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Behavioural Studies of Cowpea Seed Bruchid, *Callosobruchus maculatus* (F.) Against Volatile Leaf Extracts of Lemongrass, Neem and Curry Leaf

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ABSTRACT. *The leaf volatiles of lemongrass (Cymbopogon citratus), neem (Azadirachta indica) and curry leaf (Murraya koenigii) were investigated for their Electroantennogram (EAG) responses against cowpea seed bruchid; Callosobruchus maculatus. Dichloromethane extract of cowpea seed was used as the standard stimulant. During the EAG assay of cowpea seed extract the highest responses of 0.992±0.124 mV and 0.595± 0.045 mV were observed at the dose of 3.0 mg for female and male insects, respectively. All responses of male bruchids were significantly higher than the responses of the female ($p<0.05$). In the olfactometer assay for seed extract, the minimum dose of 5 mg showed a response of 65.5% whereas the higher dose of 25.0 mg resulted in 89.6%. At the dose of 0.20 mg of lemongrass volatiles, the highest EAG response of 1.186± 0.074 mV and 0.631±0.071 mV were observed for both male and female bruchid, respectively. For the leaf volatile of lemongrass, male bruchid always displayed significantly higher response than the female ($p<0.05$). Neem and curry leaf volatiles showed comparatively low responses of 0.41±0.048 mV and 0.36±0.031 mV at the dose of 0.25 mg, respectively. However, there was no significant difference between male and female responses against both neem and curry leaf volatiles. Behavioural bioassay with Olfactometer and choice chamber clearly indicated that the increase in the dosage of volatile oils decreased the bruchid responses. At the highest dosage of 160 mg, minimum responses were observed in olfactometer and choice chamber, respectively.*

INTRODUCTION

Cowpea (*Vigna unguiculata* Valp.) is one of the main sources of protein for people in developing countries. However, the inhabition or infestation of *Callosobruchus* species results in heavy qualitative and quantitative post harvest losses of the crop. A loss of up to 50% of cowpea substrate could result during 3-4 months storage due to infestation by *C. maculatus* (Caswell, 1981).

Phosphene, methyl bromide and pirimiphos methyl are the most popular synthetic pesticides currently used in controlling stored grain insects (Pesticide Manual, 1991; Hill, 1992; Waterford *et al.*, 1994). As methyl bromide has been recognized as a potent ozone

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depleter and pirimiphos methyl is toxic to mammals (Pesticide Manual, 1991), phosphene (PH_3) is widely used as a gas fumigant at present to control stored grain pests. The development of phosphene-resistant insect species is one of the major problems faced by stored grain industry (Waterford *et al.*, 1994). Therefore, the usage of semiochemicals to manage stored grain pests is one of the alternative strategies to overcome problems associated with grain storage. Although the essential oils are mainly used for the development of products in the flavour and fragrance industry, the insecticidal and repellent properties of these volatile oils are more popular among the farmers involved in grain production.

Many of the insect behaviour modifying chemicals are derived from plants and these phytochemical extracts have a variety of actions such as insect repellent and attractant (Bedoukian, 1992). Gunawardane *et al.* (1998) reported that the volatiles of stem bark of coconut could be used as an attractant for red weevils (*Rhynchophorus ferrugineus*). Behavioural activity of banana stem borer (*Odioporus longicollis*) against volatiles of banana stem has also been used to protect banana plants (Dissanayake, 1999). The repellant properties of volatile constituents of *Alpinia calcarata* rhizomes and *Azadirachta indica* seeds against American cockroach *Periplenata americana* has been reported using electroantennogram (EAG) studies and Olfactometer bioassay (Ekanayake and Paranagama, 1999; Kodikara, 2001).

The objective of this study was to investigate the behavioural activity using Olfactory and EAG responses of *C. maculatus* in stored cowpea against volatile constituents extracted from the leaves of lemongrass (*Cymbopogon citratus* D.C. Stapf), neem (*Azadirachta indica* A. Juss) and curry leaf (*Murraya koenigii* Spreng).

MATERIALS AND METHODS

Insect rearing was carried out at the Department of Chemistry, University of Kelaniya, under the prevailing environmental conditions (28±3°C and 75±5% R.H.) using the method described by Bandara and Saxena (1995).

