# Evidence for a male-produced sex pheromone in sesame leaf webber, *Antigastra catalaunalis* Duponchel (Pyraustidae, Lepidoptera)

**U. Sankar Narayanan[1,2] and L. Nadarajan[1,*]**

[1]Department of Agricultural Entomology, Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal 609 602, India
[2]Present address: Centre for Ecology and Rural Development, Pondicherry 600 010, India

**Sesame leaf webber *Antigastra catalaunasis* D. is a serious pest attaching sesame. It feeds on tender foliage by webbing the top leaves, and bores into the pods and shoots. Control of the pest using pesticide cannot be recommended due to the concealed feeding behaviour of the insect as well as due to the fact that sesame is an oil-seed crop. Hence eco-friendly pest management is recommended. We have studied the reproductive biology and sex communication of *A. catalaunalis*. Laboratory bioassay studies were done using the olfactometer and pheromone extraction chamber. It was found that the males produce sex pheromones. The pest can be managed by sex pheromones after isolation and chemical characterization, Thus, the use of chemical pesticides may be reduced.**

SESAME is one of oldest oilseed crops, mostly cultivated by marginal farmers. It is grown in Aden, Burma, France, Russia, Italy, Spain, Cyprus, East and West Africa and Malta. In India, sesame is cultivated in Rajasthan, Maharashtra, Gujarat, Madhya Pradesh, Andhra Pradesh, Karnataka, Uttar Pradesh, West Bengal, Orissa, Punjab and Tamil Nadu[1].

In the tropics and subtropics, sesame leaf webber *Antigastra catalaunalis* is a serious pest among the 29 insect species attacking sesame[2]. The shoot and leaf webber attacks all parts of sesame, except the roots. It feeds on the tender foliage by webbing the top leaves and also bores into the shoots and pods[3].

Management of this pest is restricted to the use of insecticides. But one of the difficulties in controlling the pest with insecticides is the concealed feeding behaviour of the insect[4]. In addition, since sesame is an oil-seed crop, organochlorine insecticides are not normally recommended due to high residue problems. Hence, eco-friendly pest management would be the future strategy for managing this key pest on sesame. Perusal of the existing literature on pheromone communication in this pest revealed that no published work is available on this aspect. Thus research on this aspect was carried out to know the sexual communication in this species so as to develop a management strategy for it.

*For correspondence. (e-mail: l_nadarajan@yahoo.co.uk)

Field-collected sesame leaf webber larvae were reared in the laboratory at 12 h L: 12 h D, 25 ± 1°C and 80–85% RH conditions.

Adults were subjected to behavioural bioassay using an all-glass *y*-tube olfactometer (Figure 1 *a*). The central, main part of the olfactometer is a *y*-tube consisting of two arms that are fitted to broad tubes serving as a test chamber (A) in which the materials to be tested and compared were kept. The middle portion of the *y*-tube is fitted with a broad conical chamber called as release chamber (B), where the insects to be tested for responsiveness were released. Air was blown from the other end of the *y*-tube (C) using a motor (Neikkei, 2000, 230 V, 50–60 Hz). Airflow was regulated by a valve situated in the release chamber. Using the olfactometer, selecting the arm containing either the live adults or their volatile extract is possible. In the test chambers, inward glass projections were provided to place meshes, so that the adults were allowed only to reach the test chamber but were not able to touch the confined material. The adults were examined for the presence of all appendages and were acclimatized in the release chamber by allowing a reasonable time (15 min). The entire olfactometer was washed thoroughly using soap solution (Labolin) and the unit was oven-dried before each experiment.

