# Electrophysiological responses of both sexes of groundnut leaf miner, *Aproaerema modicella* (Lepidoptera: Gelechiidae) to synthetic female sex pheromone blend

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**The efficacy of female-produced three-component pheromone blend of groundnut leaf miner (GLM) indigenously synthesized at the Indian Institute of Chemical Technology, Hyderabad, was bioassayed by Electroantennogram Recording (EAG) and Coupled Gas Chromatographic–Electroantennographic detection (GC–EAD) techniques. The female sex pheromone blend of GLM consists of (Z)-7,9-decadienyl acetate, (E)-7-decenyl acetate and (Z)-7-decenyl acetate in the ratio 10 : 2.0 : 1.4. EAG recordings of male antennae indicated significant responses to different doses of female-produced synthetic pheromone blend. Female antennae were found to be non-responsive to their conspecific pheromone blend by both EAG and GC–EAD techniques. However, characteristic positive, positive negative deflections were recorded in EAG with female antennae. When the three-component synthetic blend was subjected to GC–EAD analysis, strong EAD response was elicited from the major component, (Z)-7,9-decadienyl acetate at retention time 13.6 min, and two smaller bioactive peaks from (Z)-7-decenyl acetate and (E)-7-decenyl acetate at the retention times 12.0 and 12.6 min respectively.**

**Keywords:** Antenna, groundnut leaf miner, olfactory response, pheromone.

THE groundnut leaf miner (GLM), *Aproaerema modicella* Deventer (Lepidoptera: Gelechiidae) is a serious pest of groundnut and soybean found throughout India, South and Southeast Asia[1] in both rainfed and irrigated crops. Yield loss due to this pest is up to 51% every year. The damage is characterized by the leaf mining of larvae between the epidermis and longitudinal folding of individual leaves. Immediately after hatching, the larva burrows into the leaf and produces blotches by feeding on the green tissue of the leaf. As the larva grows, the tunnel expands; the affected leaves get distorted and finally dry up. In severely affected fields, the infested plants exhibit a scorched appearance. To protect the groundnut crop from pest attack, farmers use 5–6 pesticide sprays during the crop season[2]. However, insecticidal control of GLM is difficult as it is confined within the stem and leaves. Hence, any number of pesticide applications may become insignificant for the control of GLM. Therefore, the use of sex pheromones can play a significant role in protecting the crop from GLM infestations.

Nandagopal and Reddy[3] have identified the existence of sex pheromones in virgin females of *A. modicella*. The chemical composition of female *A. modicella* sex pheromone was described[1] as a blend of three components, i.e. (Z)-7,9-decadienyl acetate, (E)-7-decenyl acetate and (Z)-7-decenyl acetate in the ratio of 10.0 : 2.0 : 1.4. As a part of an on-going programme on the synthesis of insect pheromones of various crop pests, Yadav and his group[4,5] have synthesized the three-component blend of *A. modicella* through the most feasible stereo-selective synthetic routes. Large-scale field trials covering 100 ha of groundnut crop in Nalgonda and Mahaboobnagar districts, Andhra Pradesh with indigenously synthesized pheromone blend of GLM have given encouraging results with excellent trap catches. Moderately greater number of male moths (>150/trap/week) were trapped from the beginning of the trial (from 32nd to 35th std. week). The highest number (>425) of males/trap/week was recorded in the middle of the trial, i.e. from the 37th to 40th std. week. Thereafter, the data indicated a constant decline in trap catches (<75 males/trap/week) in the subsequent weeks (40th to 44th week)[6].

The perception of olfactory stimuli in insects is mediated largely through their antennal receptors. Electroantennogram recording (EAG) of insect olfactory responses has been an asset for the bioassay for chemical components of pheromones and for the development of synthetic attractants[7]. EAG is essentially the sum of many olfactory receptor potentials recorded more or less simultaneously by an electrode located in the sensory epithelium[8]. The principle of EAG is to record voltage changes between the tip and the base of an antenna during stimulation by a volatile[9]. Coupling of Gas Chromatogram (GC) and EAG (GC–EAD) has led to the development of extremely sensitive and specific detection systems for pheromone components, which fully utilize the tremendous analytical capabilities of the two techniques[10]. The GC–EAD takes the EAG to a higher level of sophistication and utility using the antenna of an insect as a detector for a capillary-column gas chromatogram. Using this stimulation technique, impurities of a synthetic sample can be distinguished from the test compound[11]. Furthermore, with a chiral GC column, enantiomers can be separated before being tested on the insect antennae[12,13]. Here, we report the electrophysiological responses of both male and female GLM to the indigenously synthesized pheromone blend using the EAG and GC–EAD techniques.