Leaves of neem and curry leaf were collected from plantations at Anuradhapura and Gampaha districts in Sri Lanka. Steam distillation was used to extract the plant volatiles (Paranagama, 1991). Essential oil of lemongrass was purchased from EOAS Organics (Pvt) Ltd., Rathmalana, Sri Lanka.

Fresh cowpea seeds (100 g) were soaked in 200 ml of CH_2Cl_2 for 24 h and the CH_2Cl_2 extract was dried on anhydrous Na_2SO_4 and concentrated to 1 ml using the Rotavopour (BUCHI, R-114, Bricman Instruments Inc, Westbury, N.Y.). The cowpea seed extract was used as a known attractant in the Electroantennogram (EAG) assays. Insecticide-free, newly harvested cowpea seeds (*V. unguiculata*) were used to prepare cultures of *C. maculatus* and as the substrate for all bioassays.

Electroantennogram (EAG) studies

Five to ten hours old male and female bruchids were used separately for the EAG studies. The head of the bruchid was dissected in the middle and the section with the antennae was used for the study. The rear end of the antennae was cut and removed using a scalpel blade. The antenna was fixed on to two Ag-AgCl electrodes, which were placed in glass capillary tubes filled with insect ringer solution. The basal end of the antenna and the rear end were connected to the reference electrode and the recording electrode, respectively. The recording electrode was connected to a high impedance amplifier.

Appropriate amounts (0.05–0.30 mg of lemongrass and 0.10–0.40 mg of neem and curry leaf) of the volatile oil samples were delivered as 100 µl aliquots on to filter paper strips (3 × 30 mm), which were placed inside glass, pasture pipettes (5 mm ID and 100 mm long). Solvents of the samples were allowed to evaporate for 5 min. The pasture pipette was then connected to a syringe, containing 1 ml of air locked inside. The antenna was directly exposed to air with volatiles. An exhausting pipe was positioned behind the antennal settings in order to prevent saturation of volatile surrounding of the setting. The time interval during each stimulation was at least 20 seconds. Antennal responses were recorded as peaks. An external signal of strength of 1 mV was used to convert the peak height to millivolts (Gunawardane, 1994).

Cowpea seeds extract was used as standard stimulant and the response of the antenna to reference substance (plant volatile) was corrected using a correction factor as described by Dissanayake (1999).

Olfactometer bioassay

The Olfactometer was used for the bioassay (Bandara, 1997). A filter paper strip (2.5×3.0 cm) separately treated with different doses (10, 20, 40, 80 and 160 mg) of test volatile oil was placed in a plastic container (300 ml) connected to one arm of the tube (baited arm). A filter paper strip treated with the same amount of ethanol was placed in another plastic container connected to the second arm (non-baited arm) of the Olfactometer. A round-bottomed flask (100 ml) with 50 unsexed adult bruchids was connected to the third arm of the tube. The forth arm, which was at the intersection of the tube, was connected to a vacuum pump to regulate the airflow inside the Olfactometer. The baited and the non-baited arms of the tube were positioned towards a 40 W fluorescent light source. The arm containing round-bottomed flask was kept in darkness by placing inside a cardboard box. All the bioassays were conducted between 7–11 a.m. The baited and the non-baited arms were interchanged in subsequent replicates. Thirty minutes after introduction of the bruchids, the number of test insects moved to the baited arm and non-baited arm were counted. Each dose of volatile oil was replicated six times.

Choice chamber bioassay

The Choice chamber consisted of eight transparent plastic bottles (300 ml), placed equidistant to each other. The bottles were connected to a large transparent

bottle (1 l) placed in the centre of the chamber through glass tubes (1 cm diameter and 8 cm long). The experimental apparatus was placed in a plastic basin having a diameter of 42 cm and the height of 18 cm and the sidewalls were covered with black paper.

Five different doses (10, 20, 40, 80 and 160 mg) of the volatile samples were placed on filter paper strips (2.5×5.0 cm) separately and the solvent was allowed to evaporate. Each strip was placed in an appropriate bottle containing 50 cowpea seeds. Two bottles containing 50 cowpea seeds without any treatment and a filter paper strip treated with only ethanol were considered as the controls. Two hundred and fifty adult bruchids (unsexed, 1-3 d old) were introduced to the central bottle, and the chamber was placed in a dark room. After 24 h, the number of bruchids moved in to each bottle was recorded. The bioassay was replicated four times.

