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A feasibility study on the use of insect pheromones to replace large-scale use of insecticides

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Netherlands Programme International Nature Management 2000 (PIN)
Project WW192101 – KNIP – 2004/2006

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Plant Research International B.V., Wageningen
April 2006

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Preface

During the late 1990s it became evident that incidence and damage of a clearwing moth in plantations of cinnamon in Sri Lanka was increasing. Field surveys in the Cinnamon Belt (districts of Galle and Matare) show yield reductions up to 40% which alarms the cinnamon sector. To maintain the income of 90,000 small farm households and to protect the environment in Sri Lanka, the Department of Export Agriculture reviewed different methods for the control of this insect pest. One of these was the option to develop a biological method for sustainable pest control based on insect pheromones. Such a method could possibly replace the large-scale use of insecticides. For this reason Dr. K.G.C.S. Ariyadasa (Cinnamon Research Station) and Dr. M. Illangasinghe (Department of Export Agriculture, DEA) of Sri Lanka requested Plant Research International (*PHERO*BANK) in Wageningen, The Netherlands, to investigate possibilities for sustainable crop management and to supply their expertise with regard to insect pheromones to the Cinnamon Research Station of the Department of Export Agriculture of Sri Lanka.

Within the scope of this feasibility study, project activities were carried out at the Cinnamon Research Station and in different cinnamon plantations in South Sri Lanka. From March till December 2005 a research team of the Cinnamon Research Station and Plant Research International were maintaining intensive contact in executing the project activities. Principal investigator of the project was Mr. G.G. Jayasinghe who worked together with co-investigator Ms. H.L.C. Darshanee. The project was supervised by Mr. K.G.G. Wijesinghe, Research Officer in Charge. Mr. W.D.L. Gunaratne, Senior Deputy Director (Research), of the Department of Export Agriculture coordinated the project and communicated with the Netherlands Embassy in Colombo. Assistance to the project activities was supplied by Mr. D.N. Samaraweera, Mrs. K.G. Jayatilake and Mr. R.S. Munasinghe of the Cinnamon Research Station. Mr. P. van Deventer gave valuable comments on the experimental design of the field trials and reared the adult wood boring moths out of cinnamon wood. The research on pheromone identification was executed by Mrs. O. Smit-Bakker, Dr. R.W.H.M. van Tol and Dr. F.C. Griepink of Plant Research International. Dr. I. Tosevski from CABI *Bioscience* in Switzerland kindly provided his taxonomical expertise for the identification on this new clearwing pest.

This feasibility study was financially supported by the Netherlands Programme International Nature Management 2000 (PIN) of the Netherlands Ministry of Agriculture, Nature and Food Quality. The support of Mr. Arnold Parzer, Counsellor for Agriculture, Nature and Food Quality of the Royal Netherlands Embassy in New Delhi, India, and of Dr. P.J. Wickramasinghe, Director of the Department of Export Agriculture (DEA), is kindly acknowledged.

Summary

Laboratory and field research was conducted in Sri Lanka and in The Netherlands to study the feasibility of the use of insect pheromones to replace the large-scale use of insecticides to control wood boring moth in cinnamon. A taxonomical study was executed by CABI Bioscience to identify wood boring moth in cinnamon up to species level.

A comprehensive set of 19 synthetic female sex pheromones of clearwings species was in field and laboratory experiments tested for their biological activity. Research on pheromone identification was done in the laboratory with male and female specimens using GC-EAG equipment. Two female sex pheromones ((E,Z)-3,13-octadecadien-1ol and (E,Z)-3,13-octadecadienyl acetate) showing response to receptors of the antennae of male cinnamon wood boring moths were identified. Field experiments were conducted in five regions in the Cinnamon Belt (Southern part) of Sri Lanka. One of the sex pheromones from the set of clearwing pheromones showed biological activity on male specimens of cinnamon wood boring moth. The field activity of this specific compound was confirmed in a second experiment in three regions. A third experiment was conducted in autumn to test the efficacy of simple, low-cost methods for trapping the cinnamon wood boring moth.

An exploratory analysis of three control options using the identified pheromones suggests good opportunities when used for monitoring, as well as for the mass trapping approach, especially if cinnamon is to be marketed under eco-label.

Extensive work on the taxonomy of the cinnamon wood boring moth yielded new insights. Earlier taxonomical studies indicated that the cinnamon wood boring moth belongs to the genus *Synanthedon*; the specific species level was not determined in this study. Detailed evaluation of specimens at CABI Bioscience resulted in the conclusion that cinnamon wood boring moth is most closely related but not identical to *Ichneumoniptera cf. flavicincta* Hampson, 1893, a species that has been reported in India and Myanmar/ Burma. Dr. Tosevski of CABI Bioscience concluded on the basis of the morphological and genital characteristics that cinnamon wood boring moth is a new insect species that has not been identified and reported before.