The test with live adults using the olfactometer indicated the sex having pheromonal attraction. After this confirmation, the sex that attracted was confined in a multicapillary volatile-collecting apparatus for collection of the volatiles[5]. It consisted of an air-loading chamber (Figure 1 *b*, A) of size (42 cm × 36 cm) through which air was blown, and which opened at five exits. The exits were closed with wide-mouthed, small chambers for confining the adults, with long capillary tubes at the ends (30 cm, Figure 1 *b*, B). Air was pumped using a motor (Neikkie 2000 230 V, 50–60 Hz; Figure 1 *b*). Air from the motor was filtered by passing through activated charcoal and then onto the air-loading chamber. From this chamber, air was passed through multi-resting chambers in which the adults were confined. The air loaded with pheromones finally came out through the capillary tubes of 30 cm length. The volatiles produced by the adults settled on the inner walls of these capillary tubes after evaporation.

The pheromone collection was done during the peak pheromone-producing hours, preferably during the early hours of the scotophase or during their calling behaviour period in a dark room. Then the confined adults were removed and the resting chambers were cleaned. The deposited chemicals were collected by washing the capillary tubes several times with n-hexane after removing from the device.

Bioassay using live adults and their volatiles collected was carried out using the olfactometer. The response of each sex of live adults to the opposite sex and to same sex and also to the their volatiles was studied by counting the number of adults entering each arm. The solvent was used as control for volatiles and the empty arm as control for live insects.

Based on the above data the attractive index (AI) was worked out as below:

$$AI = \frac{\text{No. of insects responded to test material} - \text{No. of insects responded to control}}{\text{No. of insects released} - \text{No. of insects responded to control}}.$$

Fractionation of the biologically active, volatile compounds eluted from the males was done with column chromatography using 0.2 g Florisil ® (100–200 mesh, Floridin CO) fitted in a capillary tube (30 cm length × 0.3 cm diameter). One ml each of 5% hexane (in ether), 15% hexane (in ether) and 50% hexane (in ether) and ether alone was successively used as eluting solvent. This resulted in four fractions that were bioassayed in the olfactometer using the respective solvent as check.

The first trial conducted was to study the response of sex in live moths. In the release chamber, groups of males were released at a time and their preference to the test chamber containing male and female was noted. Moths were released in groups in the chamber. For each group, at least a 30–45 min of observation was needed. The moths entering the central arm were counted as those that responded. In this study, out of 26 males tested, only one responded and entered into the test chamber with the female; others did not respond, indicating that males were not attracted to male or female (Table 1).

In the second set of experiments, having the male and female moths in the test chamber, 30 females were released into the release chamber in groups. Here all the 30 females responded and all the 30 female moths entered the test chamber having the male moths, indicating that the males attracted the females (Table 1).

The volatiles were collected from among the males based on the outcome of this study. The response of male and female adults to the male volatiles was estimated (Table 1). The male volatile was kept in one of the arms and the solvent n-hexane was kept in the other arm of the test chamber. Three groups of five males each were confined in the release chamber. But no male was found to respond since the male volatile did not attract the males.

In the next test, three groups of five females each were released into the release chamber and their responsiveness to the male volatile and solvent kept in the test chamber was observed. There was 100% responsiveness among the females, since all the moths entered in the arm containing the male volatiles (Table 1).

This confirmed that the male volatiles attracted live females. Just to see whether the female volatiles attract males, a test was conducted using female volatiles and solvent kept in test chambers and 44 females in four groups were released in the release chamber. There was no response and the females did not enter in the arm containing female volatiles, since the volatiles collected from the females did not attract the males (Table 1).

A further test was conducted keeping both male and female volatiles simultaneously, to study the responsiveness to