Data obtained for EAG assays and choice tests were analysed statistically using one-way ANOVA and the means were compared using Tukey's pair-wise comparison test.

RESULTS AND DISCUSSION

A dose response curve based on EAG assay was constructed between the dose ranges of 0.5-6.0 mg of cowpea seed extract (Fig. 1). The responses of both female and male bruchids gradually increased up to the dose of 3.0 mg of seed extract. The EAG responses of both male and female were constant after the dose of 3.0 mg. The highest response of female bruchid against cowpea seeds extract was 0.992±0.124 mV and the response shown by the male was 0.595±0.045 mV. Generally, the electrophysiological responses of females were significantly higher than that of the male ($P < 0.05$).

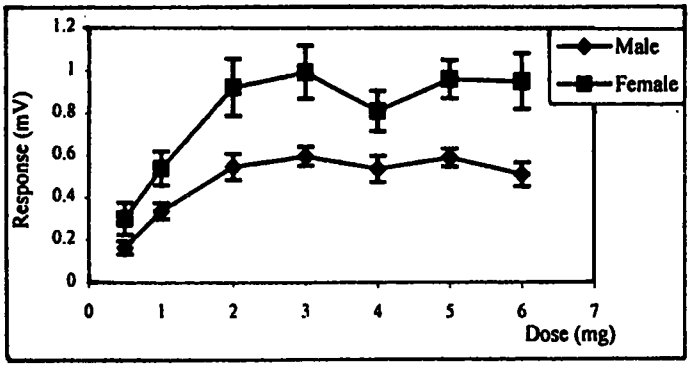

Fig. 1. EAG response of *Callosobruchus maculatus* male and female against different doses of cowpea seed extract.
[Note: Data presented are the means of 4 replicates. The vertical bars represent the standard error of means].

The EAG studies were followed by Olfactometer test to investigate the behavioural activity of the seed extract. The dose range of the seed extract used was 5.0–25.0 mg and the results of the behavioural bioassay are shown in Fig. 2. The seed extract showed a significantly higher activity and attracted significantly higher number of bruchids to the baited arm than the non-baited arm ($P<0.05$). A higher percentage response (65.5%) was observed at the lowest dose of 5 mg. Further, the bruchid response significantly increased to 89.6% with the increasing dose (up to 25.0 mg) of the seed extract.

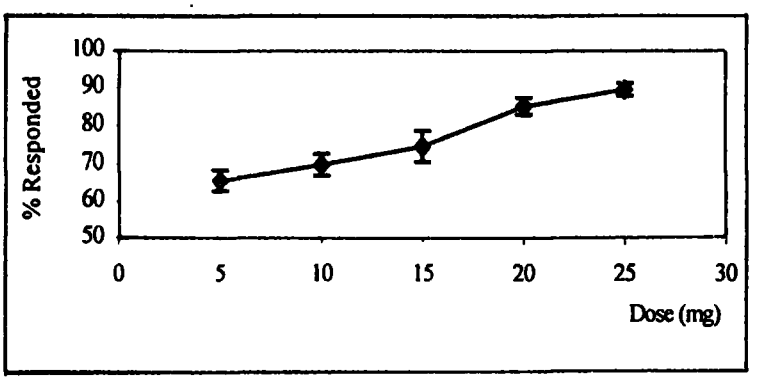

Fig. 2. Percentage bruchids responded during Olfactometer bioassay against cowpea seed extract.
[Note: Data presented are the means of 5 replicates. The vertical bars represent the standard error of means].

Fig. 3 illustrates the corrected EAG responses of bruchids for lemongrass oil in a dose range of 0.05-0.30 mg. The responses of both male and female bruchids gradually increased until the dose of 0.2 mg and a response of 1.186±0.074 mV and 0.631±0.071mV were observed for males and females, respectively. After the dose of 0.2 mg a decrease in the responses were observed. In all doses of lemongrass volatile oils tested male bruchid responses were significantly higher than the female responses ($P<0.05$).

Figs. 4 and 5 illustrate the EAG responses of bruchids for neem and curry leaf volatiles, respectively. Highest responses were observed at the dose of 0.25 mg of the two volatiles. At this dosage, the EAG responses of male and female bruchids for neem volatiles were 0.411± 0.048 mV and 0.416±0.041 mV, respectively, while for curry leaf oil were 0.378±0.042 mV and 0.362±0.031 mV, respectively. In male and female bruchids, the responses for stimuli of the volatiles of neem and curry leaf were not significantly different from each other ($P>0.05$).

