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"HOST ATTRACTANTS FOR REDUCING *Leptocorisa acuta* ATTACK ON RICE"

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3) EXECUTIVE SUMMARY

The rice grain bug *Leptocorisa oratorius* (Fabricius)* (Hemiptera Coreidae), is a serious pest on rice (*Oriza sativa*) An attractant specific to *L oratorius* is present in the panicle of the developing rice grain In this investigation, the volatile chemicals of rice plants, the preferred host of the rice bug, and two other alternative hosts, bajiri (*Pennistum specatum*) and illuk (*Imperata arundinaceae*), were characterized, and the compounds the pest utilizes to locate host plants were identified For this investigation, an instrumentation facility that enables characterizing volatile constituents present in complex mixtures was established at the Department of Chemistry of University of Kelaniya, Sri Lanka A gas chromatograph (GC), and a setup to amplify and record electrical signals produced by insect antennae, when its receptors are stimulated by signal chemicals (a technique known as electroantennography, EAG), were installed To the best of our knowledge, this is the first EAG facility in South Asia Using this equipment, it was possible to record electrical responses elicited by an isolated antenna of *L oratorius* By measuring EAG responses evoked, when volatiles of rice panicles were blown over an antenna, it was revealed that only the last segment of the antenna is vital for chemo-reception Olfactometer bioassays were conducted to evaluate the attractivity of four different varieties of rice (BG94/1, BG1492, BG 303, and AT 303) to the rice bug The results show that all varieties are attractive, however the attractivity of the AT 303 variety is slightly less than those of BG varieties

Gas chromatography in conjunction with electroantennography (EAG) and olfactometer bioassays were used to recognize the compounds which appeared to account for the attractivity EAG results showed that volatiles from milky-rice grains and flowers are better stimulants to the pest than those from green and yellow leaves, and mature grains The EAG responses evoked by volatiles of milky-grain stage of nonpreferred hosts, bajiri and illuk, were significantly lower than those elicited by rice volatiles Moreover, EAG signals recorded from leaf volatiles derived from erabadu (*Erithrina variegata*), mango (*Mangifera indica*), and pepper (*Piper nigrum*), three plants not susceptible to rice bug attacks, were substantially lower than those elicited from host and alternative host volatiles

From GC-EAG and gas chromatography-mass spectrometric (GC-MS) analyses of milky rice grain volatiles, *n*-octanal, *n*-nonanal, *n*-decanal, *n*-undecanal and (*Z*)-2-pentenol were identified as the most effective stimulants In addition, three trace constituents, 3-octanol, β-cyclocitral and linalool also evoke considerable EAG activities The presence and concentration of all five major attractants, especially that of decanal, are important for the high EAG activity Olfactometric assays of different synthetic formulations showed that the mixture which represents quantitatively the proportions of these compounds in milky-rice elicits the highest attraction although the activity was somewhat less than that of the extracts of the natural milky-rice grain Although some minor compounds that might enable the synthetic mixture to show similar activities as that of milky-rice remain unknown, it is most likely that rice varieties without, or at least with low concentrations, the major stimulants will be less susceptible to attacks by the rice bug Therefore, attempts to develop of such rice varieties as future research should be encouraged

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* Previously, this pest had been named *Leptocorisa acuta* The correct identification of the species as *Leptocorisa oratorius* was done by consulting scientists at the Rice Research Institute, Philippines

4) RESESARCH OBJECTIVES

Sri Lanka is mainly an agricultural country (of 17 million total population, over 8 million is agricultural) where rice is the staple food Rice is grown in all parts of the country in two seasons, namely Yala (April-September) and Maha (October-March), which begin parallel to the two rainy seasons The total area of rice growing land in Sri Lanka (1984-1989) is 301360 ha (Yala) and 529329 ha (Maha) which comprises respectively 4 6 and 8 0% of the total land Unfortunately, the total rice production of the country is insufficient at present due to many reasons such as inadequacy of efficient irrigation systems, droughts, diseases, and pest attack

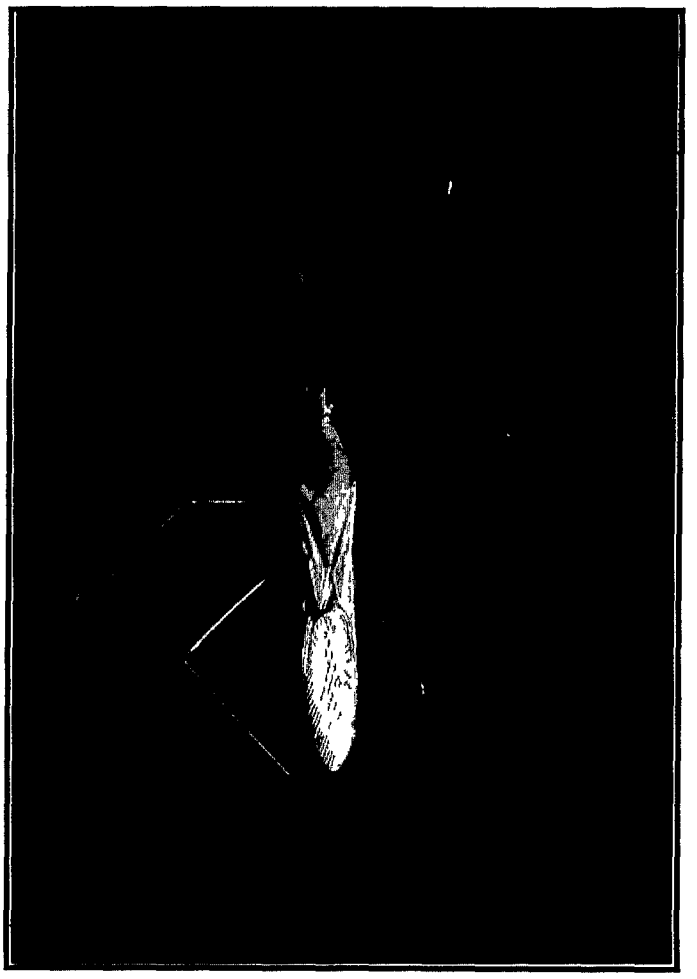


Figure 1 Adult rice bug *Leptocorisa oratorius*

Grain sterility is identified as one of the major reasons for low production of rice in Sri Lanka and in other tropical countries (Morita and Dhanapala, 1990) Studies carried out in Sri Lanka have shown that application nitrogen fertilizers during reproductive growth of the panicle and lack of pollination are the major factors causing gain sterility (Ota and Yamada, 1965a, 1965b, Ota and Devasuderarajah, 1966) A study conducted in 1985/86 has shown the existence of another type of grain sterility which occurs after normal pollination and fertilization Partially filled spikelets is the characteristic feature of this grain sterility, as opposed to unfertilized spikes

caused by the two major factors mentioned above (Morita and Dhanapala, 1990) One of the main reasons for this new type of grain sterility is infestations of the developing rice panicles by pests, mainly stink bugs of the genus *Leptocorisa* (Kobayashi and Nugaliyadde, 1988) *Leptocorisa oratorius* (Hemiptera Coreidae) (Figure 1) is one of the most devastating rice pests in Asia and the Pacific region In south Asia, *L oratorius* has on some occasions caused crop losses as high as 50% (Banerjee, 1957) In some areas of non-flooded fields of Indonesia, this bug becomes most destructive and causes sometimes losses up to 100% (Dresner, 1955) There is currently no environmentally acceptable method of control for this pest The existing method is blanket spraying or dusting with conventional insecticides such as BHC, DDT or Malathion (Grist and Lever, 1969) Field evaluations have shown that, although not gentle to the environment, Malathion (1 kg/ha) (Pangtey, 1985, Krishnakumer and Visalakshi, 1989), Cytrolane (0 75 kg/ha) (George et al ,1978), and Fenitrathione (1125 mL/ha)(Krishnamurthy et al , 1977) are effective in the control of this pest The usual practice among the farmers of Sri Lanka is to dust or spray insecticides at two week intervals throughout the development of rice grain which lasts about 1-2 months This practice not only enforces a heavy financial burden on poor farmers but also subjects them to health hazards due to exposure It is generally known that some pesticide residues do move along the foodweb exposing virtually all living organisms to these hazardous chemicals

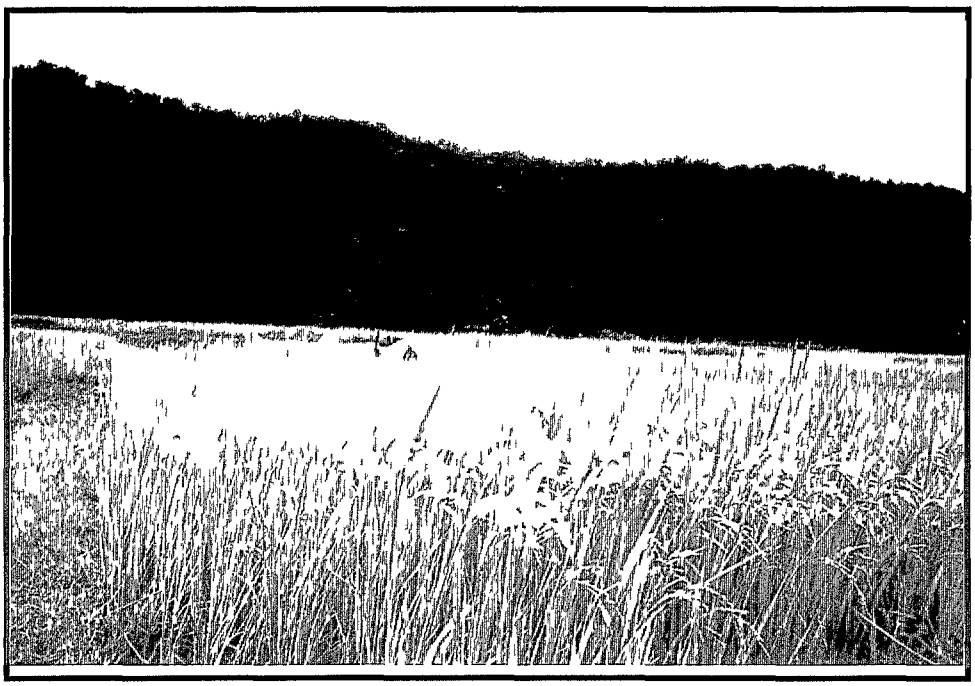


Figure 2 Rice fields, milky panicle stage

Although rice is the preferred host of *L oratorius*, some weeds of the family Graminae such as *Pennistum specatum* (Sinhala bajiri) and *Imperata arundinaceae* (Sinhala illuk) are also acceptable as alternative hosts for this pest Particularly, when rice plants are out of season, the pest spends time on any other available host (Litsinger et al , 1993) The life cycle of *L oratorius* starts with eggs (about 1 mm long), and nymphs emerge after an incubation period of 6-7 days

There are five nymphal stages which lasts about 17-27 days The adult bugs can live up to 115 days under favorable environmental conditions The pest which shows a low life profile on alternative hosts usually finds its way to rice fields during the flowering and milky panicle stages (Figure 2) Once migrated to rice fields, the pest population increases tremendously After rice plants reach maturity (Figure 3), and grains are harvested, the pest returns to land and continues its lifecycle on secondary host plants During this period the bug population reduces to a minimum, and the pest appears paler in color and smaller in size

Figure 3 Rice fields, mature panicle stage

In a previous study, it had been established that the steam volatiles of the rice panicles are highly attractive to *L oratorius* (Gunawardena, and Ranatunga, 1989) Interestingly, only the developing grain in its milk stage, which lasts only about two weeks, produces this attractant Ether extracts obtained from steam distillates of milk-stage developing grain were highly attractive to both sexes when tested against those obtained from blooming and mature stages In laboratory bioassays, using an olfactometer, nearly 81% of the total insects flew or crawled to the arm of the olfactometer baited with an active extract In field trials about 40 insects were caught per trap baited with an attractive extract

The main objective of this project was to characterize the chemicals (kairomones) involved in the attraction process Although some investigations on aggregation pheromones of Coreidae have been undertaken (Blatt, 1994, Ishiwatari, 1976), comprehensive chemical ecological studies on host-plant recognition based on olfaction have not been conducted

The chemical characterization of milk stage rice volatiles could open the way for a variety of novel applications Eventually researchers will develop unique rice varieties which do not produce these attractants leading to a departure of the popular practice of using pesticides as the only effective means control of *L oratorius* Although techniques involving semiochemicals are getting more recognition in developed countries, this technology has not made a big impact yet in poor developing countries

5) METHODS AND RESULTS

Our approach to characterize kairomones of rice was first to isolate the volatile compounds present in Gramminae plants (illuk, bajiri, rice) The extracts were tested for activity by behavioral and electroantennographic (EAG) bioassays Most active extracts were subjected to gas chromatography (GC)-EAG assays, GC retention times evaluations and microchemical reactions Compounds recognized as active constituents were characterized by gas chromatography-mass spectrometry (GC-MS) Finally, authentic samples of the chemical compounds which were identified were tested by EAG and behavioral assays

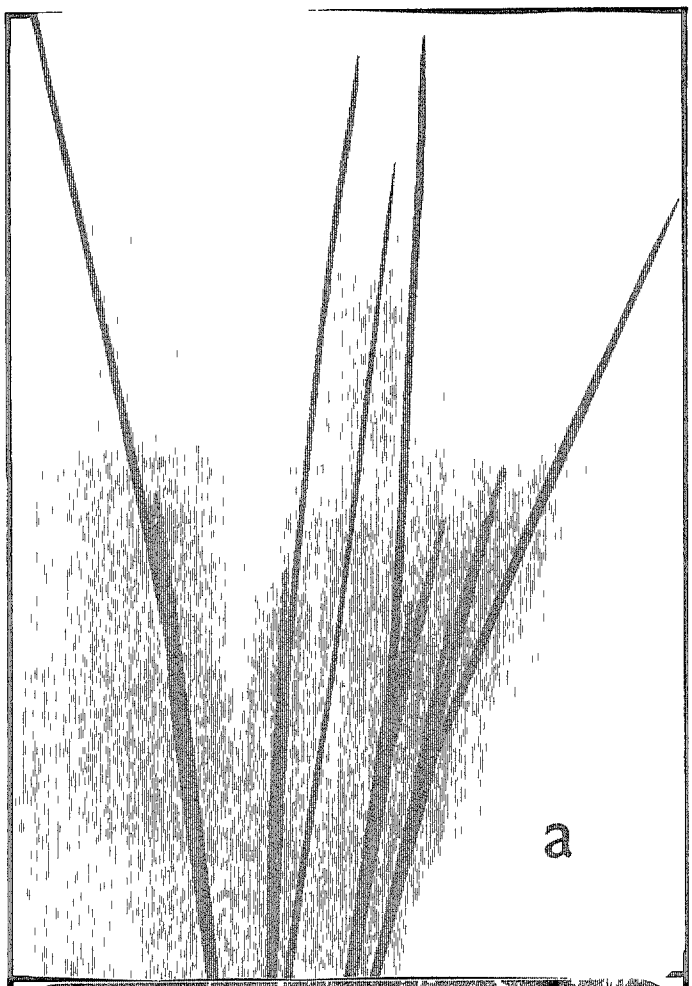
5 1 METHODS

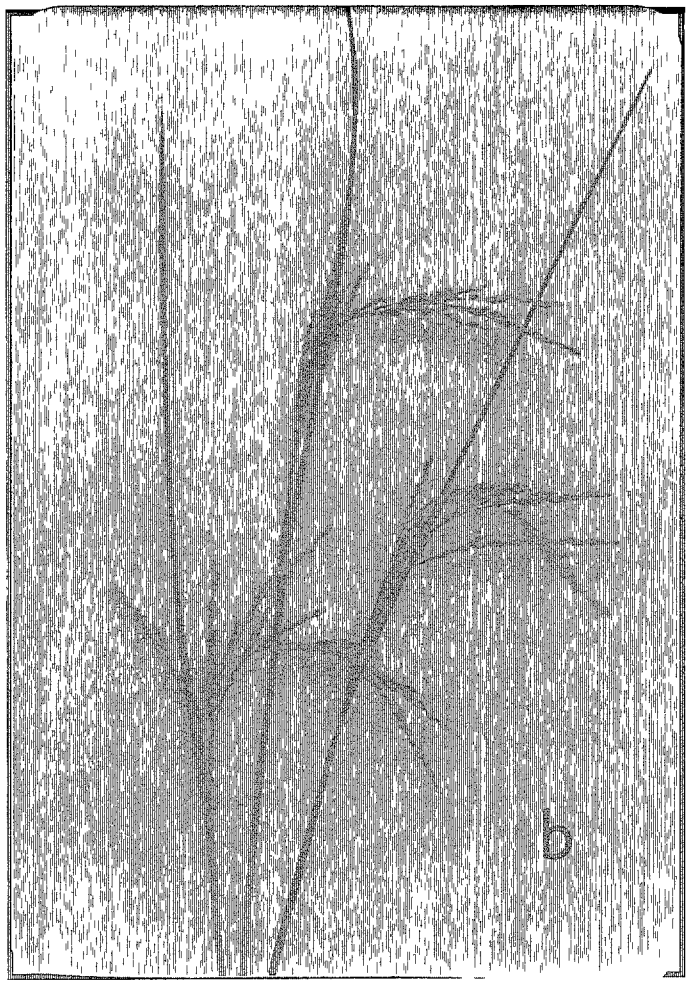
5 1 1 Biological Samples

For the collection of volatiles, the following samples, **S-1** to **S-5**, were used