EAG measurements were made using a commercially available electroantennographic system (Syntech, Hilversum, The Netherlands) consisting of a dual electrode probe for antenna fixation, a CS-05 stimulus controller, and

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an IDAC box for data acquisition. The antenna were excised along with the head and fixed between the two stainless-steel electrodes (head at one end and tip of the antenna onto the recording electrode) using electrically conductive gel. Signals generated by the antenna were passed through a high-impedance amplifier and displayed on a monitor using Syntech software for processing the EAG signals. A current of filtered pure air was constantly directed onto the antenna through an 8 mm-diameter glass tube. Stimuli were provided by connecting the pipette for 1 s into the airstream flushing over the antenna.

Serial dilutions of the synthetic blend were prepared in HPLC-grade hexane (50 µl containing different doses ranging from 0.5 to 15.0 µg), which were applied on Whatman No. 1 filter paper (7 × 16 mm). After complete evaporation of the solvent, the filter paper strips were inserted into glass Pasteur pipettes. New cartridges were prepared for each insect. The puff was delivered into the continuous air stream, after placing the pipette tip into the hole of the glass tube carrying the air stream. Continuous flow of clean air through the airflow tube and over the preparation ensured that the odours were removed from the vicinity. An equal volume of solvent alone (hexane) spread on the filter paper served as the control. Control stimulation was made at the beginning and after every 2–3 EAG recordings. EAG responses were evaluated by measuring the maximum amplitude of depolarization triggered by the stimuli. At least 30 s duration was allowed between two continuous stimuli for recovery of the antenna. EAG responses were recorded from five male and female insects individually to different doses of the pheromone blend. For data analysis, the mean solvent signal was subtracted from each mean stimulus signal.

For GC–EAD recordings, the antennal set-up was similar to that for EAG. However, in GC–EAD, the effluent from the end of the column was split into two, with one portion delivered to a flame ionization detector (FID) and the other passed into a stream of purified air blown across the insect antenna. Electrodes attached to the tip and to the base of the antenna (or alternatively, to the head or body of the insect) conduct voltages from the antenna to a high-impedance DC amplifier in a signal-connection interface box. Synchronous voltage changes by both the FID and the antenna indicate olfactory sensitivity by the insect to the compound eluting at that particular retention time. The FID output can be used to confirm the presence, identity and quantity of compounds exposed to the antenna, while the antennal (EAD) output indicates presence/absence of olfactory sensitivity to eluting compounds and provides a relative measure of the intensity of olfactory stimulation.

Recordings were performed with a GC1000 II Tech comp, gas chromatogram, with a column split and an extra outlet which allows simultaneous flame ionization (FID) and electromagnetic detection (EAD). A capillary column of 15 m × 0.53 mm i.d., SPB$^{TM}$-1701 was used with hydrogen as the carrier gas (4 ml/min). Injector temperature was 275°C, detector temperature 275°C, EAD-outlet 200°C and the split-less injection was made at 50°C oven temperature (0.3 µl of 1 mg/ml GLM pheromone blend). The temperature programme started after 2 min with a rate of 10°C/min to 225°C and was held for 5 min. The column effluent was split between the FID and EAD at a ratio of 1 : 1.

The column of the EAD outlet was introduced into an 8 mm diameter glass tube with a constant air stream filtered through activated charcoal (flow 0.5 l/min). The mounted antenna was placed 0.5 cm from the end of the glass tube (30 cm from the EAD outlet on the GC). FID and EAD signals were analysed and monitored on a personal computer using GC–EAD software (Auto spike, IDAC 2/3 Syntech, The Netherlands).