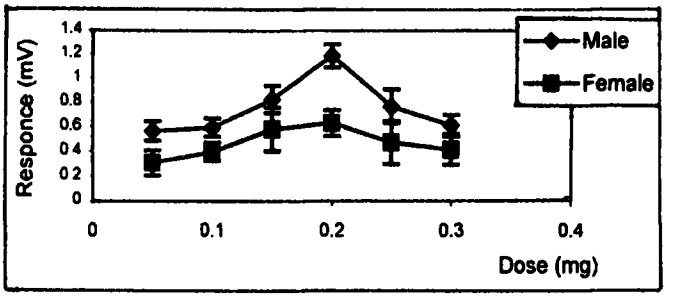

Fig. 3. EAG response of *Callosobruchus maculatus* male and female against different doses of lemongrass leaf volatile.
[Note: Data presented are the means of 4 replicates. The vertical bars represent the standard error of means].

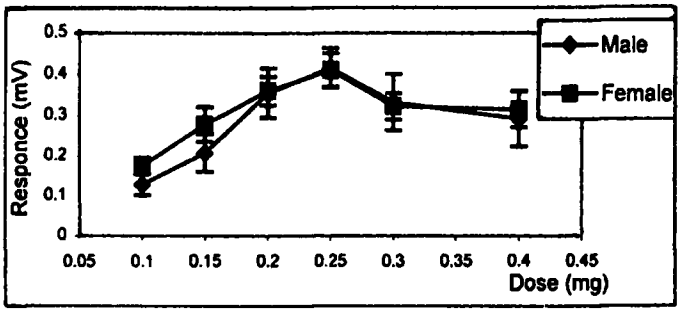

Fig. 4. EAG response of *Callosobruchus maculatus* male and female against different doses of neem leaf volatile.
[Note: Data presented are the means of 4 replicates. The vertical bars represent the standard error of means].

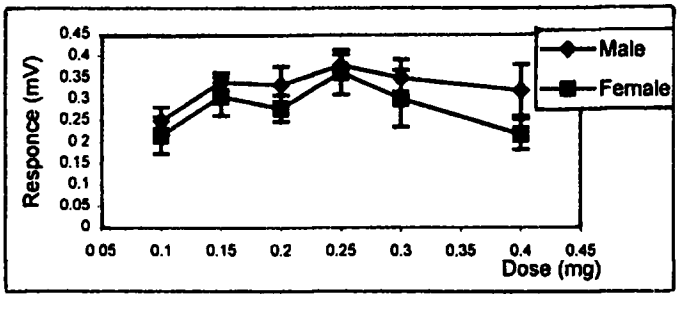

Fig. 5. EAG response of *Callosobruchus. maculatus* male and female against different doses of curry leaf volatile.
[Note: Data presented are the means of 4 replicates. The vertical bars represent the standard error of means].

During the Olfactometer bioassay, at the minimum dose of 10 mg, the maximum bruchid response of 48% and 50% was observed for neem and curry leaf volatiles whereas 41.5% response was observed for lemongrass volatiles. At this level, the response for lemongrass was significantly lower than neem and curry leaf ($P<0.05$) (Fig. 6).

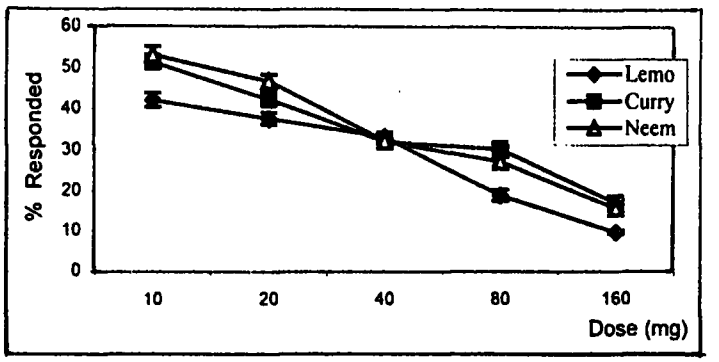

Fig. 6. Percentage bruchids responded during Olfactometer bioassay against different plant volatiles.
[Note: Data presented are the means of 6 replicates. The vertical bars represent the standard error of means].

