E. Recorder.

METHODS AND MATERIALS

Insects: Pupae of *L. orbonalis* were air lifted to Germany from Sri Lanka. They were sexed and male pupae were kept at 28 ± 2^{0}C on a 10:14 light-dark cycle. After emergence, insects were fed on a 5% sucrose solution.

Test substances: (E)-11-HDA and (E)-11-hexadecenol were synthesized as described previously by us.[9] From a stock solution of 10 mg/ ml of the test substance in analytical grade hexane (Merck Chemical Co. Germany), serial dilutions of 1000 μg/ ml, 100 μg/ ml, 10 μg/ ml and 1 μg/ ml were prepared. In case of the test mixture, (E)-11-HDA and (E)-11-hexadecenol, first the neat liquids of the above two compounds were mixed (ratio 9:1, w/w respectively) to give a stock solution of 10 mg/ ml. Dilutions were made from the stock as described before. Aliquots of 20 μl of serial dilutions were pipetted out onto filter paper strips (20 x 7 mm) and these were allowed to dry in the air (1 min). After this they were inserted into glass cartridges (54 mm long, 7 mm ID). A blank was prepared using 20 μl of hexane.

EAG assay with male antennae: 2-3 day old males were anesthetized with a stream of CO_2 and the antenna cut immediately as close to the base as possible and isolated. Following the methodology of recording electroantennograms described by Schneider,[7] two glass capillary Ag-AgCl electrodes filled with insect Ringer[10] solution were used. The tip of the antenna was cut off and the recording electrode was inserted into the open end. The indifferent electrode was inserted into the base of the isolated antenna. In order to prevent leakage of receptor lymph or the electrolyte and the drying up of the antenna, both ends of the antenna were sealed with vaseline. Total time of mounting did not exceed 10 min. The glass cartridge loaded with test substance each time, was kept about 1 cm in front of the antenna and a stream of filtered air (0.5 l/min) was purged through the cartridge for 1 sec. A vacuum outlet was positioned approximately 10 cm behind the setting of the antenna in order to remove pheromone containing air from the area surrounding the antenna. Between stimuli of 1 sec, a stream of filtered air (0.5 l/ min) continuously bathed the antenna for at least 4 min. A minimum period of 2 min between stimulations was allowed for adequate receptor recovery of the antenna. All three test stimulants, (E)-11- HDA, (E)-11 hexadecen-1-ol and the mixture of above two compounds were chosen at a random basis and then test doses for each compound were presented sequentially from lowest to highest. For each concentration, a minimum of a 6 and a maximum of 12 recordings were made. The response of the antenna was recorded as peaks and the distance between the base and the highest point of the peak was measured. Interspersed between every 10 test odour stimulations were control stimulations (see later) with odour cartridges with filter paper impregnated with 2 μl of hexane. Life time of an isolated antenna of *L. orbonalis* was about 40 min and hence for the required number of stimulations by test substances four antennae were used. Each fresh antennal EAG response was evaluated by offering the control as an internal standard and corrections were made for individual and time dependent alterations in responses. Following this, all test odor responses accompanying each "corrected control" response were subjected to correction. This method permitted comparisons within and between antennal responses. Finally responses of the test compounds were converted to percentages of the highest response.

RESULTS

In the EAG assay, the isolated male antenna remained active for a period of about 40 min. A relative EAG response of 38.0% was obtained for the control. A plot of EAG response versus concentrations of (E)-11-HDA showed an increasing antennal response with increasing concentrations from 0.02 μg upto 20 μg and a decrease thereafter. Thus EAG values of 50.5%, 59.2%, 76.6%, 92.8% and 84.3% were obtained for the concentrations of 0.02 μg, 0.2 μg, 2.0 μg, 20 μg and 200 μg respectively (Fig. 2). Highest activity of 92.1% was elicited at the concentration of 20 μg. The EAG response profile of the mixtures of (E)-11-HDA/ (E)-11-hexadecen-1-ol also was similar to that of (E)-11-HDA in that the activity increased from 0.02 μg to 20 μg and decreased thereafter. Thus EAG values of 60.3%, 66.1%, 79.4%, 100% and 90.6% were obtained for the same series of concentrations respectively, indicating the male antenna of *L. orbonalis* may be slightly more responsive (although not significantly so, $P > 0.05$, t-test) to the mixture.

The pure alcohol, showed lowest response of all test compounds elicitng only 44.3% and 38.8% EAG activities for the concentrations 20 μg and 200 μg respectively. These EAG responses were significantly different from those of the corresponding doses of (E)-HDA or the mixture (t-test, $P < 0.01$ for both cases).