Representative EAG recordings obtained from male and female antennae reveal that the shape of the EAG evoked by pheromone blend was different for males and females. EAG signal elicited by male antenna was characterized by a fast negative potential and slow return to the base line, which is a commonly observed phenomenon in response to stimulation with pheromones/plant volatiles (Figure 1 *a*). A clear effect of the dose of synthetic pheromone blend on EAG response of male antennae was observed (Figure 1 *b*). The results indicated that at doses below 0.1 µg the EAG responses were insignificant. However, at 0.1 µg dose, the response was quite significant and the EAG values ranged between 0.25 and 0.6 mV, and a dose-dependent increase in EAG activity was observed with increasing dose of synthetic pheromone blend (Figure 1 *b*). EAG responses did not increase significantly after 15 µg dose. The amplitude of the response, which correlates to the frequency of the generated nerve impulses, was found to increase with increasing concentrations of the chemical stimulus until a saturation level was reached.

The female antenna did not evoke any measurable EAG responses at doses that elicited significant olfactory responses in the male antenna. However, the shape of the EAG signals elicited by female antennae was characterized by large positive potential (Figure 2 *a*). EAG with positive deflections did not produce measurable EAG values. A few females also produced EAGs that exhibited large positive peaks followed by a smaller negative peak at dosage range 10–15 µg (Figure 2 *b*). EAG showing both positive and negative deflection was described by Pavis and Renou[14] as complex. Such complex EAG produced responses ranging between 0.3 and 0.75 mV, which were almost equivalent to solvent responses. Contreras *et al.*[15] reported that it was possible to predict whether a chemical is an attractant or repellent based on the EAG pattern displayed by it. They found that in *Periplaneta americana*, chemicals showing negative EAG were attractants at lower concentrations, while those showing positive EAG were repellents. Similarly, Ramachandran *et al.*[16] re-

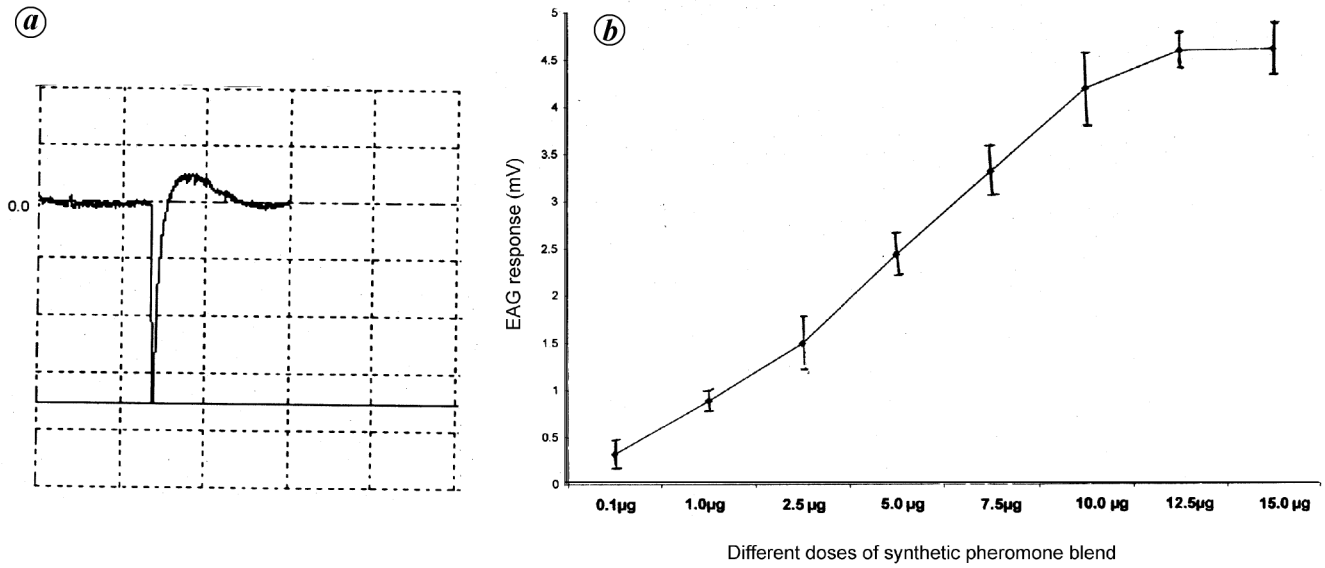


**Figure 1.** ***a***, Typical electroantennogram (EAG) of male groundnut leaf miner (GLM) towards female synthetic pheromone blend. ***b***, EAG activity of male GLM – different doses of female synthetic pheromone blend.