- **S-1** Green leaves, hay, and rice panicles at flowering, milky, and mature stages (see Figures 4a-c) of the host plant (Rice, *Oriza sativa* L, variety BG 94-1)
- **S-2** Different varieties of the host plant (Rice, *Oriza sativa* L), BG 94-1, BG 1492, BG 303 and AT 303
- **S-3** Alternative host plant (bajiri , *Pennisetum spicatum* L and illuk , *Imperata arundinaceae*) panicles at milky stage
- **S-4** Non-host plant (Pepper, *Piper nigrum*, Mango, *Mangifera indica*, Erabadu, *Erithrina variegata*) leaves (no panicles are present in these plants)
- **S-5** Males and females of *L oratorius*

Samples of plant material, **S 1-4**, were collected from the District of Kurunegala (Sri Lanka) during 1992 to 1994 They were collected between 6 00-7 00 A M and kept at 0 °C during transportation (2 - 4 h) to the laboratory to minimize the loss of volatiles For **S -5**, insects were collected from Kurunegala District and were raised in the laboratory at 25 ± 2 °C The insects were fed with sugar solution and water





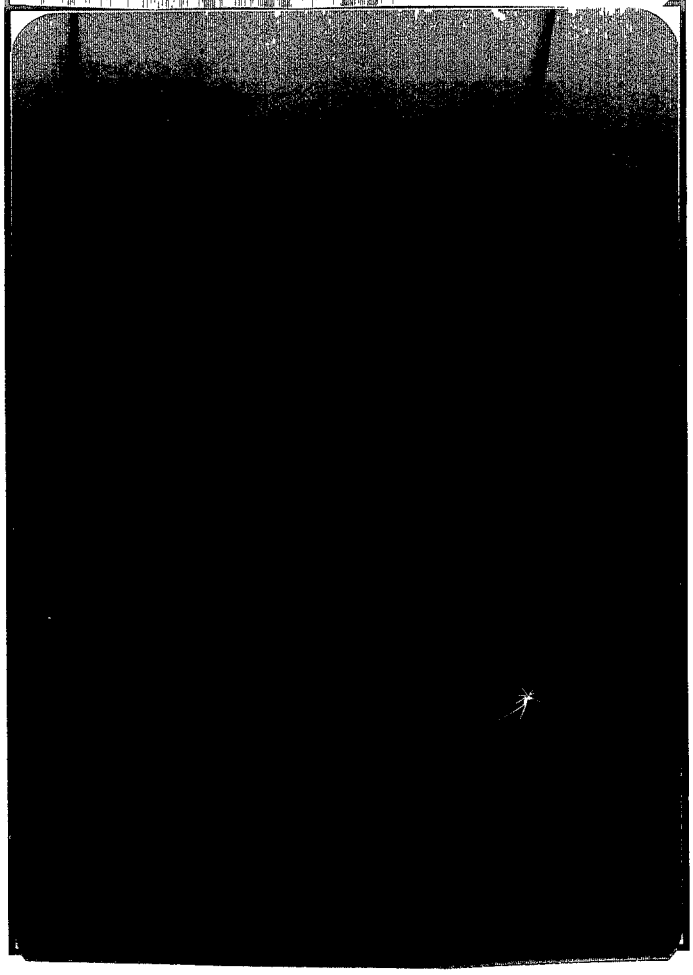


Figure 4 Rice panicles at flowering (a), milky (b), and mature (c) stages

5 1 2 Trapping Volatiles

Using milky stage rice grains as the source of volatiles, the efficacy of three methods generally used for volatile trapping was evaluated For all experiments oven-dried glassware, cleaned by washing with chromic acid, soap water, acetone and distilled water, were utilized For all extractions, unless otherwise stated, analytical grade solvents (BDH, England) were used

5 1 2 1 Volatile Trapping using Porapak Q and Super Q as Adsorbents

Volatile organic compounds of milky-stage-rice panicles (**S-1** milk-stage rice panicles) were trapped onto Porapak Q and Super Q adsorbents, separately, by the procedure described below A batch of milky-stage rice panicles (1 kg) was aerated for 72 h (3 days) with an air flow of 2 L/min Adsorbed volatiles were extracted using either pentane or ether, and the extracts were concentrated to 2 mL Concentrated extracts were stored at -5 °C for subsequent GC and EAG analyses

Using Porapak Q as adsorbent Porapak Q (10 0 g, ethylvinylbenzene-divinylbenzene copolymer, 80/100 mesh, Supelco, Inc Bellefonte, PA) was preconditioned by heating at 150 °C for 24 h, followed by Soxhlet extraction with diethyl ether for 24 to 48 h periods until the solvent was shown by GC analysis to be free of contaminants The preconditioned material was stored in a dark glass bottle and kept in a desiccator until use

Volatiles were captured on preconditioned Porapak Q following the method described by Byrne et al (1975) Plant materials or insects were placed in a 5 L three-necked round bottom flask (Figure 5) One neck of the flask was connected to a Porapak Q (1 0 g) filled glass tube, while the inlet neck was attached to an activated-charcoal (15 g) filled filter (both traps 1 0 cm ID x 12 cm) The charcoal-filled trap served as an air purifier Air was drawn using a water aspirator, at a rate of 2 0 L/ min through a water trap and a scrubber, and then over the plant material/insects, and through the Porapak Q filter

After aeration, the adsorbed compounds were eluted from Porapak Q (1 0 g) by extracting with pentane (40 mL) in a Soxhlet extractor for 5 h The pentane extract was concentrated to 2 ml by evaporation of the solvent through a glass Dufton column (30 cm) at atmospheric pressure The sample was stored at -5 °C

Using Super Q as adsorbent Super Q (5 0 g, Ethylvinylbenzene-divinylbenzene copolymer 80/100 mesh, Alltech Associates, Inc , IL) was conditioned (Oehlschlager et al , 1992, Bartelt et al ,1994) by washing with 50 0 mL of distilled ether Subsequently the material was heated in an oven for 3 hr at 150 °C and stored in a dark-glass bottle and kept in a desiccator

Volatiles were captured on Super Q adsorbent in a similar manner as described for Porapak Q After aeration, the adsorbed compounds were eluted by passing distilled ether (3 mL) through the Super Q packed tube

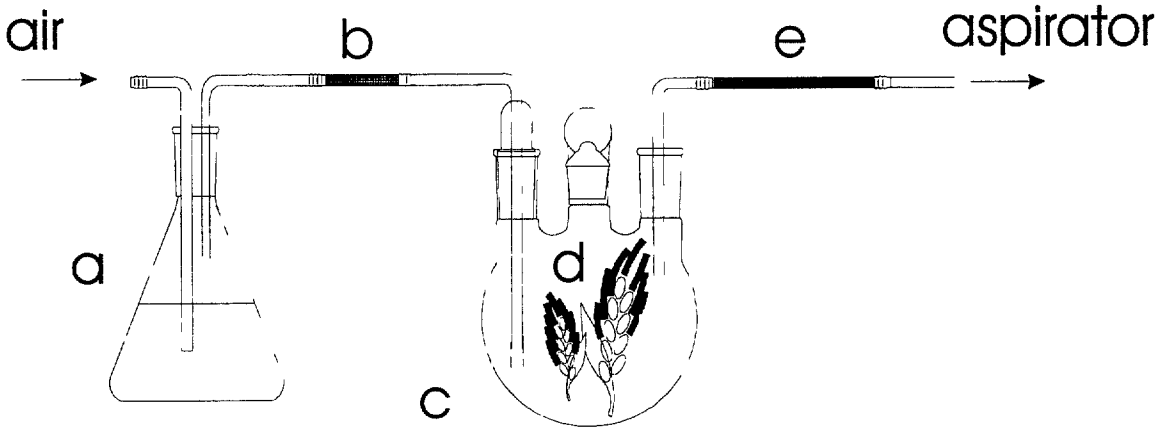


Figure 5 Apparatus used for aeration of biological samples Volatile emitting material (live insects, or plants) was kept in chamber C (3L - 5L), and a water aspirator was used to draw charcoal-filtered air through the Porapak Q/Super Q filter (e) in which the volatiles were trapped [a - air moisturizer, b - charcoal trap (air scrubber), c - chamber for the volatile emitting material, d - volatile emitting material (plant or insects), e - Super Q or Porapak Q trap]

5 1 2 2 Volatile trapping in an open capillary tube

Volatile organic compounds present in the sample of S-1 milky grains were trapped on an open capillary tube trap using the headspace analytical method described by Grob and Habich (1985) Apparatus used for collecting volatiles by this method is shown in Figure 6 Chopped rice panicles (100 g) were placed in a flask (Figure 6, c) at 60 °C and allowed to equilibrate for 3 min Charcoal-purified air was drawn over rice panicles in flask (c) and the volatiles were trapped in the capillary trap [HP-1, methyl-silicon-coated capillary column (90 cm x 0 53 mm ID, 2 65 μm film thickness)] (e), for three hours The trapped volatiles were extracted by flushing the capillary trap with a small amount of diethyl ether and was stored at -50 °C until further use

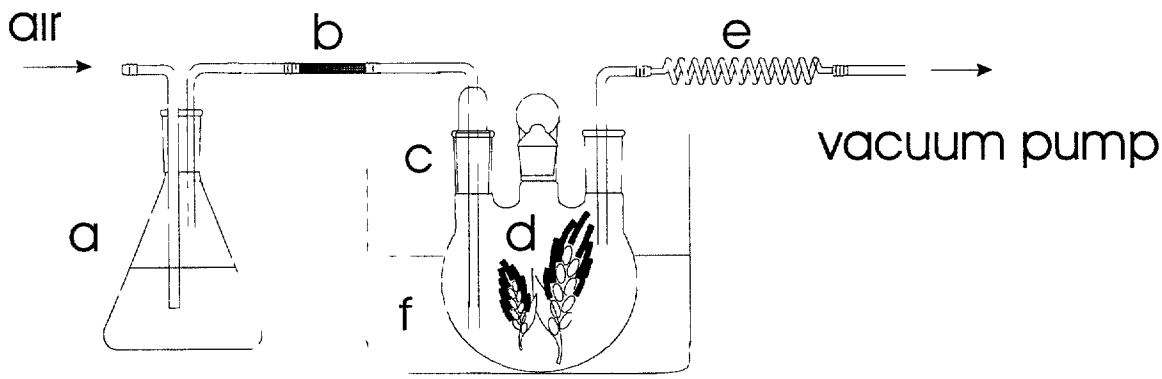


Figure 6 Set up for trapping headspace volatiles in an open capillary tube (a- water trap, b - charcoal filter, c - chamber for host plants, d - plant material, e - open capillary tube, f - water bath)

5 1 2 3 Collection of volatiles by steam distillation

Plant materials (1 kg) were subjected to steam distillation (4 h), and the condensate was collected at a rate of 50 mL/hour (Figure 7) The resulting aqueous solution was saturated with NaCl and extracted with diethyl ether (50 mL x 3) The ether phase was dried over anhydrous $MgSO_4$ and concentrated to 2 mL The required amounts for bioassay, EAG and GC experiments were withdrawn from this solution

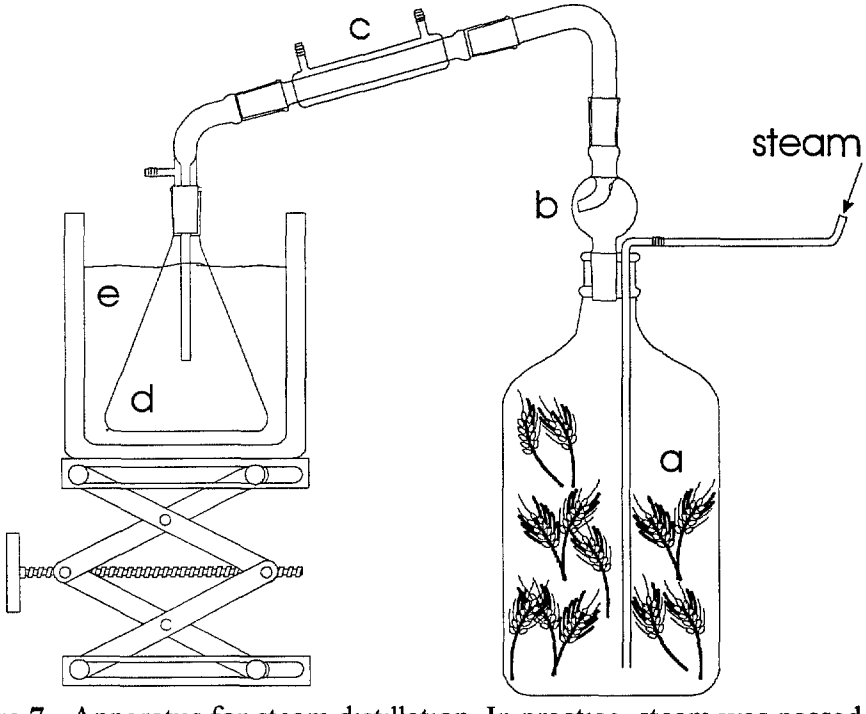


Figure 7 Apparatus for steam distillation In practice, steam was passed through the plant materials, and the plant volatiles together with water was collected as the distillate (a - chamber for the host plant, b - steam head, c - condenser, d - collecting flask, e - ice bath)

5 1 3 Collection of Test Samples

5 1 3 1 Steam distillation of host plants (rice, *Orisa sativa*)

According to the procedure described in Section 5 1 2 3, 1 0 kg samples of host plant material (sample **S-1**, green leaves, hay, rice panicles at flowering, milky and mature stages) were subjected to steam distillation