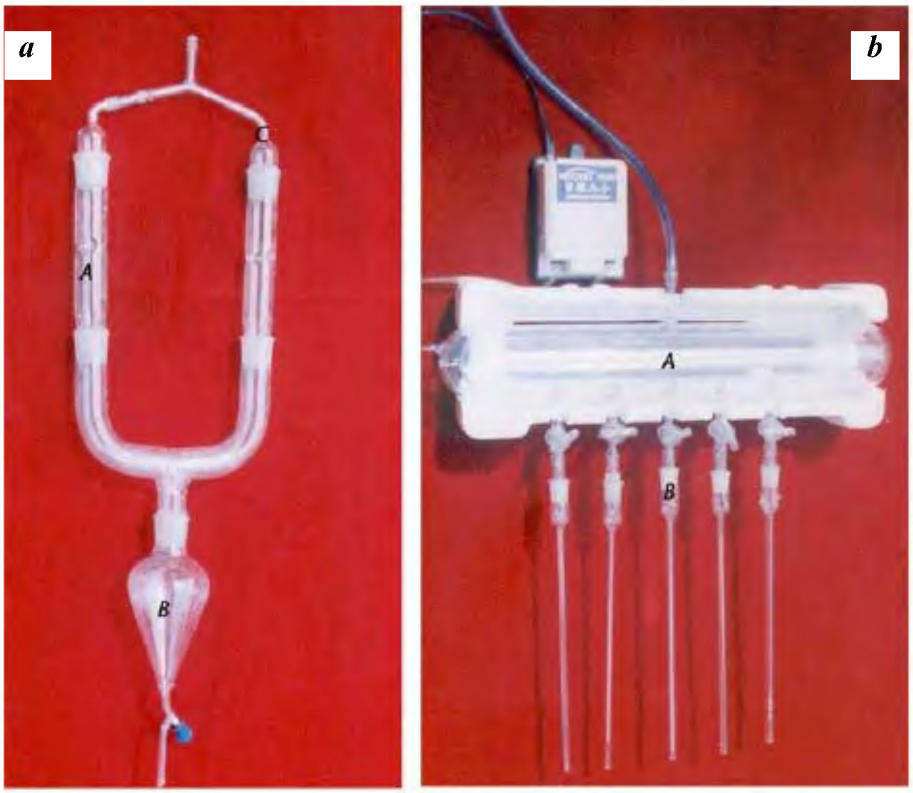


**Figure 1.** ***a***, Olfactometer. A, Test chamber; B, Release chamber; C, Y tube. ***b***, Pheromone extraction chamber. A, Air loading chamber; B, Insect confinement chamber.

different sexes of sesame leaf webber. Out of 40 males released in four groups, not even a single male moth entered either of the arms having the male or female volatiles, indicating that both male and female volatiles did not attract males (Table 1). When the same test was repeated with 12 live female adults in three groups, ten moths responded and entered into the arm with the male volatiles, thus confirming that male volatile attracted female moths (Table 1). This indicated clearly that male volatiles attracted only female moths. Statistical analysis using $\chi^2$ goodness-of-fit also confirmed that female moths were attracted to male moths and volatiles from males (Table 1).

The four fractions of male volatiles obtained as mentioned earlier were bioassayed using the *y*-tube olfactometer. Since the attraction by males to females was confirmed in earlier studies, all tests using the fractions of male volatiles were conducted only to test the response of the females. Fraction I, when tested with 15 females in three groups, attracted seven females and all these females entered the test chamber containing fraction I. This amounted to 46.67% response to fraction I of male volatiles (Table 2).

In a similar test with fraction II, with 15 female moths released in three groups, 14 moths responded; all the 14 moths preferred the test chamber containing fraction II, since

**Table 1.** Olfactometer study in live adults and volatiles

| Test no. | Adults in release chamber | | | Preference | | Choice combination | $\chi^2$ value and significance | Remarks |
|---|---|---|---|---|---|---|---|---|
| | Sex | Number | No. responded | Percentage | For sex | | | |
| A1 | Male | 26 | 1 | 3.8 | – | M vs F | 10.5 NS | Males are not attracted by males and females |
| B1 | Female | 30 | 30 | 100 | M | M vs F | 0.0* | Females are attracted by males |
| C1 | Male | 15 | 0 | 0 | – | Mv vs Sol | 15.0 NS | Males are not attracted by male volatiles or solvent |
| D1 | Female | 15 | 15 | 100 | M | Mv vs Sol | 0.0* | Females are attracted by male volatiles |
| E1 | Female | 44 | 0 | 0 | – | Fv vs Sol | 44.0 NS | Females are not attracted by female volatiles |
| F1 | Male | 40 | 0 | 0 | – | Mv vs Fv | 20.0 NS | Males are not attracted by females or male volatiles |
| G1 | Female | 12 | 10 | 83.3 | Mv | Mv vs Fv | 6.0* | Females are attracted to male volatiles |

M, Male; F, Female; Mv, Male volatile; Fv, Female volatile; Sol, Solvent; *, Significant; Ns, Non-significant.