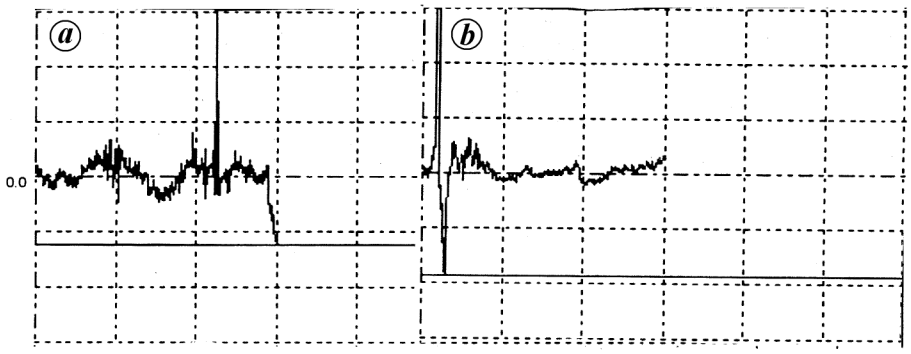


**Figure 2.** Shape of EAG elicited by GLM female antennae in response to stimulation with female-produced synthetic pheromone blend. ***a***, Positive deflection; ***b***, Quick positive–negative deflection.

ported that thymol and carvacrol, which elicited a positive EAG in both sexes of rice leaf folder species, were found to have strong antifeedant activity. *Ceratitis capitata* also responded with a positive or complex EAG when stimulated with organic acid[17]. However, EAG is described as the sum of the peripheral neuronal activity, but not the message arriving at the central nervous system[14]. The authors envisaged that at high doses, some molecules can change the properties of the membrane of the nerve cells for a short period[14]. In the present investigation the female antenna did not elicit any electrophysiological activity to the three-component blend by GC–EAD analysis (Figure 3 *a*). Generally, the female moth is considered to be anosmic for its own attractant[18]. However, examples where female moths detect their own pheromone are well documented in some species of Noctuidae and certain other families[18]. Several authors have proposed possible functions for pheromone auto-detection. For example, female pheromone can function as a dispersal trigger[19,20], as a repellent[21] or as attractant[22] and may influence the calling and oviposition behaviour of conspecific females[23].

Figure 3 illustrates GC–EAD responses of male and female GLM to synthetic pheromone blend. Analysis of three-component, female-produced synthetic pheromone blend by combined GC–EAD showed that the male antenna of GLM responded to all the three components. A notable and conspicuous EAG response was observed towards the major component (Z)-7,9-decadienyl acetate. Relatively smaller bioactive EAG peaks towards the minor components, i.e. (Z)-7-decenyl acetate and (E)-7-decenyl acetate were detected in the chart (Figure 3 *b*), perhaps due to the presence of low number of olfactory sensilla for the minor components. Generally, moth species use two or more compounds as the attractive blend of their pheromones. Sometimes, a major component is singly responsible for male attraction, whereas in most species minor component is needed for attraction. Our field-