For comparison purposes, 1 0 kg of different varieties of host plant (sample S-2, BG 94-1, BG 1492, BG 303 and AT 303) were also subjected to steam distillation Each extract was concentrated to 2 0 mL and stored at -5 °C for GC, EAG, GC-EAD experiments and laboratory bioassays

5 1 3 2 Steam distillation of alternative host (Family, Graminae) and non-host plants

Samples **S-3** and **S-4** (Section 5 1 1) (1 0 kg each) were also subjected to steam distillation as described in Section 5 1 2 3 The ether extracts were concentrated to 2 mL and stored at -5 °C until further use

5 1 4 Gas chromatographic analysis of volatiles

Gas chromatographic (GC) analyses were performed using a Hewlett Packard 5890 Series II chromatograph equipped with a DB-5 stationary phase coated capillary column (30 m x 0 25 mm ID, 0 25 μm film thickness, J & W Scientific, California) Helium was used as the carrier gas at a flow rate of 1 mL/min, and nitrogen was used as the make-up gas for the detector at a rate of 30 mL/min

5 1 4 1 Comparative analysis of rice volatiles

In order to select the best method for the collection of rice volatiles, a comparative chemical analysis of the above samples was carried out by gas chromatography using a temperature program of 5 min at 40 °C, from 40 °C to 170 °C (at a rate of 5 °C/min), from 170 °C to 250 °C (at a rate of 20 °C/min), and finally 10 min at 250 °C

5 1 5 Evaluation of samples by behavioral bioassays

Behavioral bioassays were conducted using a Y-shaped olfactometer modified to satisfy our requirements (Gunawardena et al , 1989) (Figure 8) Air was passed through arms A and B of the olfactometer by an air pump adjusted to maintain an air displacement rate of 0 2-0 4 L/min Each sample under investigation was placed in arm A, while control samples were kept in arm B of the Y-tube Samples were prepared by applying an appropriate amount of test substances in diethyl ether to a plug of cotton wool and allowing the solvent to evaporate Controls were prepared by applying diethyl ether only Immediately prior to the experiment, the air circulation was stopped for a short time, and the insect chamber containing about 10 insects was fitted in to arm C of the Y-tube After a few minutes, the air circulation was re-commenced through the system

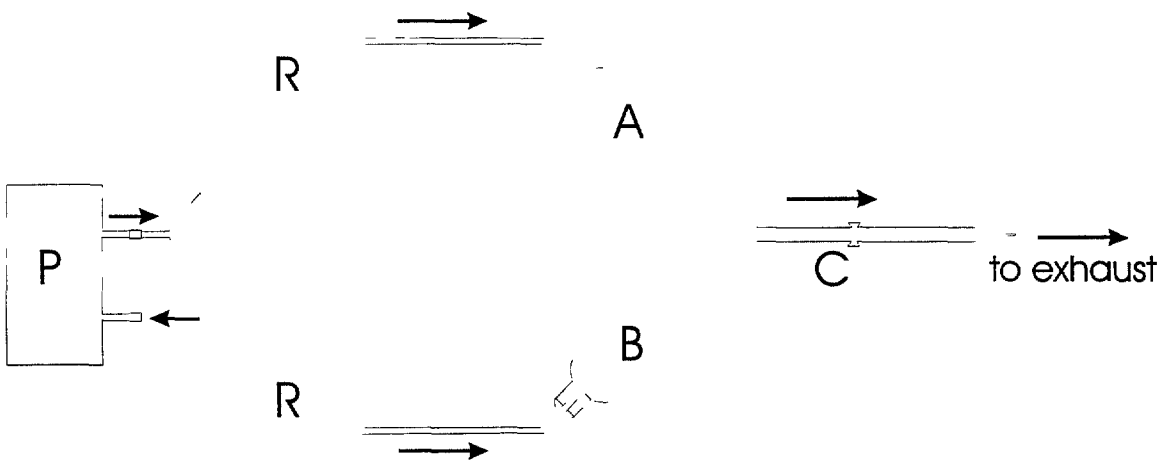


Figure 8 Y-shaped olfactometer used for bioassay experiments (A - arm for test material, B - arm for control material, C - insect chamber, P - air pump R - rubber tube)

The ability to select the baited arm, and other behavioral responses of insects moving against the direction of the air flow, were noted Each bait was tested with 5 to 6 batches of insects In general, the test insects were not used repeatedly A pre-run of each bioassay was

carried out to ensure equal conditioning of each arm of the Y-tube All parts of the apparatus were cleaned by washing and blowing air for 15 min after each bioassay

5 1 5 1 Behavioral bioassays with host volatiles

Behavioral bioassay experiments were performed to evaluate the activities elicited by extracts of the steam-distillate of milky rice samples (Section 5 1 3 1)

Dose response curve Eight doses (0 10, 0 20, 0 30, 0 50, 0 75, 1 00, 1 2, 1 5 mL) of the ether extract of steam-distillate of milky-stage-rice (Section 5 1 3 1) were tested for activity using males and females of *L oratorius* in the Y-tube olfactometer as described in section 5 1 5 Experiments were conducted between 7 00-8 00 h and 16 00-18 00 h

5 1 5 2 Behavioral bioassays with volatiles of different varieties of rice

Following the procedure described in section 5 1 5, behavioral bioassays were carried out using 0 75 mL of the ether extract of steam-distillates of different varieties of rice, BG 94-1, BG 1492, BG 303 and AT 303 (Section 5 1 3 1) grown in Sri Lanka

5 1 6 Electroantennogram (EAG) assay

According to the procedure described by Schneider (1957), an antenna of *L oratorius* was excised immediately below the joint between 1st and the 2nd segments The tip of the isolated antenna was cut off and the antenna was fixed between two glass capillary tubes filled with Ringer's solution Two gold wires were placed in the Ringer's solution for electrical contact In this way the base of the antenna was attached to the indifferent electrode and the open end (4th segment) of the antenna was connected to the recording electrode which was connected to a high impedance amplifier (Bjostad, 1988) (Figure 9) Total time required for mounting the antenna did not exceed 5 min

Chemical Stimuli were delivered to the antenna by blowing air over filter paper strips loaded with appropriate material The filter paper strip (3 x 30 mm) was placed inside glass Pasteur pipette (5 mm ID x 100 mm) connected to a syringe that contained 1 mL of air By lowering the plunger of the syringe, a puff of 1 mL of air was injected over the filter paper strip into a slow stream air (0 3 mL/min) which was passed continuously over the antennal preparation A vacuum pipe placed behind the antennal setup continuously removed air surrounding the antennal area An interval of at least 15 sec was maintained between each recording to allow adequate receptor recovery The EAG amplitudes acquired were stored in microcomputer, and changes in response were measured in millimeters An external source of 1 mV was used to calibrate the system in order to convert EAG amplitudes to millivolts The EAG amplitude evoked by a reference substance was measured in between those of the test compounds in order to make corrections for antennal fatigue

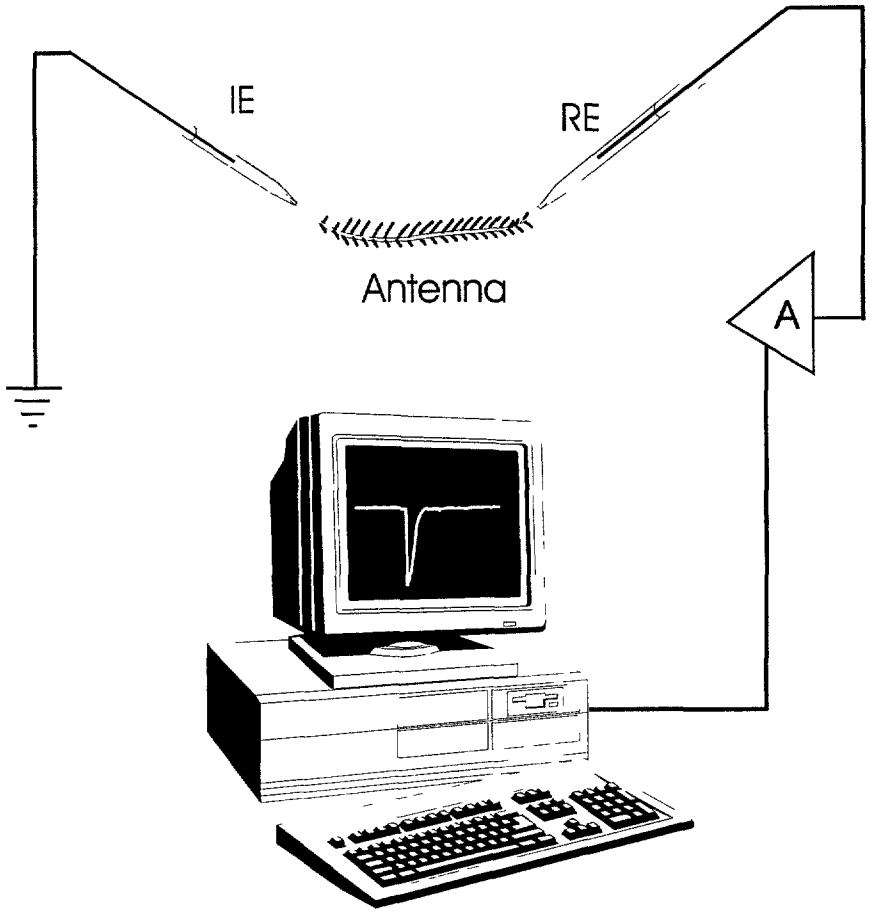


Figure 9 Setup used for recording electroantennograms (EAGs) IE - indifferent electrode, RE - recording electrode

5 1 6 1 EAG assay of host volatiles

Following the EAG procedure described above, the EAG amplitudes evoked by 100 µL doses of each extract of steam distillate of milky rice (Section 5 1 3 1), and Super Q or Porapak Q trapped rice volatile extracts (Section 5 1 2 1) were measured Each test stimulant was assayed using both male and female antennae

Dose response curve Seven doses (1, 5, 10, 50, 100, 200, 500 µL) of the extract of the steam distillate of milky rice samples (Section 5 1 3 1) were tested with male and female *L oratorius* antennae In order to maintain a constant volume of sample loading, each dose was made to a total volume of 100 µL by adding ether, or by concentrating the sample appropriately Aliquots of 100 µL were applied to filter paper strips (3 x 30 mm) and placed inside glass Pasteur pipettes (5 mm ID x 100 mm) for testing by EAG

Each series of samples was tested first using antennae of six *L oratorius* males, and then the procedure was repeated using six female antennae The EAG amplitude evoked by a

reference substance was measured in between those of the test compounds in order to make corrections for antennal fatigue

5 1 6 2 EAG assay of host plant volatiles of different life stages

EAG activities elicited by extracts of steam distillates of different life stages of rice plants (green leaves, hay, rice panicles at flowering, milky and mature stages, Section 5 1 3 1) were measured by the procedure described in Section 5 1 6 Each stimulus was delivered by blowing air over 100 µL aliquots applied to a filter paper strips An external source of 1 mV was used to calibrate and convert the EAG amplitudes to millivolts

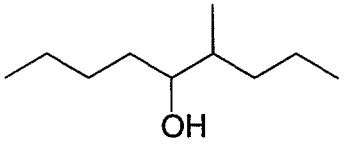
5 1 6 3 EAG assay of alternative host and non-host volatiles

Following the procedure discussed in section 5 1 6, EAGs were recorded for steam-distilled extracts of alternative host (bajiri, *Pennisetum spicatum* L, and illuk, *Imperata arundinaceae*) and nonhost (pepper, *Piper nigrum*, mango, *Mangifera indica*, and erabadu, *Erithrina variegata*) volatiles (Section 5 1 3 2) Each stimulus was delivered as 100 µL aliquots on to a filter paper strip Ether (100 µL on a filter paper strip) was used as the control and 100µL of steam-distilled extract of milky-stage-rice was used as the reference

5 1 7 Combined Gas Chromatographic and Electroantennographic (GC-EAD) Assay

GC-EAD analyses were conducted using a Hewlett Packard 5890 Series II capillary gas chromatograph (GC) fitted with flame ionization (FID) and electroantennographic detectors (EAD) (Roelofs, 1984, Arn et al , 1975, Struble, and Arn, 1984) A capillary column (30 m x 0 25 mm ID, J & W Scientific California) coated with DB-5 stationary phase (0 25µm film thickness) was used for the analyses As illustrated in Fig 10, the column effluent was split between the FID and the EAD in a ratio of 20 80 Helium was used as the carrier gas at a flow rate of 1 mL/min Nitrogen at a flow rate of 30 ml/min was introduced before the splitting device as a makeup gas to facilitate a better transfer of the GC effluent to the two detectors The high flow outlet from the splitter was passed through a heated transfer line (200 °C) to prevent any condensation of volatiles

The time for any compound eluting from the GC column to reach the FID and the antenna (EAD) was synchronized by varying appropriate parameters, such as flow rates and length of capillaries etc, by an experiment using an antenna of *Rhynchophorus ferrugineus* (Coleoptera Curculionidae) (Figure 11) The antennae of *R ferrugineus* respond well to ferrugineol, its aggregation pheromone (Hallett et al , 1993)



ferrugineol

An antenna of the *R ferrugineus* was cut close to its base as possible, and mounted immediately in the EAD setup The recording electrode was inserted into the antennal cone and the indifferent electrode to the base of the antenna as described in the previous section In this way, the optimal distance for an antennal preparation to be placed was estimated to be ~2 mm away from the GC-outlet The total time required for mounting the antenna did not exceed 5 min

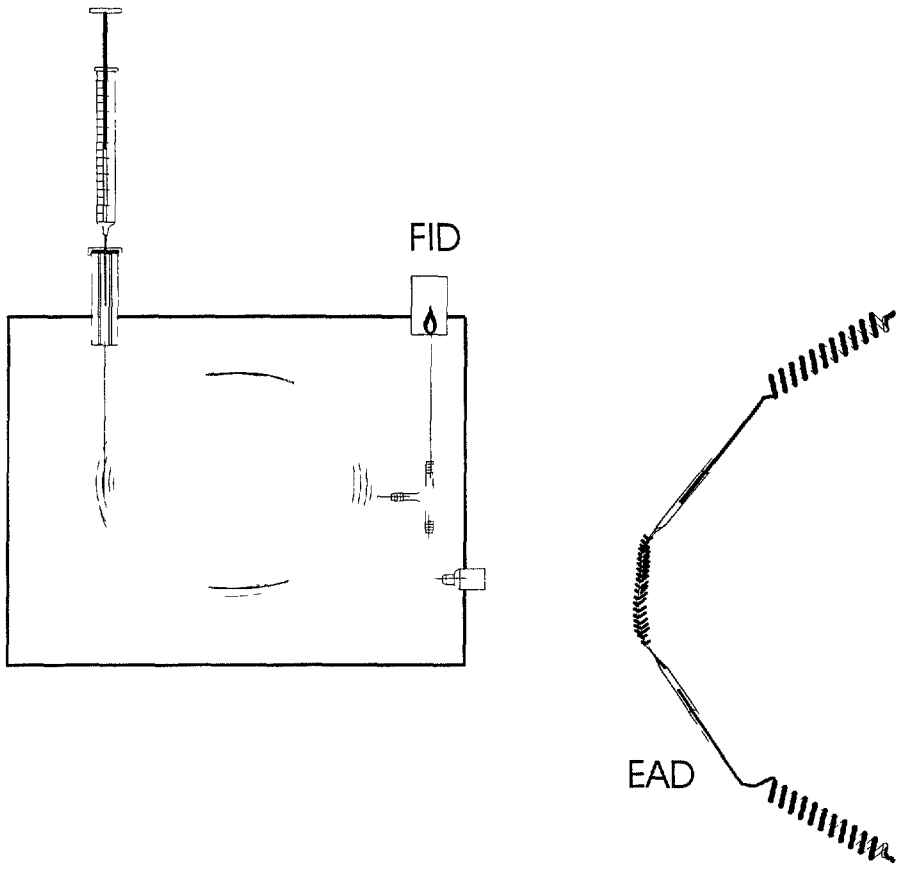


Figure 10 Set up for simultaneous recording of flame ionization and electroantennographic signals FID - flame ionization detector, EAD electroantennographic detector