**Table 2.** Testing fractions I to IV of male volatiles

| Test no. | Adults in release chamber | | | Preference | | Choice combination | $\chi^2$ value and significance |
|---|---|---|---|---|---|---|---|
| | Sex | Number | Responded | Percentage | For sex | | |
| a2 | Female | 15 | 7 | 46.67 | FI | FI vs Solv | 4.4 |
| b2 | Female | 15 | 14 | 93.33 | FII | FII vs Solv | 0.2* |
| c2 | Female | 15 | 1 | 6.67 | – | FIII vs Solv | 13.2 Ns |
| d2 | Female | 15 | 1 | 6.67 | – | FIV vs Solv | 25.0 Ns |

F, Fraction; Solv, Solvent; *, Significant; Ns, Non-significant.

**Table 3.** Attractive index and responsiveness of adults of *Antigastra catalaunalis*

| Sex of adult in release chamber | Combination in each arm of test chamber | Responsiveness to test material | Attractive index |
|---|---|---|---|
| Male | Live male vs live female | Live female | $0.05^{d}$ |
| Female | Live male vs live female | Live male | $1^{a}$ |
| Male | Male volatile vs solvent | MV | $0.0^{d}$ |
| Female | Male volatile vs solvent | MV | $1.0^{a}$ |
| Male | Male volatile vs female volatile | MV | $0.0^{d}$ |
| Female | Male volatile vs solvent | MV | $1.0^{a}$ |
| Male | Male volatile vs female volatile | MV | $0.0^{d}$ |
| Female | Male volatile vs female volatile | MV | $0.833^{b}$ |
| Female | Fraction I vs 5% hexane in ether | Fraction I of MV | $0.47^{c}$ |
| Female | Fraction II vs 15% hexane in ether | Fraction II of MV | $0.93^{ab}$ |
| Female | Fraction III vs 50% hexane in ether | Fraction III of MV | $0.07^{d}$ |
| Female | Fraction IV vs ether alone | Fraction IV of MV | $0.0^{d}$ |
| Control | Respective solvents | Respective solvents | $0.0^{d}$ |

MV, Male volatile; CD ($P - 0.05$ level) 0.13*. *Significant. The values with common letters (a, b, c, d) are statistically on par.

93.33% females were attracted to fraction II of male volatiles (Table 2). Further, testing with fraction III in which 15 females in three groups were released, and only one female moth 6.67% responded among the lot for fraction III. The same test was repeated using fraction IV of male volatiles to study the responsiveness of 15 female moths in three groups of five moths each released into the release chamber. None of the female moths responded positively, clearly indicating that fraction IV of the male volatiles was not attractive to the females (Table 2). The $\chi^2$ test for goodness-of-fit also confirmed that fraction II of the male volatiles was highly attractive to females than fraction I and the rest were not attractive (Table 2).

The responsiveness of the adult moths was converted to AI and subjected to statistical analysis (Table 3).

A maximum value of 1 was arrived for the females, to live males and male volatiles. An AI of 0.93, equivalent to male volatiles and live males was shown by the females to fraction II. Hence further investigations to isolate, identify and characterize the compounds in fraction II of the male volatiles will be indicative of the pheromones secreted by the males of *A. catalaunalis*.

Studies regarding reproductive biology and sexual communication in this serious pest of sesame indicated that the males produced sex pheromones.

Attractions of females to males in sesame leaf webber is well established. A similar phenomenon was also seen in another lepidoptera like the beehive pest, *Achroia innotata* and in Greater wax moth, *Galleria mellonella*[6]. Such pheromonal attractions were tested in wind tunnels[7,8] and using the olfactometer[8] by various workers. This study also gave similar results. Methylene chloride extraction of pheromonal glands from females of *Earias vitella* was done in the last hour of scotophase and the extract was tested for attraction to males[9].