The lowest response of bruchid (< 9.5%) was observed at the highest dose of lemongrass oil (160 mg). There was no significant difference in the responses of bruchid when exposed to the same dose of neem and curry leaf volatiles ($P>0.05$).

During the choice chamber assay, bruchid responses in volatile treated samples were significantly lower than that of the control and the ethanol treatments ($P<0.05$). The percentage of bruchids responded significantly decreased ($P<0.05$) with the increasing test volatile treatments. At the doses of 80 and 160 mg, lemongrass treatment showed significantly low responses compared to responses shown against neem and curry leaf volatiles. The results indicated that lemongrass is the best repellent out of the three volatiles tested (Fig. 7).

Based on the data obtained it could be concluded that the cowpea seed extract could be used as a standard stimulant during EAG assays and as an attractant during behavioural assays.

The antennal response of bruchid was the highest against lemongrass volatile during EAG assays. The results of both Olfactometer and choice chamber indicated that the test volatiles are acting as repellents against cowpea bruchid. The use of phytochemicals to modify the behaviour of insects, would supplement existing pest control method with chemicals of low toxicity (Bedoukian, 1992). Many of the plants, which are used as protectants, have a strong smell, is believed to repel or kill insects. As EAG shows the semi activity of antennal receptors, this technique could be used to identify the most suitable behaviour modifying chemicals in plant volatiles. Shu *et al.*

(1999) have conducted bioassays to determine the activity of sex pheromones of *Callosobruchus sabinnotatus* using Olfactometer and EAG studies. During this survey, 80% of test insects were attracted to the pheromone treatments (during the Olfactometer assay) and 2.1 mV response had been detected against an identified sex pheromone of 3-methyl 2-heptenoic acid. Gunawardane *et al.* (1998) identified 4-nonalactone and 4-hydroxy-3-methoxystyrene as the red weevil attractant using EAG and behavioural bioassays. The toxicity and repellent effect of volatile oils of lemongrass, neem and curry leaf have been reported against *Callosobruchus chinensis, Sitophilus zeamais,* tsetse fly, houseflies and mosquitoes (Jilani and Su, 1983; Namarata *et al.*, 1997; Jayasinghe *et al.*, 1999).

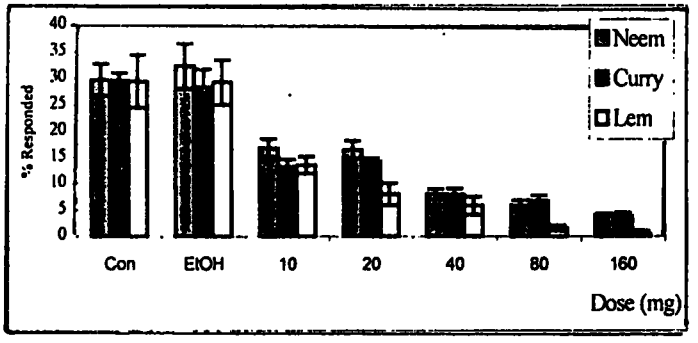

Fig. 7. Percentage bruchid responded against different plant extracts during choice chamber bioassay.
[Note: Data presented are the means of 4 replicates. The vertical bars represent the standard error of means].

The present study demonstrated the potency of the plant volatiles as repellents for *C. maculatus*. In the EAG assay stimulation of antenna of bruchids showed positive peaks. These peak patterns were reproducible and were characteristic for each volatile. Further, EAG assay results indicated that the EAG amplitudes of lemongrass are similar to that of the seed extract. Generally, repellent effects of volatiles of curry leaf and neem were less effective than that of lemongrass oil.

Volatile constituents of lemongrass, neem and curry leaf could be potential sources to develop repellents to control insect pests in cowpea and other similar grains. The botanicals used in this study are used as flavouring agents and for medicinal purposes in indigenous medicine and provide a cheap and easy control method against bruchids. Experiments are being conducted to identify the active components in the volatiles using GC-EAG and GC-MS studies and field studies will also be carried out to evaluate the activity of the volatiles against bruchids.