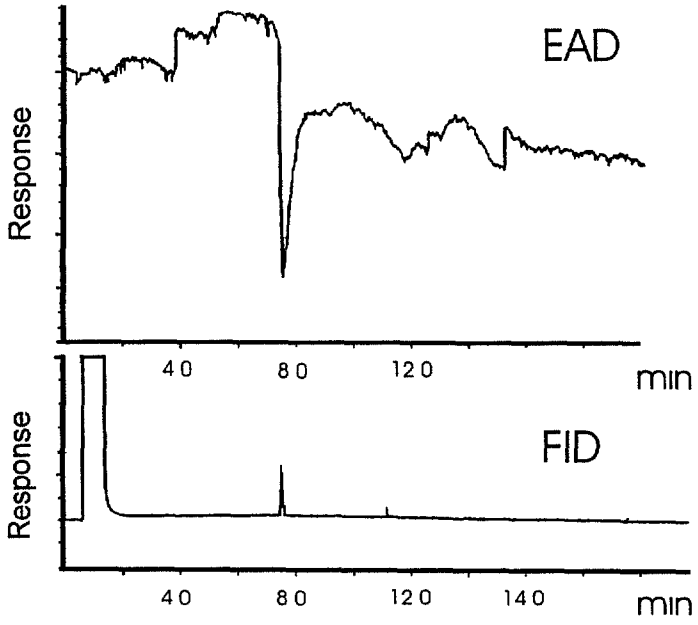
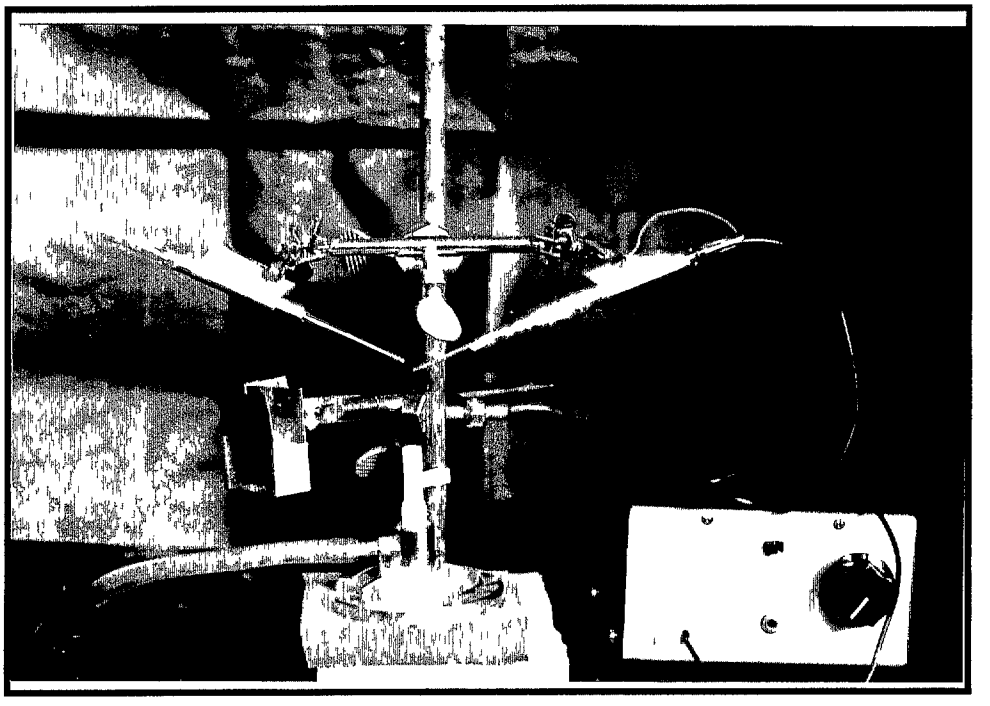
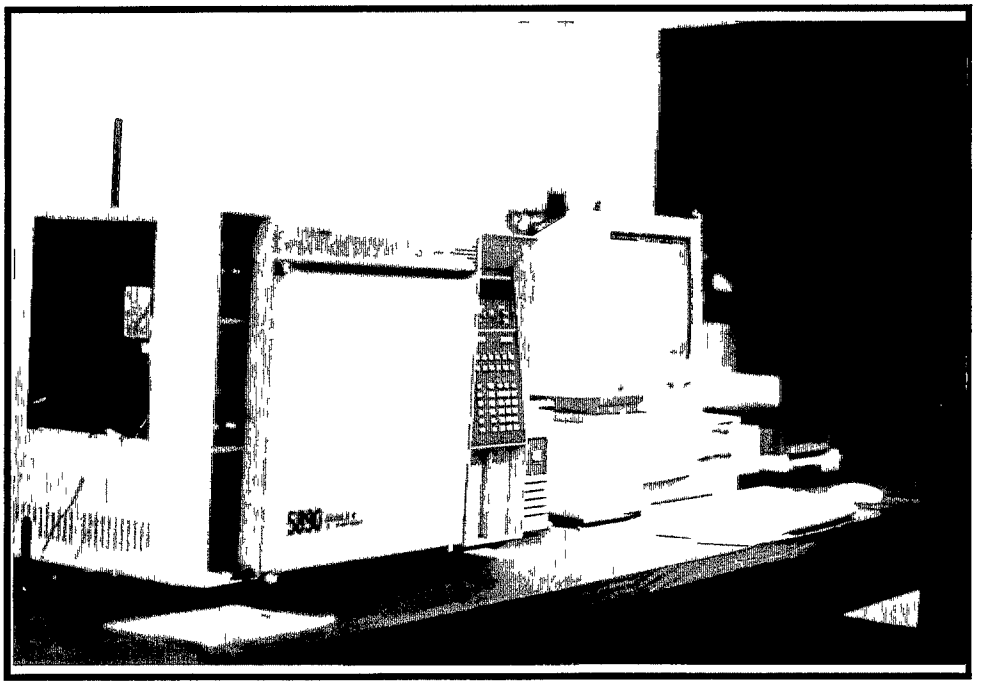


Figure 11 GC-EAD response of *R ferrugineus* antenna to ferrugineol EAD - electroantennographic detector signal, FID - flame ionization detector signal



A



B

Figure 12 EAG setup (**A**) and GC-EAD apparatus (**B**) at the Department of Chemistry, University of Kelaniya The GC oven temperature program started at 40 °C for 5 min, increased to 170 °C at a rate of 5 °C/min, them from 170 °C to 250 °C at a rate of 20 °C/min, and finally the temperature was held at 250 °C 10 min The column parameters are those described in Section 5 1 7 The most appropriate distance for *L oratorius* antennal mounting was 3 mm away from the GC outlet

5 1 7 1 GC-EAD assay of host volatiles (rice)

An extract of the steam distillate of milky stage rice (sample described in Section 5 1 3 1) was concentrated 10 times and assayed by the GC-EAD procedure using an antenna of *L oratorius* as described in Section 5 1 7

5 1 8 Chemical identification of attractants

5 1 8 1 Micro-chemical reactions

An extract of the steam distillate of milky stage rice obtained according to the procedures described in Section 5 1 3 1 was subjected to the following microreactions The EAG activity evoked by samples before and after the chemical treatment was estimated

Bromination A sample of the extract of steam distillate of milky stage rice (100 μL) was placed in a glass vial (5 mL) was treated with a mixture of Br_2/H_2O until the brown color persisted The resulting mixture was applied to a filter paper strip and tested by EAG The untreated extract (100 μL) was used as the standard, and a mixture of Br_2/H_2O (500 μL) was used as the blank

Acetylation An extract of the steam distillate of milky stage rice (100 μL) in a glass vial (5 mL) with a Teflon-lined cap was treated with excess acetyl chloride (300 μL) After allowing to react for 45 min, distilled water (200 μL) and ether (1 mL) were added After the reaction mixture was neutralized with Na_2CO_3, the organic layer was extracted and the extract was applied to a filter paper strip for EAG assay To estimate the change of activity, an untreated sample was prepared without the addition of acetyl chloride, and a control was made without the addition of the extract of the steam distillate of milky stage rice EAG recordings were made for the acetylated, untreated and control samples as described in Section 5 1 6

Hydrolysis KOH in methanol (150 μL) was added to a sample of the extract of the steam distillate of milky stage (100 μL) and the mixture was heated at 100 ^{0}C for 20 min After cooling, the solution was transferred to a filter paper strip for EAG assay For the comparison of change of EAG activity, a sample of the extract of the steam distillate of milky stage (100 μL) mixed with methanol (150 μL) was used as the reference standard, while a mixture of ether (100 μL) and KOH in methanol (150 μL) was used as a control

1,1-Dimethylhydrazone preparation An EAG-active sample of the extract of the steam distillate of milky stage (100 μL) in glass vial (5 mL) was treated with 100 μL 1,1-dimethylhydrazine solution (30% in hexane) and allowed to stand for 5 min The reaction mixture was exposed to air for a few minutes to allow excess 1,1-dimethylhydrazine to evaporate, concentrated to 100 μL, and applied to a filter paper strip for EAG assay The untreated extract (100 μL) was used as the standard, and 100 μL of hexane treated with 1,1-dimethylhydrazine solution (30% in hexane) as a control

5 1 8 2 GC and GC-MS analyses

Samples were analyzed by GC-MS using a Hewlett Packard 5890 Series II gas chromatograph coupled to a Hewlett Packard 5970 Series mass spectrometer operated in electron-ionization mode at 70 eV A DB-5 stationary phase coated (0 25 μm film thickness) capillary column (30 m x 0 25 mm ID, J & W Scientific, California) was used for the analyses The temperature of the GC oven was held at 40 °C for 5 min and increased to 170 °C at a rate of 5 °C/min From 170 °C to 250 °C, the oven temperature was increased at a rate of 20 °C/min and held at 250 °C 10 min

Six constituents, named as A, B, C, D, E, F, and G, were recognized by GC-EAD experiments as compounds that may act as attractants to *L oratorius* The mass spsectra of these compounds were obtained by GC-MS In order to characterize these compounds, authentic samples of candidate compounds were subjected to the GC and GC-MS analyses under identical conditions

5 1 9 Further Studies on the host attractants

5 1 9 1 Determination of concentrations of active constituents

According to the procedure described in Section 5 1 3 1, 1 0 kg each plant material (sample S-1, green leaves, rice panicles at flowering, milky and mature stages), alternative host (sample S-3) and non hosts (sample S-4) were subjected to the steam distillation, and after extraction each sample was concentrated to 0 25 mL The concentration of each constituent, A, B, C, E, F and H, was estimated by comparing GC peak areas with those obtained from tridecane used an internal standard

5 1 9 2 EAG and Behavioral bioassays of synthetic samples of active constituents

EAG assays According to the procedure described in Section 5 1 6, EAG responses evoked by synthetic samples of (*Z*)-2 pentenol, 3-octanol, octanal, linalool, nonanal, decanal, β-cyclocitral (Sigma) and undecanal were recorded Samples (100 μL) were loaded to filter paper strips from standard solutions of 1 mg/mL in hexane Ether (100 μL) and hexane (100 μL) applied to filter paper strips served as controls

Behavioral Bioassays Behavioral bioassays were carried out using a mixture of decanal and nonanal (100 μg each, 1 1, w/w), the ether extract of rice steam distillate (100 μL), and a synthetic mixture (100 μg) of the major constituents [(*Z*)-2-pentenol, nonanal, octanal and decanal] admixed in proportions similar to those found in the milky stage rice volatiles Further experiments were carried out by adding the trace constituents linalool (2 μg) and 3-octanol (2 μg) to the above five-component mixtures Since (*Z*)-2-pentenol was found only in the milky stage rice volatiles, it was bioassayed as an individual component (17 μg)

5 2 RESULTS

5 2 1 Gas chromatographic analysis of volatiles

Comparative GC analysis of milky-stage-rice volatiles from Porapak Q Super Q, open-capillary tube, and steam distillation isolation methods GC analysis of the solvent extract of milk-stage-rice volatiles adsorbed on Porapak Q showed approximately 10 chromatographic peaks under the chromatographic conditions mentioned in Section 5 1 4 1 (Figure 13) Under the same conditions, about 20 chromatographic peaks were observed for that adsorbed on Super Q [Figure 14], while over 50 peaks were observed for that obtained from steam distillation [Figure 15] In contrast, the sample obtained from trapping volatiles on an open capillary tube trap using the headspace analytical method showed no peaks Therefore, we concluded that the steam distillation method is the best technique for sample preparation

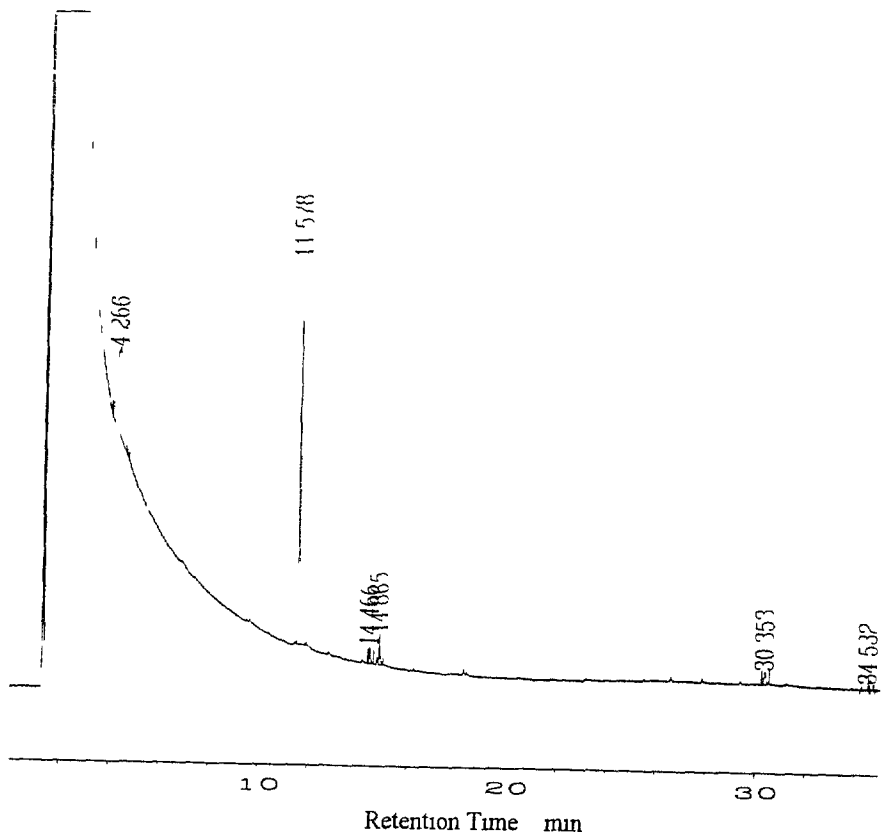


Figure 13 A gas chromatogram obtained from volatiles of milky-stage-rice panicles trapped on Porapak Q