The project created new opportunities for sustainable and environmentally sound insect control in cinnamon, which may replace the large scale use of insecticides. This in turn may support the production of organic cinnamon, which may fetch premium prices on the world market. The project also created valuable cooperation between Sri Lanka and Dutch scientists, and a Sri Lankan television programme raised awareness of the research to the general public and policy makers.
Further work on the practical application of the current results involves limited agronomic, technical and economic research, and the development of business models for the production and use of pheromone traps.

1. Introduction

The export of spices makes a significant contribution to the export of agricultural crops (EAC) from Sri Lanka. Annual earnings of export of agricultural crops reached a total foreign exchange value of about US$ 97 million and the annual export volume was about 27,483 Mt in 2002 (Anonymous, 2002). Cinnamon (*Cinnamomum verum* Presl.), the most prominent spice crop grown in Sri Lanka, contributes 50% to the foreign exchange of EAC (4500 – 5000 million SL Rupee per year) and 40% to the export volume (around 11,000 metric tonnes of cinnamon bark per year). The production of true cinnamon in Sri Lanka represented over 75% of the world production. The total acreage of cinnamon is more than 26,000 ha. Cinnamon production is the primary source of income of more than 60,000 smallholder farmers with less than 0.25 ha, 24,000 holdings with an acreage between 0.25 and 1.0 ha, and 2,500 holdings with more than 1.0 ha. The Cinnamon Belt is situated in the western and southern parts of Sri Lanka, mainly in the Galle, Matara, Kalutara and Ratnapura districts. More than 100,000 inhabitants of Sri Lanka derive their income from growing, peeling, packing and trading true cinnamon (Dr. M. Illangasinghe, personal comment).

Until the 1970's, pest and disease problems in cinnamon were negligible. After that, with the rapid population increase and intensifying land cultivation due to the application of fertilizers and other agricultural practices, pest and disease incidences increased dramatically. Despite the potential damage to environment and public health, cinnamon farmers integrated agrochemicals into pest, disease and weed management in order to increase crop production (Darmadasa, M., 2000). Residues of agrochemicals may, however, affect the quality and image of produce from which 90% is exported. Future export of agricultural crops (EAC) may be influenced by an increasing world-wide demand for products without residues of agrochemicals produced under organic production methods. Toxic compounds like carbofuran and dimethoate are currently used for agrochemical pest control, products with an adverse effect on the ecosystem. Besides target organisms, these insecticides also kill many non-target organisms like pollinators, natural enemies and insects which contribute to agro-biodiversity and to a stable natural environment. Excessive use of agrochemicals also causes direct environmental damage to soil and groundwater and health problems among farmers and farm animals. Moreover, the use of agrochemicals adds significantly to the expense of farming and negatively affects the intrinsic quality of agricultural export production.

In the context of this feasibility study, a socio-economic workshop on 'Environmentally Sound Insect Control in Cinnamon' was held on 21 March 2005 in Galle, Sri Lanka. In this workshop the problem of wood boring moth and the demand for sustainable production of cinnamon was reviewed with participation by local scientists, growers, traders and exporters (Chapter 2). After the workshop and field visits, arrangements were made for field experiments in Sri Lanka and laboratory research in the Netherlands.

In April 2005 field trials were established at five locations (at the Cinnamon Research Station, in Galle (2 locations) and in Matara district (2 locations)) for empirical research with 19 known pheromone compositions for *Synanthedon spp*. After the first experiment, a second experiment was established in June 2005 to gain more insight in the pheromone composition in the Galle and Matara districts. To gain more information on trap placement and trap type suitable for Sri Lankan conditions, a third field experiment was set up in October 2005 in a severely affected cinnamon field in the Matara district (Chapter 4.1).

In August 2005 a shipment of cinnamon wood infested with wood boring moth was sent to Wageningen to set up an artificial rearing programme. This material yielded enough moths for laboratory research on pheromone identification (Chapter 4.2). For the identification of the clear wing moth contact was established with a researcher from CABI *Bioscience* who identified the clear wing moth (Chapter 4.3) on the basis of prepared specimens obtained from the artificial rearing programme.

Possibilities for control of wood boring moth and future implementation are described in Chapter 5 and Chapter 6.

2. Ecology and damage of Cinnamon Wood Boring Moth

Cinnamon wood boring moth is the most destructive insect occurring in the cinnamon crop. The insect has been identified only up to the genus level: *Synanthedon* sp. The life cycle of the insect is as yet neither been completely clear, mainly because it is not easy to investigate the larval stage in cinnamon wood at the collar region of the bushes. According to current knowledge at the Cinnamon Research Station, under laboratory conditions larvae live for 2 to 3 months in cinnamon wood and then turn into pupae. Adult insects are emerging from the wood after ten to twenty days. Further investigation should be done on eggs; especially on the time needed for adults to lay eggs. Under laboratory conditions adults only live two days. Another area of research is the development of an artificial diet to facilitate research into the ecology of the adult insects. Availability of an artificial diet would make it possible to collect male and female insects in the field and to obtain eggs in the laboratory since eggs are very difficult to see in the field.