CONCLUSIONS

The leaf volatiles of lemongrass, curry leaf and neem reduced the responses of the bruchid *C. maculatus* in the behavioural bioassays with Olfactometer and choice chamber. The results of the EAG assay revealed that the volatiles of lemongrass shows higher activity than those of neem and curry leaf. However, further studies are needed to identify the active compound(s) and their effect on non-target organisms before recommending this for practical use.

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REFERENCES

Bandara, K.A.N.P. and Saxena, R.C. (1995). A technique for handling and sexing of *Callosobruchus maculatus* (F.) adult (Coleoptera; Bruchidae). J. Stored. Prod. Res. 31(1): 97-100.

Bandara, K.A.N.P. (1997). Insecticidal compounds from Sri Lankan plants. Unpublished PhD thesis, University of Peradeniya, Peradeniya, Sri Lanka.

Bedoukian, R.D. (1992). Regulation of some classes of phytochemicals, flavour and fragrance ingredients related to insect behaviour modifying chemicals. pp. 79-91. *In*: Ridgway, R.L., Incoe, M. and Arn, H. (Eds). Insect Pheromones and Other Behaviour Modifying Chemicals. Proceed. Brigton Crop Protection Conf. - Pest and Diseases, 1990.

Caswell, G.H. (1981). Damage to stored cowpeas in northern Nigeria. Samaru J. Agric. Res. 1: 11-19.

Dissanayake, S.A.S.P. (1999). Studies on aggregation pheromones and host attractant of the Banana Steam Borer-*Odoiporus longicollis* (Colieoptera: Curculionidae). Unpublished MPhil thesis, University of Kelaniya, Kelaniya, Sri Lanka.

Ekanayake, E.M.D.S. and Paranagama, P.A. (1999). Repellent activity of *Alpinia calcarata* volatiles for *Periplanata americana*. Proc. Sri Lanka Assoc. Adv. Sci. 55: E2-02.

Gunawardane, N.E. (1994). Electroantennogram response of the coconut pest, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) to alcohols. J. Nat. Sci. Coun. Sri Lanka. 22(1): 25-33.

Gunawardane, N.E., Kern, F., Janssen, E., Meegoda, C., Schafer, D., Otto, V. and Bestmann, H.J. (1998). Host attractant for red weevil, *Rhynchophorus ferrugineus*: identification, electrophysiological activity and laboratory bioassay. J. Chem. Ecol. 24(3): 425-435.

Hill, D.S. (1992). Pest of stored products and their control. CBS Publishers and Distributors, Delhi. pp 109-112.

Jayasinghe, P., Warnasooriya, D. and Dissanayake, H. (1999). Lemongrass (*Cymbopogon citratus*): A literature survey. Medicinal and Aromatic Plant Series, No 9, Information Service Centre, ITI, Colombo, Sri Lanka.

Jilani, G. and Su, C.F.H. (1983). Laboratory studies on several plant materials as insect repellent for protection of cereal grains. J. Entomol. Soc. Amer. 76: 154-157.

Kodikara, K.A.B.C.H. (2001). Effect of Azadirachtin and other polar components of neem, *Azadirachta indica* on specific tissues on American cockroches, *Periplanata americana*. Unpublished MPhil thesis, University of Kelaniya, Kelaniya, Sri Lanka.

Namarata, P., Yadav, T.D., Jha, A.N. and Vasudevan, P. (1997). Contact and fumigant action of volatile essential oil of *Murraya koenigii* against *Callosobruchus chinensis*. Ind. J. Entomol. 59(2): 198-202.

Paranagama, P.A. (1991). Analysis of Sri Lankan essential oil by gas chromatography and mass spectroscopy. I.T.I., Colombo, Sri Lanka.

Pesticide Manual. (1991). A World Compendium. Pp. 694. *In*: Wothing, C.S. and Hance, R.J. (Eds). 9th Edition, British Crop Protection Council, 49, Downing Street, Farnham, Surrey GU9 7PH UK.

Shu, S., Mbata, G.N., Cork, A. and Ramaswamy, S.B. (1999). Sex pheromone of *Callosbruchus subinnotatus*. J. Chem. Ecol. 25(12): 2715-2727.

Waterford, C.J., Whittle, C.P. and Winks, R.G. (1994). New aluminium phosphide formulation for controlled generation of phosphine. Proceed. 6th Int. Working Conf. on Stored Product Protection. 1: 226-229.