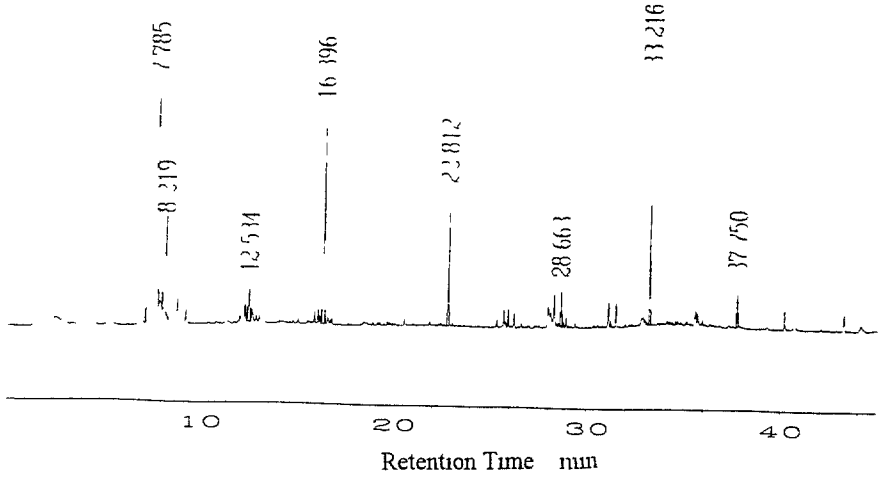


Figure 14 A gas chromatogram obtained from volatiles of milky-stage-rice panicles trapped on Super Q

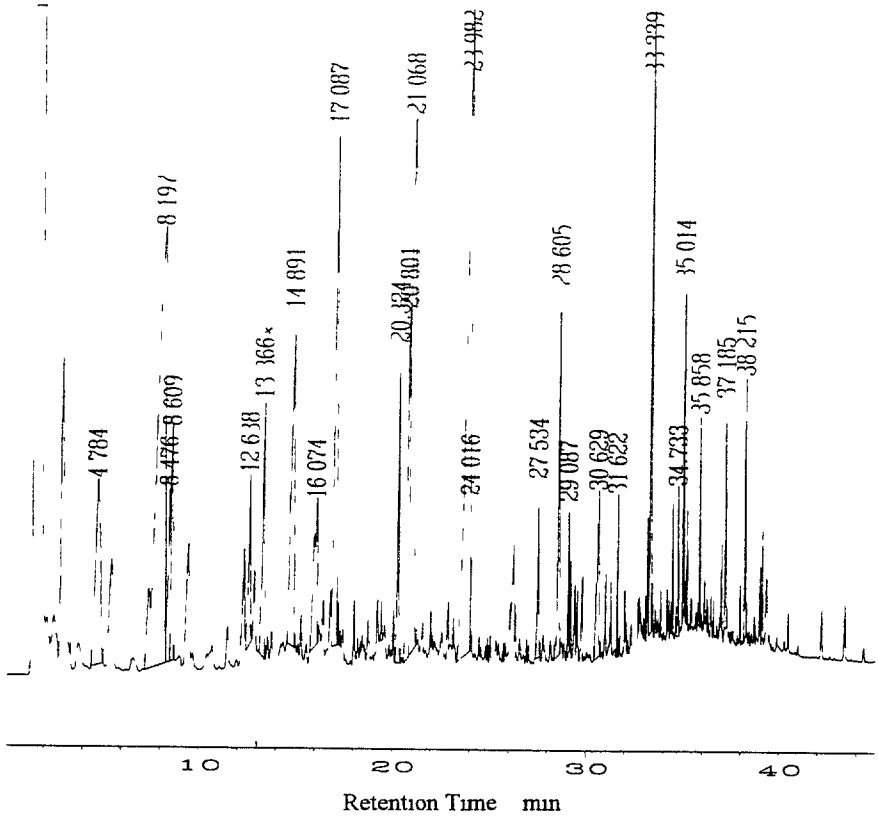


Figure 15 A gas chromatogram obtained from volatiles of milky-stage-rice panicles obtained by steam distillation

5 2 2 Evaluation of samples by behavioral bioassay

A behavioral bioassays based on a Y-shaped olfactometer was used to evaluate the efficacy of samples to attract *L oratorius* in the laboratory The ability of insects to select the baited arm was counted as a positive response

5 2 2 1 Olfactometric estimation activity of host volatiles

The pest attractant activity of milky-stage rice volatiles was evaluated by an olfactometric bioassay As shown in Figure 16, the activity elicited by samples to attract insects to the appropriate arm of the olfactometer baited with different amounts of test sample increased as the dose applied to cotton wool baits increased For example, the activity of about 20% insects selecting the baited arm at a dose of 0 1 mL increased to about 75 % when a dose of 0 75 mL was used Apparently, the increase of activity reaches a maximum at a sample dose of about 0 75 mL After this concentration, no significant increase of activity was observed with further increase of sample amount

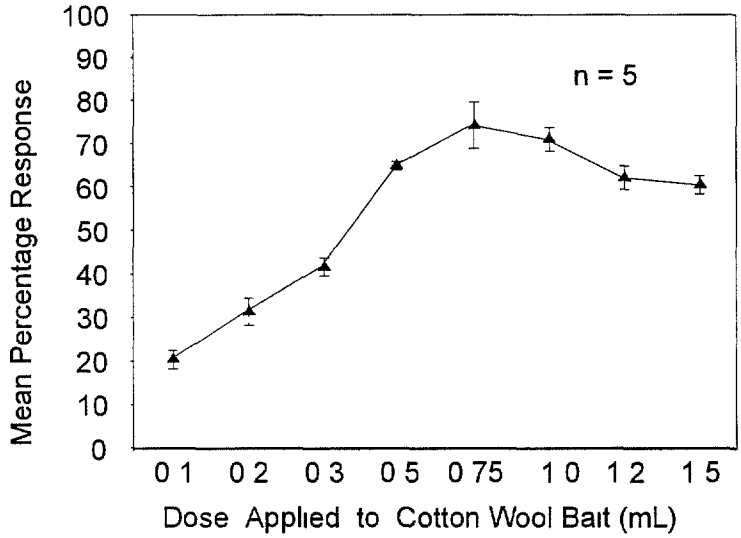


Figure 16 Dose response relationship obtained from olfactometer experiments using different amounts of the extract of steam distillate of milky-stage rice

5 2 2 2 Activity of volatiles from different varieties of rice

A slightly higher mean percentage response was observed when the activities of extracts of BG-type rice varieties (94/1, 1492 and 303) were compared to that of AT 303, however, this difference was not statistically significant (Figure 17)

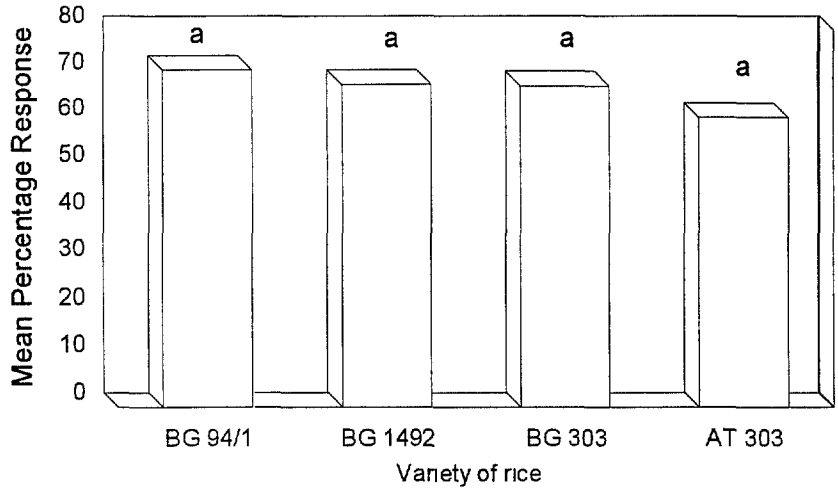


Figure 17 Behavioral response of *L oratorius* to extracts of different varieties of milky-stage-rice [0 75 mL of the concentrated steam-distilled-extracts (Section 5 1 3 1) were used as the bait Bars headed by same letters are not significantly different, one way ANOVA & Scheffers test p<0 05]

5 2 3 Evaluation of samples by electroantennogram assay

5 2 3 1 Electroantennogram assay of host volatiles

EAG studies conducted with host volatile samples prepared by two different techniques showed remarkable differences in activity depending on the method of extraction For example, the extract of the steam distillate of milky-stage-rice evoked a mean EAG response of 1 02 mV whereas the volatiles trapped on Super Q elicited only a response of 0 41 mV (Table 1)

Table 1 EAG responses evoked by *L oratorius* antennae when stimulated by samples prepared by two different techniques

Stimulus[a]	Mean EAG response (mV) ± S D[b]
Extract of steam distillate of milky-stage rice panicles	1 02 ± 0 08
Extract of Super Q trapped volatiles	0 41 ± 0 05

[a]Samples (100 µL) prepared as described in Sections 5 1 3 1 and 5 1 2 1 were used
[b]The EAG values obtained for the two samples are significantly different (one way ANOVA, and Scheffers test p<0 05)

An experiment using antennae obtained from males and females of *L oratorius* indicated that antennae from both sexes show similar EAG responses to host volatiles The dose response profiles obtained from antennae of males and females were similar over the dose range that was investigated (Figure 18)

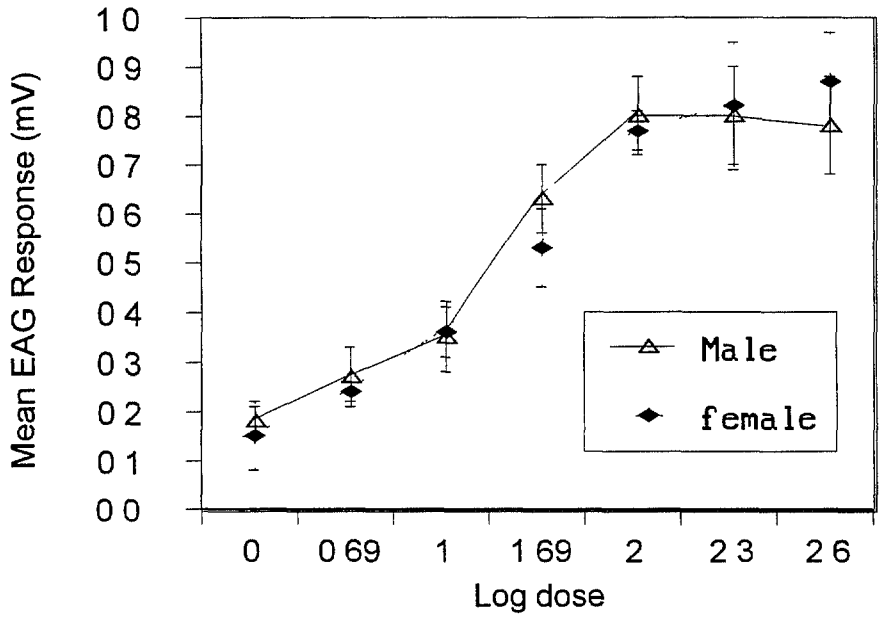


Figure 18 Dose-EAG response profiles obtained from antennae of males and females of *L oratorius* [Samples made from the concentrated extract of the steam distillate of milky-stage rice (Section 5 1 3 1) were applied to filter paper strips maintaining the total volume that was applied constant (100 µL)]

5 1 3 2 Electroantennographic assay of volatiles obtained from different parts and life stages of rice plants

Extracts of steam distillates obtained from different parts and life stages (green leaves, flowering stage, milky grain, mature grain, and yellow leaves) of rice were evaluated by electroantennography The EAG response obtained for volatiles present in milky grains was significantly higher and different from those of the rest of the samples (Figure 19) Volatiles emitted from extracts of steam distillates of flowering rice panicles evoked the second highest EAG response (0 83 mV) The EAG values for volatiles present in milky grains and that of flowering rice panicles were significantly higher than the EAG values obtained for green leaves, mature grains and yellow leaves of the rice plants

5 1 3 3 Electroantennographic assay of alternative host and non-host steam volatiles

Among all the samples tested, the volatiles present in extracts of steam distillates of milky grains evoked the highest EAG amplitude (1 004 mV) Samples obtained from bajiri and illuk the alternative hosts, also showed relatively high EAG values (0 79 mV & 0 8 mV, respectively) On the other hand, volatiles from steam distillates of leaves of pepper, mango and erabadu, the non-host plants, showed the lowest EAG responses (0 49 mV, 0 41 mV and 0 20 mV, respectively) (Figure 20)

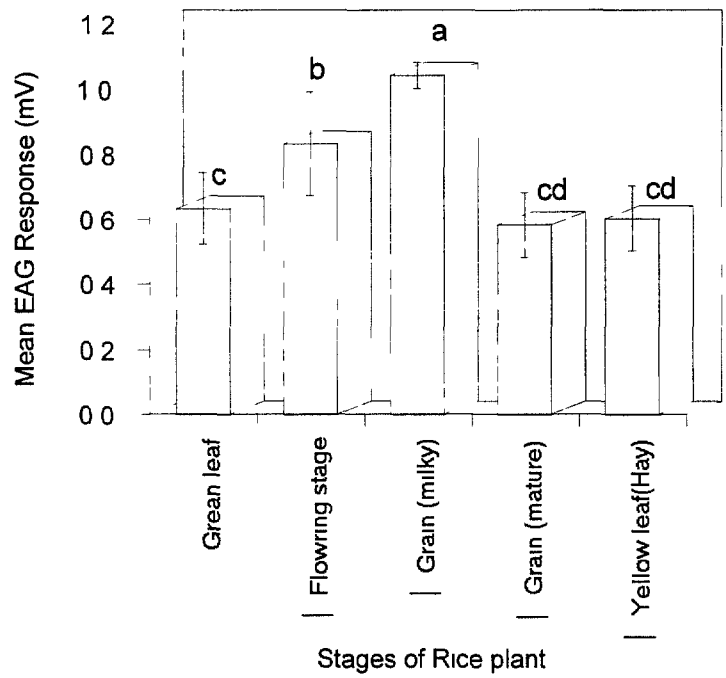


Figure 19 Mean EAG responses (mV) of *L oratorius* to extracts of steam distillates obtained from different parts and life stages of rice plants Samples made from the concentrated extracts of the steam distillates (Section 5 1 3 1) were applied to filter paper strips as 100 μL aliquots Bars labeled with the same letter are not significantly different (one way ANOVA & Scheffers test, p<0 05)

5 2 4 GC-EAD assay of rice volatiles

Figure 21 illustrates GC-EAD and GC-FID responses obtained from extracts of steam distillate of milky stage rice The profile obtained from the GC-EAD assay of milky-stage-rice steam distillate showed five significant responses from antennae of males and females of *L oratorius* The experiments were repeated several times, and the EAD responses obtained were reproducible The EAD responses could be correlated with major GC peaks that eluted at the retention times, 4 69 (A), 13 24 (C), 16 97 (E), 20 19 (F) and 23 11 min (H) (Figure 21) In addition, some minor GC peaks (B, D, G) were also recognized as significant for olfaction since these peaks correlated to significant responses in the GC-EAD trace Significant EAG responses are marked with asterisks and corresponding FID peaks are marked with letters A to H in Figure 21