Of the many pests and diseases that may occur in cinnamon, the clearwing moth, *Synanthedon sp.* (Dharmadasa & Jayasinghe, 2000) causes the most severe crop yield loss. The larvae of the clearwing moth bore into the stem bases and feed on the fleshy tissues between bark and woody stem (cambium feeder). The damage caused by such feeding is commonly known as pink stem borer damage, worm damage, or as damage due to stem boring moth. The damage is caused by the voracious feeding of the caterpillar of the adult moth (Figure 1 and 2). The excreta of the caterpillar can be found as brown tiny granules appearing from the feeding holes. This damage, if extensive, can cause plants to collapse due to plant base rotting. Rotten stems do not produce new shoots, and eventually the clump may be destroyed.

Figure 1. Adult of the cinnamon wood boring moth.

 

Figure 2. *Pupa and larva of the cinnamon wood boring moth.*

When damage is severe, insecticides such as carbofuran can be applied at the base of the plants as a quick treatment or the base of the cinnamon bush can be covered with soil which will partly prevent insects laying eggs. However, carbofuran is expensive and there are many negative effects on the environment, including animals and mammals. Proper crop management, weed control and fertilizer application are cheaper and more effective methods to reduce the incidence of wood boring moth. The most effective control method is found to be the frequent earthing up of plant bases (Jayasinghe, G.G. & P.J. Wickramasinghe, 2001). This (very labour intensive) practice will prevent adult moths to lay their eggs at the base of cinnamon stems, the preferred spot for this activity. Earthing up of cinnamon stems can reduce damage by up to 95%.

In order to reduce the use of chemicals and to maintain final product quality, it is essential to develop an alternative method for management of the wood boring moth. The new methods should be cost-effective for farmers and sustainable from an environmental point of view. Investigation of the possible application of sex pheromones for integrated pest management would be a first option for further research. This could be a good alternative for other methods such as cultural, physical, biological and chemical strategies to control the wood boring moth in cinnamon.

3. Workshop 'Environmentally sound insect control in cinnamon'

A workshop entitled 'Environmentally sound insect control in cinnamon' was organized by the Department of Export Agriculture (DEA) of Sri Lanka with the Collaboration of Plant Research International, Netherlands. On 21 March 2005, scientists, extension officers from the government and cinnamon growers, traders and exporters from the private sector (Appendix II) met at the Closen Berg Hotel, Galle, Sri Lanka.. Six presentations were made in the morning and open discussion was held in the afternoon session (Appendix I).

The first presentation was given by Dr. P.J. Wickramasinghe, Director, Department of Export Agriculture (DEA), Sri Lanka. Up-to-date information on cinnamon cultivation in Sri Lanka and of the research and extension program of DEA was given. The development plan of DEA was presented after introducing the cinnamon crop, the contribution of cinnamon to national income and export earnings and volume, acreage, harvesting and processing, quality and international standards of cinnamon.

The present situation and possible future trends in the cinnamon industry were presented by Dr. Anura Herath, Economist of the DEA. During his presentation Dr. Herath presented the acreage distribution of cinnamon plantations, the age of cinnamon cultivations, plant density, as well as current and potential yield. After explaining the effects and costs of fertilizer, weed control and other factors on yield and profit, Dr. Herath reviewed possible future trends, export sharing, market access and strategies for development of the industry.

The current use of pesticide in cinnamon was presented by Mr. W.D.L. Gunaratne, Senior Deputy Director (Research) of DEA. He explained the agro-ecological environment for cinnamon, current practices in use by the cinnamon industry, the major insect pests and diseases with current management tools and the use of pesticides in each situation. Finally, he gave an overview on the effect of proper weed control on the yield of cinnamon.

The demand for sustainable production of cinnamon was presented by Dr. M. Illangasinghe, Senior Deputy Director (Development). He explained different definitions of sustainability, history, environmental imperatives, goals, economic imperatives, farming systems, value additions, market shares, promotions and the demand for organic cinnamon and strategies to increase margins.

An overview of ecology, biology and assessment of yield loss by the cinnamon wood boring moth (*Synanthedon sp.*) was presented by the Mr. G.G. Jayasinghe, Research Officer, of the Cinnamon Research Station of Sri Lanka. Subjects presented during the presentation were: botany of cinnamon and major and minor pests in cinnamon. Mr. Jayasinghe clearly pointed out that the wood boring moth is currently the most serious pest in cinnamon. Effects of cultivation practices, history, classification and life cycle of the pest, ecology of the pest, population dynamics of the pest, response to fertilizer, behaviour of pest with different spacing levels, symptoms, damage, intensity of damage, yield loss assessment, control measures (cultural, mechanical, chemical and biological), current management pest control practices and future possibilities to develop more efficient management practices through sex pheromones and natural enemies.