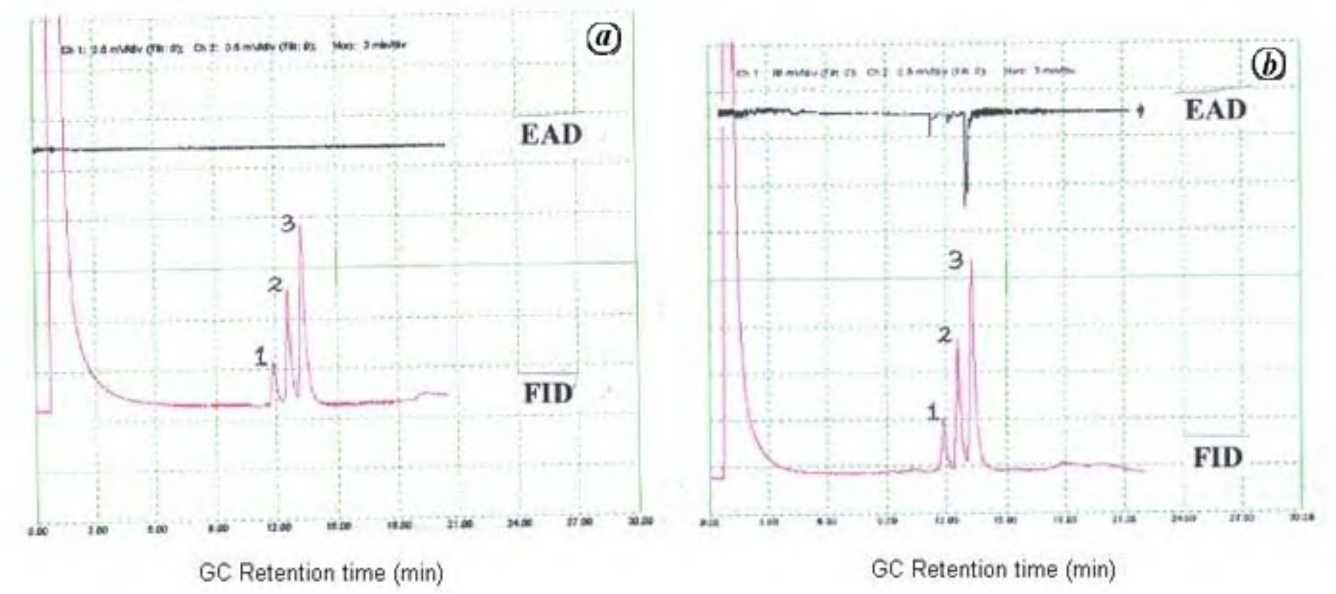


**Figure 3.** Simultaneous FID and EAD responses of an adult female GLM (***a***) and an adult male GLM (***b***) *Aproaerema modicella* to female-produced synthetic pheromone blend. 1, (Z)-7-decenyl acetate; 2, (E)-7-decenyl acetate; 3, (Z)-7,9-decadienyl acetate.

evaluation studies indicated that GLM lures containing only the major component caught reduced number of males than the three-component blend[6]. Plastic vial dispensers impregnated with 3 mg of the three-component pheromone blend showed the presence of all the three components up to three weeks of field exposure[6]. However, analysis of the lures after three weeks of field exposure indicated the presence of major component only and minor components were not detected[6]. Therefore, lure replacement is advised for every three weeks for maximum trap-catch efficiency of the blend.

Although EAG gives some information on the specificity of insect olfactory receptors, a more detailed understanding of the perception of the odour molecules by insects can be obtained by recording the response of the individual olfactory cells by single sensilla recording technique (SCR)[24]. Multicomponent pheromone systems are common in insect communication and receptor cells specialized to different components of the mixture are being increasingly implicated in pheromone research[25–27]. Currently efforts are under way to study the pheromone detection system of GLM by characterizing the pheromone sensitive sensilla using the SCR technique.

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# Genetic polymorphism of Indian tobacco types as revealed by amplified fragment length polymorphism

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**During the past five decades, a large number of tobacco varieties have been developed for different end uses in India through pure line selection from local land-races, mutation breeding and hybridization involving local selections and exotic introductions followed by pedigree selection. No systematic effort has been made to understand the existing diversity pattern in these varieties, which is crucial to define future breeding strategy in this important commercial crop. Amplified fragment length polymorphism (AFLP) analysis was used to determine genetic variation in 54 varieties of cultivated tobacco (*Nicotiana tabacum* and *N. rustica*) and three accessions of exotic germplasm. Nine oligonucleotide primer-pair combinations resolved a total of 967 AFLP fragments, of which 785 (81.2%) were polymorphic. The mean genetic distance among the 49 cultivars and three exotic accessions of *N. tabacum* was 15.35%; 22% among the five cultivars of *rustica*. Genetic polymorphism present among the cultivars of tobacco was low, as evidenced by the high degree of similarity in the AFLP profiles of different tobacco types. All the five cultivars of *N. rustica* can be readily identified using the primer pairs E-ACT/M-CAG and E-AAC/M-CTG. Two major clusters were formed on the basis of species and seven sub-clusters were formed on the basis of manufacturing quality traits in the cultivars of *N. tabacum*. Cultivated flue-cured varieties were clustered separately from other air cured types. Species-specific markers identified in this study would be useful in identification of the true hybrids and monitoring introgression of useful genes from the wild relatives. The markers found specific to the varieties can be used in correct identification of the carrier genotypes in trade and commerce.**

**Keywords:** AFLP markers, flue and air-cured varieties, genetic diversity, Indian tobacco types.

THE genus *Nicotiana* is a member of the family Solanaceae. Out of 64 recognized species[1,2] in the genus *Nicotiana*, two species, namely *tabacum* and *rustica*, which are natural amphidiploids ($2n = 48$), are grown commercially in the world. India is the only country where different

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