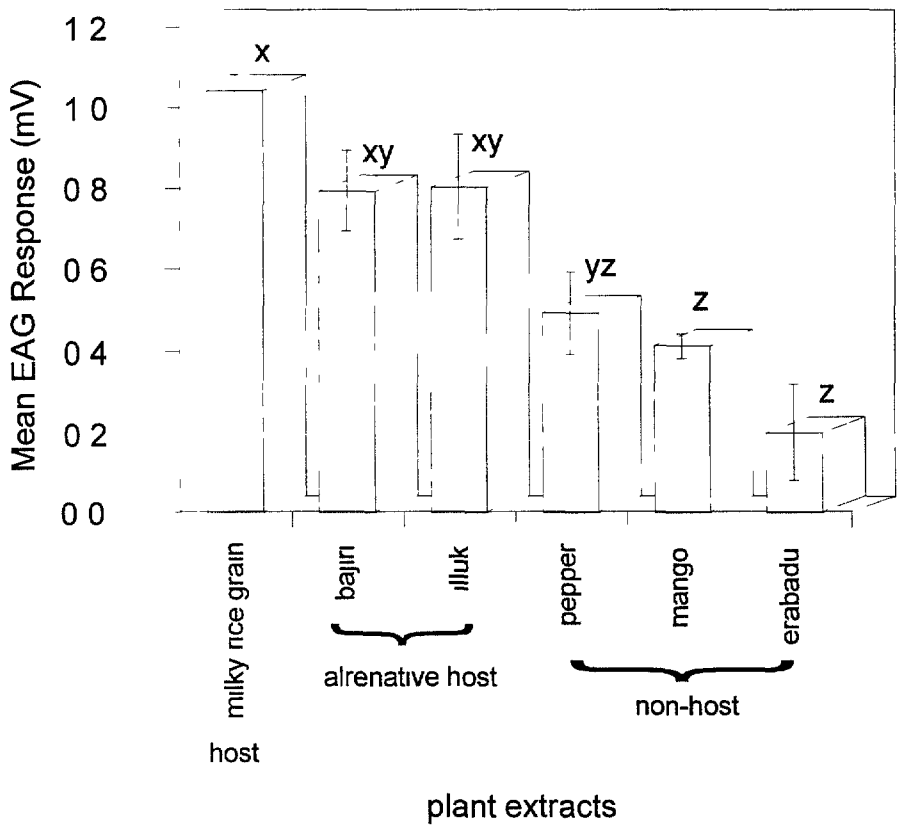


Figure 20 Mean EAG responses (mV) of *L oratorius* antennae evoked by host, alternative host, and non-host plant extracts Samples made from the concentrated extracts of the steam distillates (Section 5 1 3 1 and 5 1 3 2) were applied to filter paper strips as 100 μL aliquots Bars labeled with the same letter are not significantly different (one way ANOVA & Scheffers test, p<0 05)

5 2 5 Chemical Identification

5 2 5 1 Micro-chemical reactions

Tables 2, 3, 4, and 5 summarize the EAG responses evoked by extracts of the steam distillates of milky rice, before and after being subjected to micro-chemical reactions, bromination, acetylation, hydrolysis and 1,1-dimethyl hydrazone formation, respectively

A moderate loss of EAG activity of the steam distillate (1 00 mV to 0 81 mV) was observed after the sample was brominated (Table 2), whereas the losses of EAG activity due to acetylation and hydrolysis were considerably less (Table 3 and 4, respectively] The most significant reduction of EAG activity (0 98 mV to 0 50 mV) was observed when the steam distillate was subjected to derivatization with 1,1-dimethylhydrazene (Table 5) The above results are indicative of the presence of carbonyl groups and probably double bonds in the chemo-receptive constituents

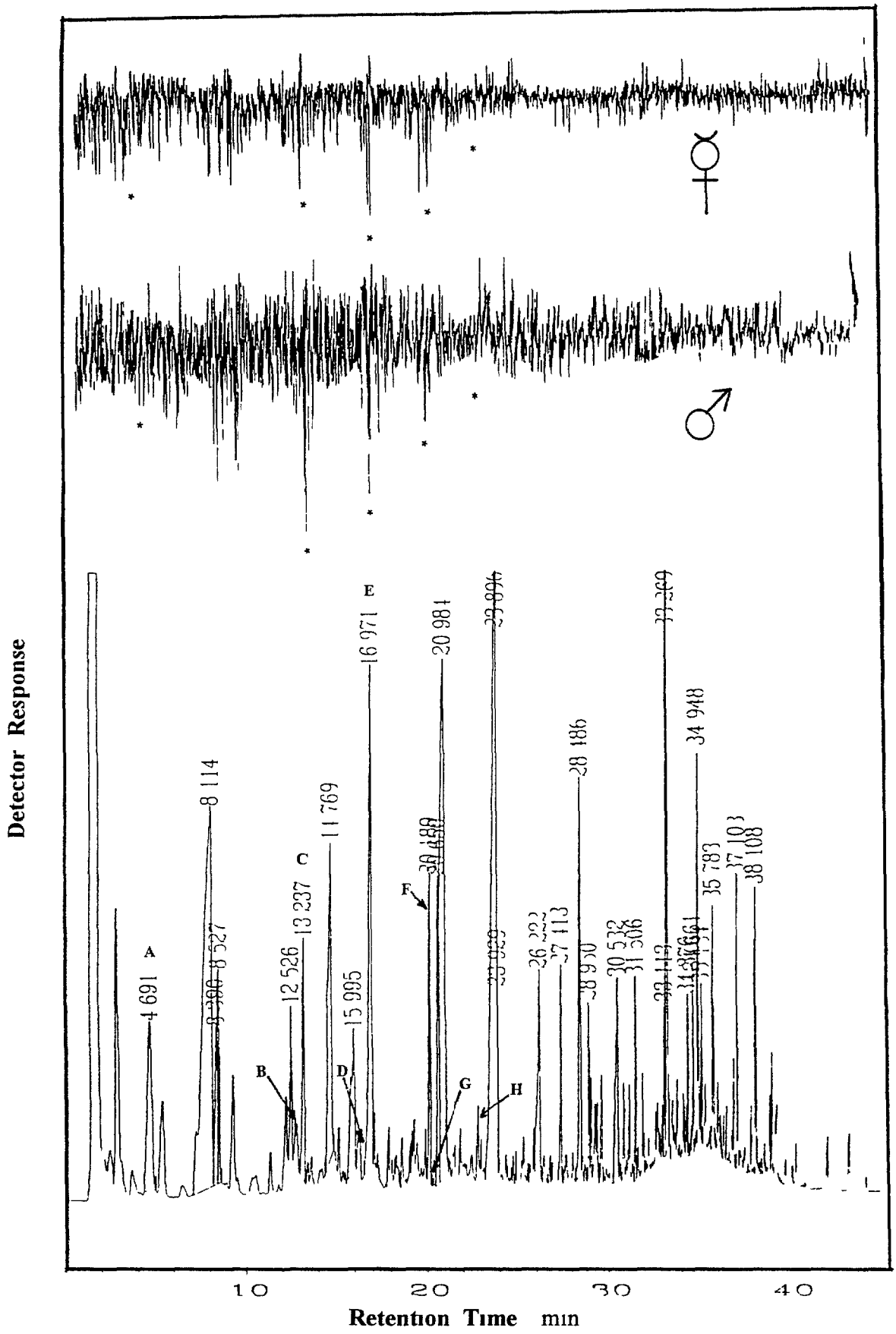


Figure 21 FID & EAD responses obtained from extracts of steam distillate of rice volatiles The antennal recording (EAD) were carried out using antennae from males and females of *L oratorius*

Table 2 EAG responses recorded from *L oratorius* antennae by stimulating with rice steam distillate, before and after bromination

Stimulus	Mean EAG response (mV) ± S D[a]	
Rice steam distillate before treatment	1 00 ± 0 04 a	difference - 0 19 mV
Brominated steam distillate	0 81 ± 0 11 a	
Control (Br_2/H_2O)	0 00 ± 0 00 b	

[a]Means followed by the same letters are not significantly different, one way ANOVA and Scheffers test $p<0\ 05$

Table 3 EAG responses recorded from *L oratorius* antennae by stimulating with rice steam distillate, before and after acetylation

Stimulus	Mean EAG response (mV) ± S D[a]	
Rice steam distillate before treatment	0 90 ± 0 05 a	difference - 0 06 mV
Acetylated steam distillate	0 84 ± 0 12 a	
Control	0 00 ± 0 00 b	

[a]Means followed by the same letters are not significantly different, one way ANOVA and Scheffers test $p<0\ 05$

Table 4 EAG responses recorded from *L oratorius* antennae by stimulating with rice steam distillate, before and after hydrolysis

Stimulus	Mean EAG response (mV) ± S D[a]	
Rice steam distillate before treatment	0 91 ± 0 04 a	difference- 0 19 mV
Hydrolysed steam distillate	0 72 ± 0 08 a	
Control	0 00 ± 0 00 b	

[a]Means followed by the same letters are not significantly different, one way ANOVA and Scheffers test $p<0\ 05$

Table 5 EAG responses recorded from *L oratorius* antennae by stimulating with rice steam distillate, before and after treating with 1,1-dimethylhydrazene

Stimulus	Mean EAG response (mV) ± S D[a]
Rice steam distillate before treatment	0 98 ± 0 09 a
Rice steam distillate after treatmenting with 1,1-dimethylhydrazene	0 50 ± 0 03 b
Control	0 00 ± 0 00 c

difference-0 48 mV

[a]Means followed by the same letters are not significantly different, one way ANOVA and Scheffers test $p<0\ 05$

5 2 5 2 GC and GC-MS analysis

The GC-EAD experiments with steam-distilled-extract indicated the presence of eight potential stimulants (Fig 21) Electron-ionization mass spectra of these components (A to H) are shown in Figures 22 - 29

By comparing the fragmentation patterns and GC retention time studies with those of the authentic samples, the compounds were identified as summarized in Table 6

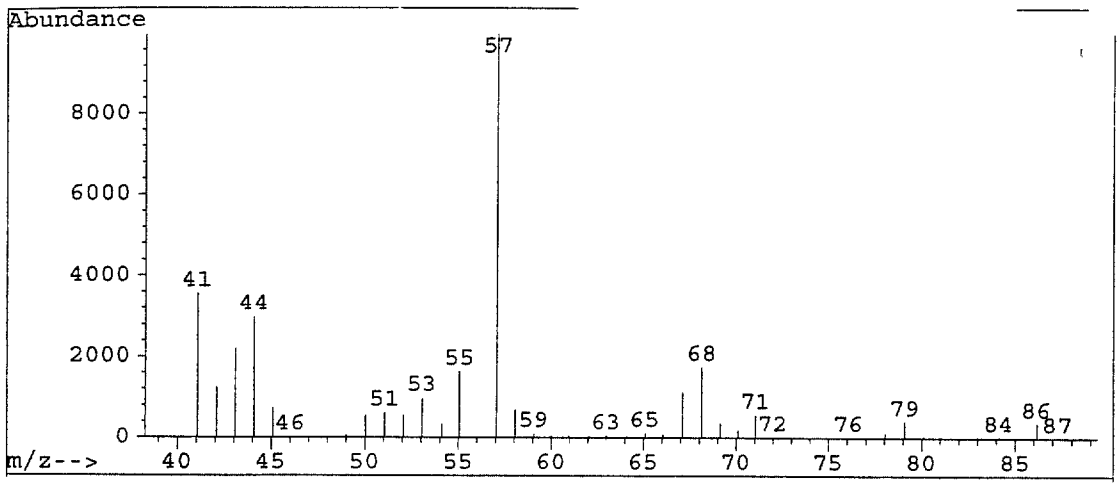
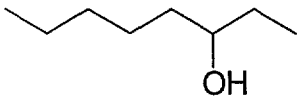
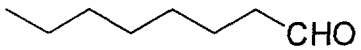
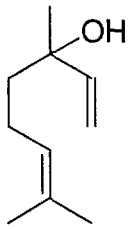
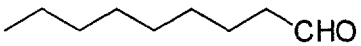
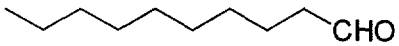
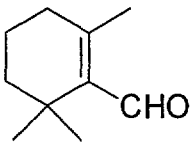
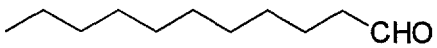


Figure 22 EI-mass spectrum of compound A present in the steam distillate of rice

Table 6 GC retention data of characterized compounds and authentic samples

	Compound	Structure	Retention time of authentic standards (min)	Retention time of rice compounds (min)
A	(Z)-2- pentenol		4 98	4 97
B	3- octanol		13 13	13 10
C	octanal		13 58	13 67
D	linalool		16 78	16 75
E	nonanal		17 27	17 26
F	decanal		20 56	20 51
G	β-cyclocitral		21 03	20 98
H	undecanal		23 20	23 17

Gas chromatographic (GC) analyses were performed using a Hewlett Packared 5890 Series II chromatograph fitted with a DB-5 stationary phase coated (0 25 μm film thickness) capillary column (30 m x 0 25 mm ID, J & W Scientific, California) and a flame ionization detector (FID) Helium was used as the carrier gas at the flow rate of 1 mL/min, and nitrogen at a flow of 30 ml/min was used as the make up gas for the detector A temperature program of 40 °C for 5 min, from 40 °C to 170 °C (at a rate of 5 °C/min), from 170 °C to 250 °C (at a rate of 20 °C/min) and 10 min at 250 °C was employed