In cotton caterpillar *Diaphania indica*, a Pyralid moth, Florisil column chromatography was used to fractionate sex pheromones and reports enumerated that in 5% fraction an active substance was found[10]. In the present study, fraction II with 15% hexane was found to be highly attractive.

Further research on this aspect to isolate, identify, characterize and synthesize pheromones will help in tackling this serious pest of sesame in an eco-friendly manner. Use of insecticides may be avoided in future to save this important oilseed crop from insecticide pollution, and instead pheromone trapping could be introduced.

1. Singhal, V., *Indian Agriculture*, Indian Council of Agricultural Research, New Delhi, 1999, pp. 1–600.
2. Weis, E. A., Sesame. In *Oil Seed Crops*, Longman, London, 1983, p. 660.
3. Rai, B. K., *Pests of Oilseed Crops in India and their Control*, Indian Council of Agricultural Research, New Delhi, 1976, pp. 77–80.
4. Ayyar, T. V. R., *Hand Book of Economic Entomology for South India*, Govt. of Madras, 1940, pp. 202–204.
5. Nadarajan, L., Isolation and identification of insect pheromones. National Seminar on Trends in Pheromone Research and Technology, NRCG (ICAR), DRR (Hyderabad) and IICT (CSIR), Hyderabad, 6–7 February 2004, pp. 133–143.
6. Singh, G., Sindhu, S. S. and Grewal, G. S., Chemical control of sesame shoot and leaf webber *Antigastra Catalunalis* (Dup) (Pyralidae : Lepidoptera). *J. Res. Punjab Agric. Univ.*, 1980, **17**, 47–54.
7. Ravi, G. and Palaniswami, M. S., Evidence for a female-produced sex pheromone in the banana pseudostem weevil, *Odioporus longicollis* Olivier. *Curr. Sci.*, 2002, **83**, 893–898.
8. Roller, H., Biamam, K., Bjerke, J. S., Norgard and McShan, D. W., Pyralid moths – Isolation and identification of the sex attractant of *Galleria mellonella* (Greater wax moth). *Acta Entomol. Bohemosiov.*, 1968, **65**, 208–211.
9. Carde, R. T., Chemo-orientation in flying insects. In *Chemical Ecology of Insects* (eds Bell, W. J. and Carde, R. T.), Chapman and Hall, London, 1984, pp. 111–124.
10. Miller, J. R. and Roelofs, W. L., Sustained flight tunnel for measuring insect responses to wind-borne sex pheromones. *J. Chem. Ecol.*, 1978, **4**, 187–198.

ACKNOWLEDGEMENTS. We thank Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal for facilities.

Received 10 May 2004; revised accepted 25 October 2004

# Nature of bank erosion along the Brahmaputra river channel, Assam, India

**P. Kotoky[1,*], D. Bezbaruah[1], J. Baruah[1] and J. N. Sarma[2]**

[1]Geoscience Division, Regional Research Laboratory, Jorhat 785 006, India
[2]Department of Applied Geology, Dibrugarh University, Dibrugarh 786 004, India

**A study on a selected stretch of the Brahmaputra river channel revealed that the mechanisms involved and responsible for riverbank erosion were basically related to aqueous flow of sediments (liquefaction) enhanced by the inhomogeneity in the bank materials in question, oversteepening and associated sub-aerial processes of weathering and weakening in relation to soil moisture content. The study revealed that the extent of erosion and deposition in not same for the period 1914–75 and 1975–98. Different scale and nature of shear failure are considered to be mainly responsible for bank erosion processes.**

THE Brahmaputra valley in Assam represents a tectonosedimentary province 720 km long and 80 to 90 km wide, with elevation ranging from 120 m at Kobo in the extreme east

*For correspondence. (e-mail: probhatk@yahoo.com)