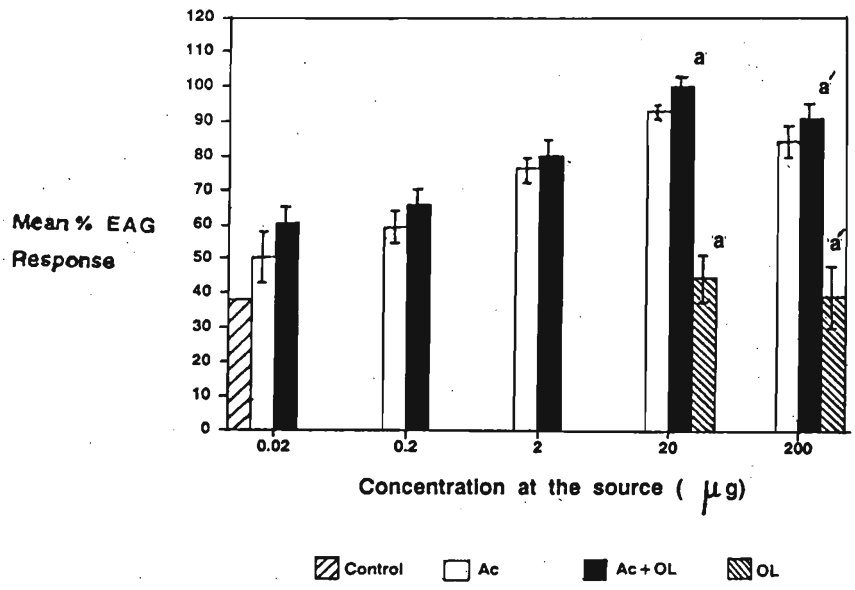

Figure 2: Mean relative % EAG response profiles of the male L. orbonalis antenna to pure (E)-11-hexadecenyl acetate, a mixture of (E)-11-hexadecenyl acetate: (E)-11-hexadecen-1-ol, 9:1 (w/w) and pure (E)-11-hexadecen-1-ol. Control = Hexane, Ac = (E)-11-Hexadecadienyl acetate, OL = (E)-11-hexadecen-1-ol and Ac + OL = the mixture of above two components. Vertical bars indicate standard errors of the mean. Number of observations = 6-12. a and a' identify compounds that elicit significantly different (t- test, $P < 0.01$) responses.

DISCUSSION

With the availability of advanced techniques in recent years it has become easy to analyze the pheromone gland contents during calling time.[5,11] It is a common observation that minor chemical components are present in the pheromone gland in addition to the pheromone itself and common fatty acids.[12-14] While the minor compounds are often precursors of pheromones they may also be a part of a multicomponent pheromone system.

(E)-11 hexadecen 1-ol, the alcohol found during the calling period of the female *L. orbonalis*, is the probable precursor of the pheromone acetate, (E)-11-HDA, because in several biosynthetic studies, pheromone acetates /and aldehydes have been shown to derive from the corresponding alcohols.[14] On the other hand (E)-11 hexadecen-1-ol itself has been recognized as a pheromone of several lepidopterans.[15] For example, *Eupsilia quadrilinea* uses (E)-11 hexadecen-1-ol as the only compound in its sex attractant pheromone.[16]

In our field evaluations with the synthetic (E)-11 HDA, the trap catches were poor when compared with the natural pheromone emitted from virgin females. When a synthetic compound is field tested in this manner and found to be less effective than the natural pheromone, it often indicates an imperfect synthetic pheromone blend[5].

Our results on the EAG assay of synthetic (E)-11-HDA are interesting firstly because it is consistent with the behavioral study reported by us previously, using the olfactometer.[4] While the EAG is not a behavioral response, in both the EAG and the olfactometer assay an increasing response with an increasing (E)-11-HDA concentrations, up to 20 μg and a decrease thereafter was observed. This shows that effective concentration of the pheromone to the male antenna of *L. orbonalis* should be 20 μg at close range. The receptors of male *L. orbonalis* are clearly insensitive to (E)-11-hexadecen-1-ol alone and therefore it is unlikely that this alcohol is a pheromone by itself.

However this study suggests a slight enhancement of the activity (although not statistically significant with the numbers of the samples used) of (E)-11-HDA due to presence of (E)-hexadecen-1-ol. Activity enhancements by the trace/ minor components have been reported in some field assays.[17] One interesting example is that of the cotton leaf worm attractant, (Z)-9, (E)-11-tetradecadienyl acetate.[18] The bait made using above attractant became extremely attractive to male *Spodoptera littoralis,* Boisd the cotton leaf worm, only when it is contaminated with traces of another geometrical isomer, (Z)-9, (E)-12-tetradecadienyl acetate. Addition of increased amounts of(Z)-9, (E)-12-tetradecadienyl acetate however reduced the activity of the attractant. In our EAG evaluations, the maximum enhancement by the minor component, (E)-11-hexadecen-1-ol was only 9.8%. The possibility that this enhancement is more prominent in a behavioral assay requires further investigation.

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