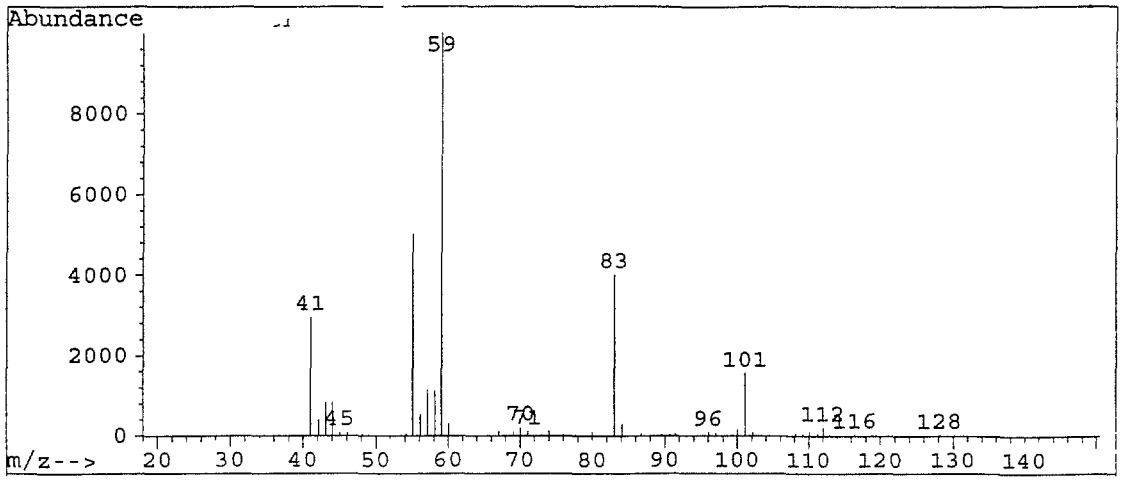


Figure 23 EI-mass spectrum of compound B present in the steam distillate of rice

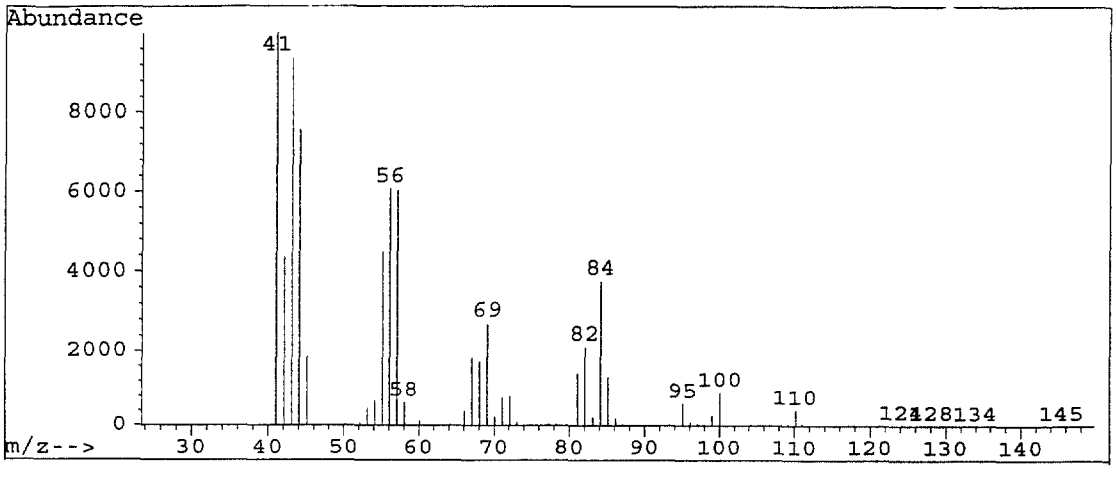


Figure 24 EI-mass spectrum of compound C present in the steam distillate of rice

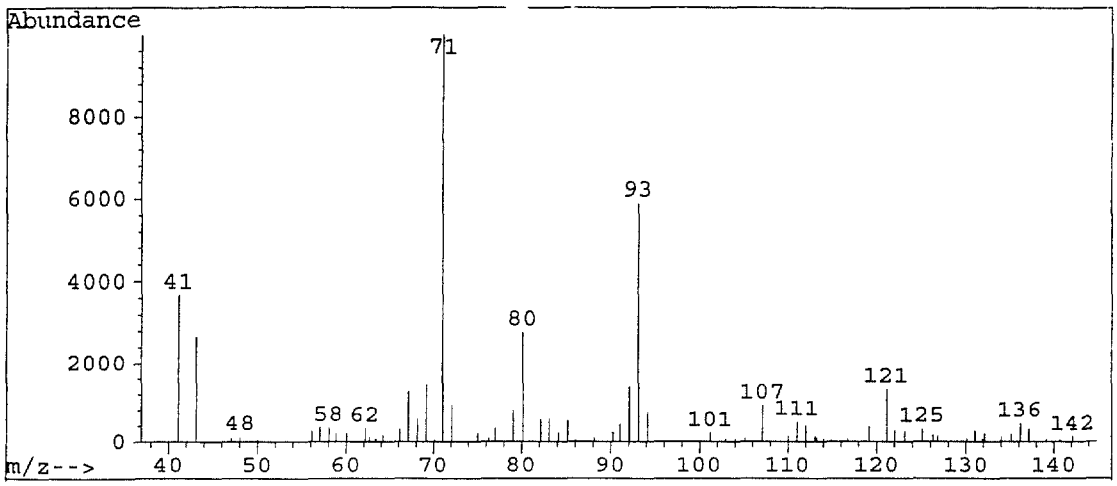


Figure 25 EI-mass spectrum of compound D present in the steam distillate of rice

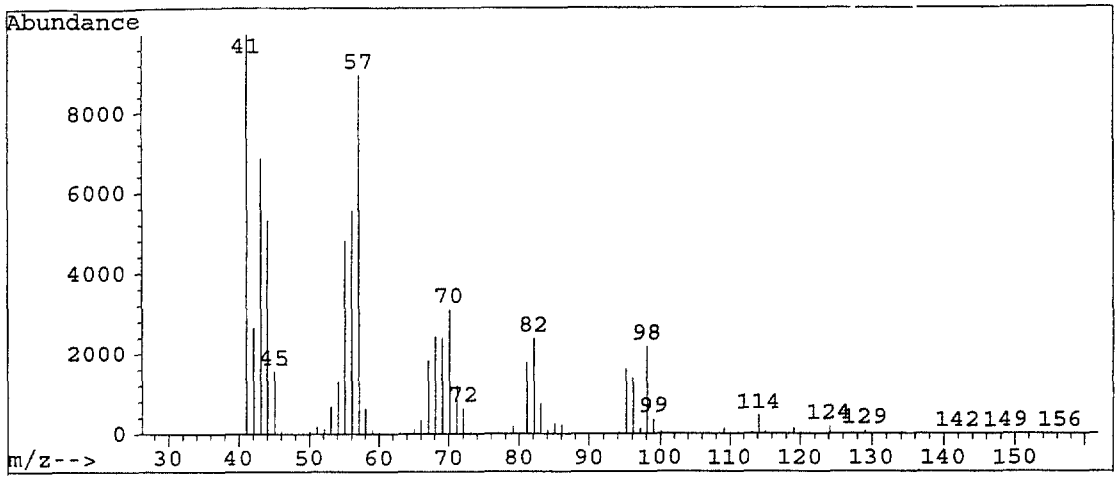


Figure 26 EI-mass spectrum of compound E present in the steam distillate of rice

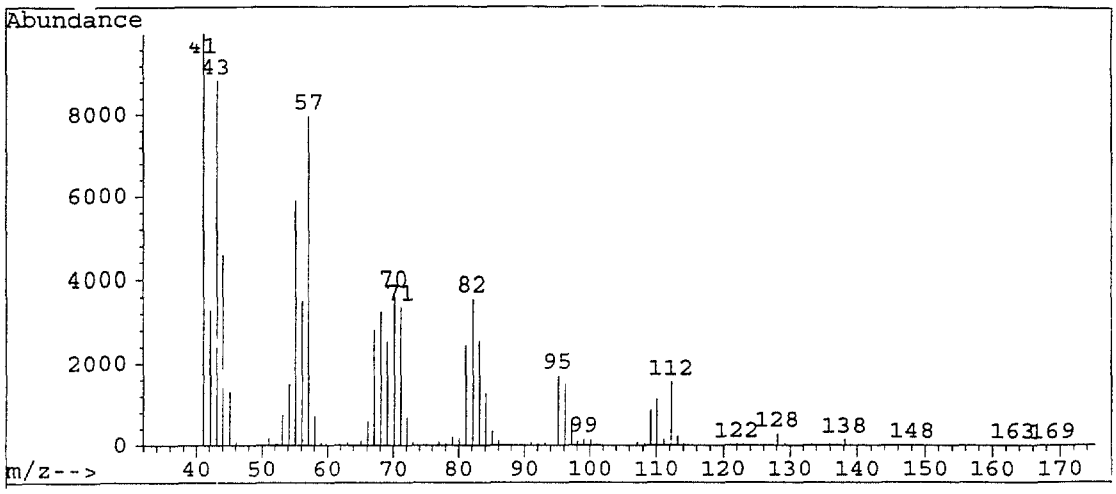


Figure 27 EI-mass spectrum of compound F present in the steam distillate of rice

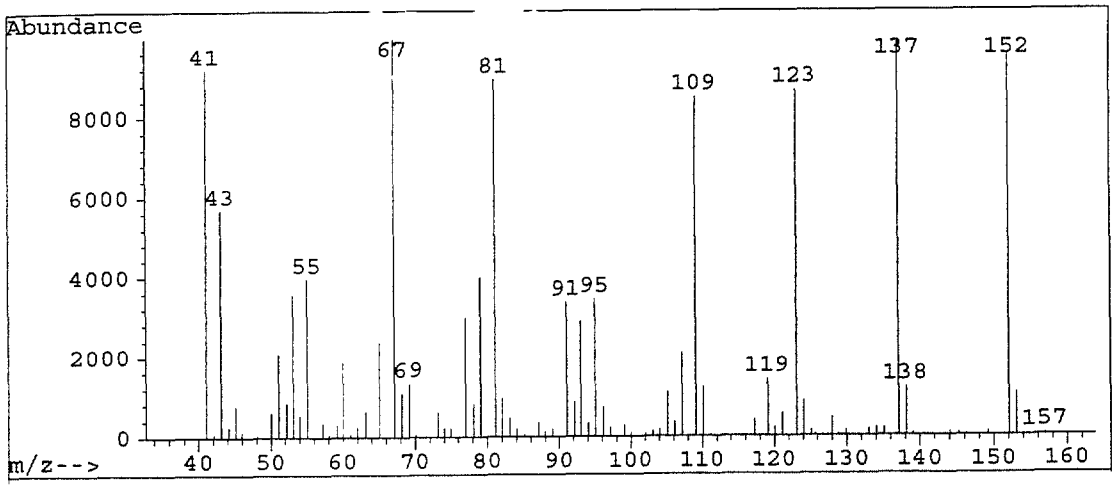


Figure 28 EI-mass spectrum of compound G present in the steam distillate of rice

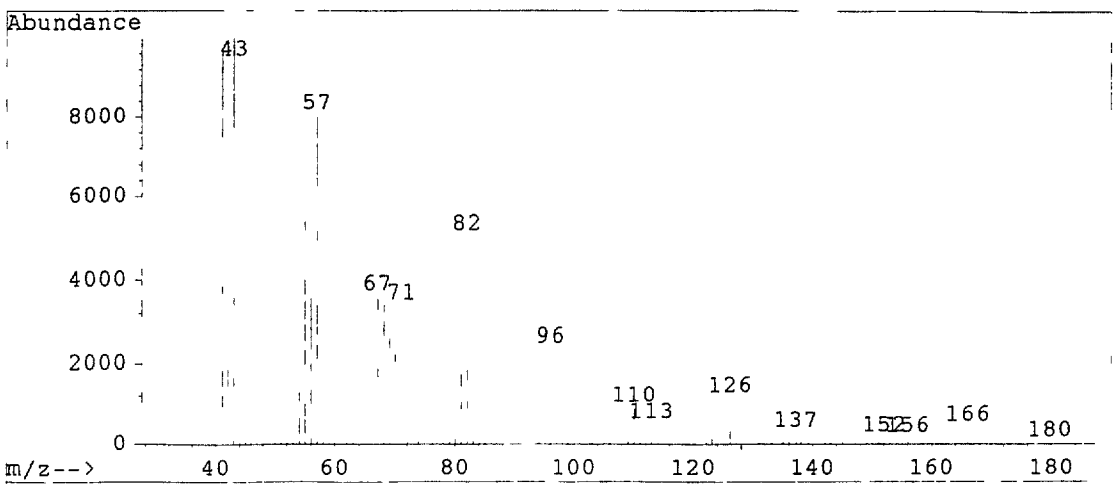


Figure 29 EI-mass spectrum of compound H present in the steam distillate of rice

5 2 6 Further Studies on candidate host attractants

5 2 6 1 Determination of the concentrations of compounds A, C, E, F, H by GC peak area measurements

The amount of components A, C, E, F, H present in 0 75 µl of each extract was estimated by comparison of GC peak areas with those obtained of standard solutions of authentic material The results are summarized in Table 7

Table 7 Amount (µg) ± S D components A, C, E, F, H present in 0 75 µL of each extract (Section 5 1 3 1 and 5 1 3 2)

Extract	Amounts of compounds (µg per 0 75 µL) ± S D				
	(Z)-2-pentenol (A)	Octanal (C)	Nonanal (E)	Decanal (F)	Undecanal (H)
Green leaves	-	0 004 ±0 070	0 042 ± 0 013	0 016 ± 0 010	-
Flowering stage	-	0 172 ± 0 061	0 643 ± 0 071	0 150 ± 0 034	0 048 ± 0 011
Grain (milky)	0 226 ± 0 130	0 141 ± 0 054	0 384 ± 0 076	0 098 ± 0 019	0 029 ± 0 040
Grain (mature)	-	0 012 ± 0 001	0 025 ± 0 016	0 008 ± 0 007	-
Bajiri	-	0 024 ± 0 007	0 104 ± 0 015	0 014 ± 0 002	0 010 ± 0 002
Illuk	-	0 016 ± 0 002	0 009 ± 0 001	0 027 ± 0 006	-
Mango	-	trace	0 048 ± 0 002	trace	-
Erabadu	-	trace	0 010 ± 0 001	0 024 ± 0 004	-

5 2 6 2 EAG assay of synthetic compounds

Among the synthetic compounds tested, the highest EAG response was elicited by decanal (0 53 mV) while nonanal, octanal, undecanal and (*Z*)-2-pentenol also showed moderate activity In contrast to these five compounds the activities of linalool, β-cyclocitral and 3-octanol were low (Table 8)

Table 8 EAG response of *L oratorius* antennae evoked by synthetic compounds

Stimulus[a]	Mean EAG response (mV) ± S D
(*Z*)-2-pentenol	0 22 ± 0 05
3-octanol	0 09 ± 0 06
octanal	0 26 ± 0 03
linalool	0 10 ± 0 06
nonanal	0 49 ± 0 02
decanal	0 53 ± 0 02
β-cyclocitral	0 11 ± 0 05
undecanal	0 32 ± 0 03

Aliquots (100 μL) were applied to filter paper strips from stock solutions of 1 mg/mL

Figure 30 shows relative relationship of EAG activities evoked by volatiles of different life stages of rice, alternative host and non-host plants, and the amount of major stimulants [*n*-octanal, *n*-nonanal, *n*-decanal, *n*-undecanal, and (*Z*)-2-pentenol)] Apparently, the presence of all five compounds in the volatile mixture is vital for high EAG-stimulant activity The volatiles of milky-grain extracts contain all five of the above compounds in significant quantities, and this mixture evokes the highest EAG responses of all the mixtures tested Moreover, second highest EAG responses were recorded from volatiles of the flowering stage which also contain the four major aldehydes but lacks (*Z*)-2-pentenol In contrast, the volatiles of milky grains of bajiri, which also have the four major aldehydes but in relatively lower quantities, show moderate EAG activity The volatiles of milky grains of illuk shows an EAG activity comparable to that of bajiri although it contains only three aldehydes This result can be rationalized since the relative concentration of decanal (a more effective EAG stimulant) in illuk is higher that that in bajiri The volatiles of green leaves and mature leaves of rice, both of which contain only three of the characterized stimulants in much lower relative quantities, show less EAG activities that those derived from volatiles present in milky- or flowering-stage rice bajiri, or illuk The lowest EAG values were recorded from non-host volatiles which contain only two of the EAG-active compounds, nonanal and decanal, in lower quantities

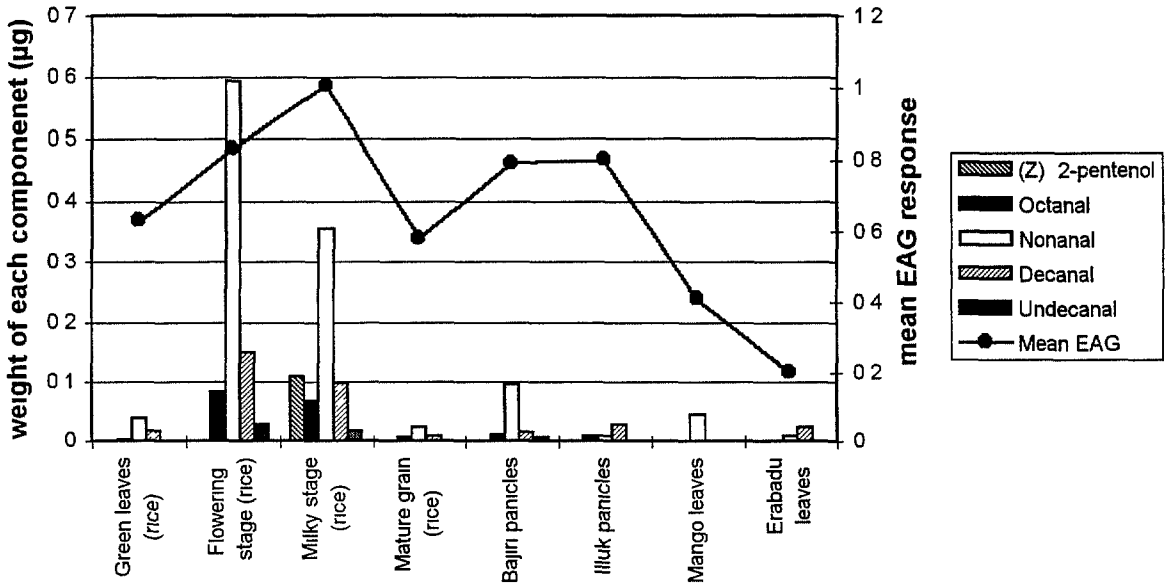


Figure 30 Mean EAG responses (mV) elicited by *L oratorius* antennae, and the weights (µg) of different components in 0 75 µl of different extracts

5 2 6 3 Behavioral bioassay of synthetic compounds and their combinations

Behavioral bioassay results obtained from olfactometer experiments using number of different mixtures as baits are illustrated in Figure 31 In this way, the activity of several synthetic mixtures were compared to that of an extract of rice steam distillate

As expected, among the samples tested, the highest mean response was evoked by the rice steam volatiles (represented by column ii in Figure 31) For this sample, 80 6% of the test insects selected the baited arm of the olfactometer The samples i, iii and iv also showed moderate activity (% insects selecting baited arm was 58 3, 61 1, and 63 9, respectively)

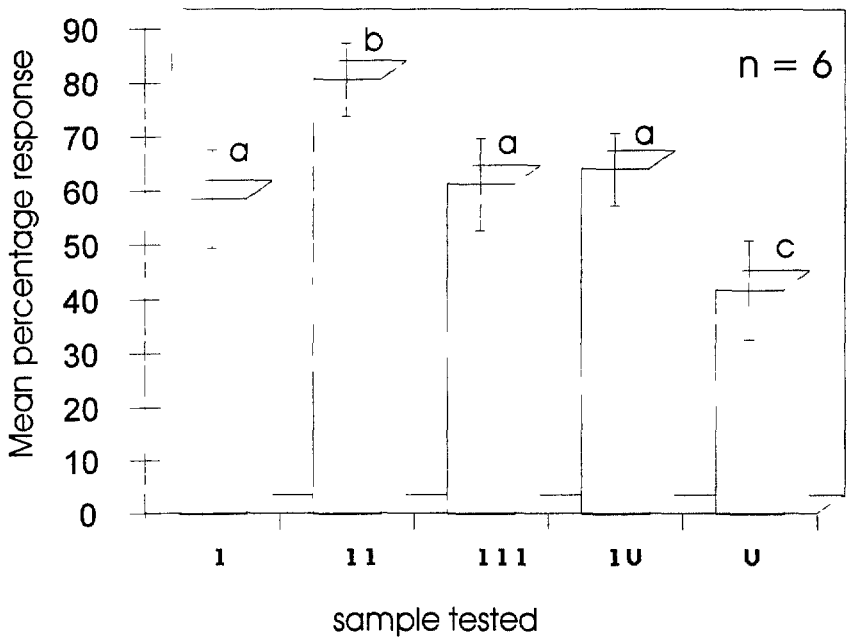


Figure 31 Behavioral response of *L oratorius* to samples i (nonanal + decanal, 100 each µg, applied from a 1 1 solution), ii (100 µL of the extract of rice steam distillate), iii (100 µg of a mixture made by combining (*Z*)-2-pentenol, octanal, nonanal, decanal, undecanal, in proportions similar to those present in the milky stage rice steam distillate), iv (100 µg of a mixture as described in iii, together with 2 µg of linalool and 2 µg 3-octanol), and v (17 µg of (*Z*)-2-pentenol, the amount present in 100 µL of the extract of the milky stage rice steam distillate) Bars headed by same the letter are not significantly different (one way ANOVA & Scheffers test, $p<0\ 05$)

6) IMPACT, RELEVANCE AND TECHNOLOGY TRANSFER

Host attractant chemistry is still a growing field unlike that involving pheromones which has been well investigated Host volatiles that act as attractants to many phytophagous insects have been shown to be blends of simple compounds usually consisting of unsaturated and saturated C_6-acetates, C_6-alcohols and C_6-aldehydes Such mixtures are often referred as "green leaf volatiles" These compounds are produced from general oxidative break down of simple fatty acids (Bullery, 1981) To the best of our knowledge, there is only one report where nonanal has been identified as a major host attractant for a hemipteran plant bug, *Lygus lineolaris* (Hemiptera, Miridea) (Chinta et al , 1994) Our results on the host attractants of the coreid bug *L oratorius* are therefore of particular importance since an aldehyde mixture of C_8-C_{11} compounds with nonanal and decanal as the major constituents has not been previously reported as an active kairomone mixture except that for a Coleopteran, which is attracted to a mixture of C_3-C_{14} aldehydes found in its host volatiles (Pierce et al , 1990) Although host attractant chemistry of insect orders such as Coleoptera have been widely investigated The order Hemiptera is one of the poorly studied areas Of the hemipterans, to the best of our knowledge, only one preliminary evaluation for a host attractant for *L oratorius* has been reported (Gunawadena and Ranatunga, 1989)

From our olfactometer bioassay results, it was evident that all varieties of rice tested (BG 94-1, BG 1492, BG 303 and AT 303) are equally attractive to *L oratorius* Therefore, we used the variety BG 94-1 for the extraction volatiles for our detailed studies Solvents extracts obtained from the steam distillation technique produced relatively higher EAG activities than those from the Super Q method although the latter technique has been one of the popular methods for the collection of volatiles due to the production of relatively "cleaner" samples

EAG responses of *L oratorius* to volatiles of rice produced at different stages showed a wide range of values demonstrating pest's sensitivity to chemoreception The volatiles of milk-producing rice grains elicited the highest EAG responses while those of the flowering stage also showed significant values Rice plants at these two life stages are significantly more attractive to this pest than all other life stages These observations agree with previous results reported from a behavioral bioassay (Gunawadena and Ranatunga, 1989) Therefore, it is likely that the mass movement of this pest from land to rice fields during the milky grain stage of rice is guided by chemical signals emerging from rice fields The results from EAG assay of rice, alternative host and non host volatiles, further indicated that *L oratorius s* host preference is based on chemoreception Since the GC-EAD profiles recorded from antennae of male and female *L oratorius* were similar, it appeared that the attractants were food derived Although the chromatograms obtained by GC-FID analyses of the milky stage rice volatiles were complex, from the responses recorded by GC-EAD analyses, eight compounds were recognized as potential candidates which appeared to account for the chemoattraction These components were identified by GC-MS as *n*-octanal, *n*-nonanal, *n*-decanal, *n*-undecanal and (*Z*)-2-pentenol, 3-octanol, β-cyclocitral and linalool Of these compounds, the highest EAG amplitudes were recorded from *n*-nonanal, *n*-decanal Moderate EAG activities were recorded from (*Z*)-2-pentenol, *n*-octanal, and *n*-undecanal, while those of β-cyclocitral, 3-octanol, and linalool, were relatively low In an investigation of an attractant to *Leptocorisa chinenesis*, Leal et al (1996) have reported that octanal and nonanal evoke EAG responses from antennae of both males and females of that species

Our investigation on the relationship EAG activities evoked by volatiles of different life stages of rice, alternative host and non-host plants, and the amount of major stimulants [*n*-octanal, *n*-nonanal, *n*-decanal, *n*-undecanal, and (*Z*)-2-pentenol)] present in these sample provided much valuable information (Figure 30) Evidently, the presence of all five compounds in the volatile mixture is essential for high chemoreception activity Since volatiles of milky-grain extracts contain all five of the above compounds in significant quantities, it is not surprising that this mixture evokes the highest EAG responses of all the mixtures tested Second highest EAG responses were recorded from volatiles of the flowering stage which also contain the four major aldehydes but lacks the stimulant (*Z*)-2-pentenol) Of the alternative hosts, the milky grains of bajiri which also have the four major aldehydes, but in relatively lower quantities, shows moderate EAG activity The volatiles of milky grains of illuk shows an EAG activity comparable to that of bajiri although it contains only three aldehydes This result can be rationalized since the relative concentration of decanal (a more effective EAG stimulant) in illuk is higher that that in bajiri The volatiles of green leaves and mature leaves of rice, both of which contain only three of the characterized stimulants in much lower relative quantities, show less EAG activities that those derived from volatiles present in milky- or flowering-stage rice, bajiri, or illuk The lowest EAG values were recorded from non-host volatiles which contain only two of the EAG-active compounds, nonanal and decanal, in lower quantities

Furthermore, from above data, it is apparent that for high EAG activity decanal concentration is more important than that of nonanal For example, the volatiles of rice flowers which have the

highest concentration of nonanal have less EAG activity than that of volatiles of milky stage Illuk volatiles which contain only three of the characterized chemo-stimulants, show comparable EAG activities to those derived from of bajiri volatiles which contain four stimulants However, the former has a relatively higher amount of decanal than that of bajiri In this way, the higher activity recorded for the three component mixture could be rationalized

The above hypothesis was supported by our behavioral bioassay results A mixture of decanal and nonanal (1 1 w/w) attracted 58% of the test insects while a five component synthetic mixture [(*Z*)-2-pentenol, octanal, nonanal, decanal, and undecanal] made according to the ratios present in the milky rice grain attracted 61% Activity of the synthetic mixture was improved by adding traces of linalool and 3-octanol which are also found in the milky rice steam distillate Although this seven-component mixture attracted 64% of the test insects, the extracts of the steam distillate of milky rice grains attracted more insects (81%) the synthetic mixture indicating that our synthetic bait is not yet optimal It is likely that some minor components are missing in the synthetic mixture

In conclusion, the present study demonstrates that rice volatiles are more effective stimulants for the chemoreceptors in the antennae of *L oratorius* than volatiles from other Graminae hosts and non-host plants The volatiles of each variety of rice tested were similar in activity to stimulate the receptors of *L oratorius* The milky-rice grains produce the most attractive volatiles to the pest Of the stimulants characterized, decanal and nonanal were the most effective, therefore we consider these two compounds as the major kairomones present in rice volatiles Since (*Z*)-2-pentenol, octanal and undecanal also elicit moderate EAG activities, these three compounds are also considered important In fact, for high chemoreceptor stimulant activity all five components are necessary, and the concentration of decanal appear to be the most vital In addition to these attractant five components, other components are also required to evoke the complete antennal response equivalent to that produced by the natural host volatiles

7) PROJECT ACTIVITIES/OUTPUTS

Dr Athula Attygalle (Cornell University) visited University of Kelaniya in March, 1993 for the installation of GC equipment The installation of the gas chromatograph and the microcomputer was completed in about 10 days For two weeks, Dr Attygalle provided instructions to research assistants on the operation of the gas chromatograph Instructions were also provided on the maintenance and basic trouble shooting Usually, it is difficult to keep very sensitive analytical instruments in running order under "third-word" conditions However, we are happy to report that the gas chromatograph and computer we have purchased from US manufacturers, Hewlett-Packard and Gateway, respectively, have been functioning properly since installation During his stay in Sri Lanka, Dr Attygalle, together with Dr Neelakanthi Gunawardena and the Vice Chancellor of Kelaniya University, visited the USAID mission in Colombo and had discussions with the AID officers about the progress of the project

The US Ambassador Hon Teresita Schaffer visited the Department of Chemistry of University of Kelaniya on September 21, 1993 She was accompanied by Mr Bill Foerserer, the environment and natural resources officer of the USAID During the visit, the Ambassador and her team were shown the equipment purchased from the funds of the USAID grant and briefed about the progress of the project The vice chancellor of Kelaniya University also attended the ceremony

Sponsored by the funds allocated to this project, Professor Louis Bjostad (Department of Entomology, Colorado State University, Fort Collins, CO 80523) visited University of Kelaniya, Sri Lanka He arrived on January 16, 1994, and left Colombo on January 26 He brought with him a setup for the measurement of electrical responses given by insect antenna This equipment fabricated by him could amplify the electrical signals evoked by antennae after chemical stimulation After testing the system with male antennae of the silkworm moth to its sex pheromone, the setup was used to record electrophysiological responses from the antennae of *Leptocorisa oratorius*, the rice bug On January 19, he delivered a lecture on "Electroantennogram (EAG) analysis of chemical compounds" to an audience of about 30 scientists including the senior and junior staff of the Chemistry Department of University of Kelaniya The lecture was very informative and the researchers could see the potential of EAG method as a research tool for investigations in insect behavior Prof Bjostad trained three research assistants, Mr Chandana Meegoda, Ms Anoma Swarnakanthi and Ms Thamara Jayesekera, on techniques of conducting electroantennographic measurements

Dr Neelakanthi Gunawardena (Collaborator, University of Kelaniya) visited Cornell University during the month of April, 1994 She brought solvent extracts attractive to *L oratorius* and analyzed them in the laboratory of Dr Athula Attygalle using the gas chromatography and mass spectrometry facilities available in his laboratory

Dr Athula Attygalle visited University of Kelaniya from December 15, 1994-January 18, 1995 During this visit samples were prepared to be taken back to Cornell for chemical analysis

Mr Kithsiri Bandara Herath, and Ms Samadara Dissanaike, the research assistants who worked at Kelaniya on this project, visited Cornell from June 20, 1994 to September 17, 1995, and June 3, 1996 to August 15, 1996, respectively During these visits, they were trained to use gas chromatography and mass spectrometry equipment

8) PROJECT PRODUCTIVITY

The main research objective was to recognize volatile kairomones present in steam distillates of rice panicles which are attractive to *L oratorius* The principle kairomones were characterized as *n*-octanal, *n*-nonanal, *n*-decanal, *n*-undecanal and (*Z*)-2-pentenol In addition, three trace constituents, 3-octanol, β-cyclocitral and linalool, appear to contribute as minor attractants

9) FUTURE WORK

In order to find out the efficacy of the identified compounds as attractants to *L oratorius*, extensive field experiments must be carried out In behavioural bioassays, a synthetic mixture of decanal and nonanal (1 1 w/w) attracted 58%, and a five component mixture of *n*-octanal, *n*-nonanal, *n*-decanal, *n*-undecanal and (*Z*)-2-pentenol of 61% of the test insects to the baited arm of the olfactometer The activity of the synthetic test mixture was further improved to 64%, by adding traces of linalool and 3-octanol which are also found in the milky rice steam distillate as minor constituents The steam distillate of milky rice grains, however, was more attractive (81%) indicating that some attractant components are missing in our synthetic mixture Although it would be a fruitful exercise to find out the missing components, practically, it would more interesting to develop novel rice varieties which lack the characterized major kairomones in the milky stage, and test whether such varieties are less attractive to the rice bug Therefore, in conclusion, we recommend the development of such rice varieties

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