Mr. Willem Stol, Plant Research International, the Netherlands held the final presentation on 'Environmentally sound insect control in cinnamon: is there a possible role for insect pheromones'. He explained the structure and function of the research institute, the tools they developed, GC-EAG techniques they use to identify insect pheromones, the different applications by means of insect pheromones that are possible for pest control, the different steps in pheromone identification, how to use sex pheromone for mating disruption. He concluded by presenting relevant experience of Plant Research International in pheromone research and an example of a project with public and private funding in Vietnam where Plant Research International is involved in a cooperation program with coffee growers.

During the interactive afternoon session, questions regarding wood boring moth damage and possible control measures were discussed in detail. During these discussions the officers of DEA, scientists of the Cinnamon Research Station and the private sector agreed that there is a clear demand for sustainable control of cinnamon wood boring moth using cost-effective and sustainable control methods instead of high-tech, cost-intensive methods like e.g. technology based on sterile insects. The cinnamon growers suggested to start field experiments, along with the laboratory research, to test the activity of the known insect pheromones from *Synanthedon spp.* available from Plant Research International.

Plant Research International agreed to supply relevant pheromones to investigate the possible response of wood boring moth to these substances. Cinnamon Research Station agreed, on behalf of the Department of Export Agriculture, to conduct the necessary field experiments.

4. Experimental results

4.1 Field experiments

Shortly after the workshop and the visit of cinnamon plantations, field experiments were designed for testing the biological activity of known insect pheromones of *Synanthedon spp.* on the wood boring moth. For this purpose the Cinnamon Research Station (CRS) received 19 synthetic sex pheromones from Plant Research International's *PHERO*BANK. The 19 different sex pheromones each have the ability to attract a different *Synanthedon spp.*.

Experiment 1

Objectives
The objective of the experiment was to test the possible biological reaction of the cinnamon wood boring moth to one or more of the 19 chemical compounds.

Method
Insect pheromones absorbed in natural rubber dispensers named S1, S2, S3, S4, … to S19 were mounted in transparent delta traps with sticky inserts. These 19 different insect pheromones were installed at five locations in the Cinnamon Belt (Kosgoda, Ahungagalle in the Galle district and Akuressa, Yatiyana and Cinnamon Research Station in the Matara district) with two replicates (Figure 3 and 4).

Figure 3. Installing the traps in Matara.

Figure 4. Adults of the cinnamon wood boring moth captured in insect glue.

The number of insects captured in the traps were counted on a weekly basis during 6 weeks. After completion, the trial was repeated at the same locations. Results are given in Tables 1 and 2.

Results
From the 19 different sex pheromones, one sex pheromone (S3) gave positive results while the other 18 pheromones showed no effect in these two trials. The number of wood boring moths captured in traps loaded with the S3 pheromone was very low. This was mainly caused by the low density of the insect population during that period. After the S3 pheromone was found to be able to attract male insects of cinnamon wood boring moth, the

experiment was repeated at same locations. Wood boring moth were captured in all S3 pheromone lures while only one wood boring moth was captured in S19R3 pheromone lure at Kosgoda in the 3rd week (Table 2).

Table 1. Number of wood boring moths captured in the 1st experiment at different locations at the initial stage.

Locations	Number of insects captured by the S3 pheromones												
	1st week		2nd week		3rd week		4th week		5th week		6th week		Total
	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	
Kosgoda (Galle)	-	-	-	-	-	1	-	-	-	-	-	-	1
Ahungalle (Galle)	1	3	-	-	-	1	-	-	-	-	-	-	5
Akuressa (Matara)	-	-	-	-	-	1	-	-	-	-	1	-	2
Yatiyana (Matara)	1	-	-	-	1	-	-	-	-	-	-	7	9
Cinnamon Res. St.	-	1	-	-	1	-	-	-	-	-	-	-	2
Total	2	4	-	-	2	3	-	-	-	-	1	7	19

Table 2. Number of wood boring moths captured in the repetition of the first experiment at different locations.

Locations	Number of insects captured by the S3 pheromone												
	1st week		2nd week		3rd week		4th week		5th week		6th week		Total
	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	R_1	R_2	
Kosgoda (Galle)			1		1	1	2	1	2	1		1	10
Ahungalle (Galle)	2	1	1	2	1	1	1		10	7			26
Akuressa (Matara)	1		1		1	1	1	1	1		3		10
Yatiyana (Matara)		14		3	5	10	1	3		2		1	39
Cinnamon R S			1			2	3			10	1	1	18
Total	3	15	4	5	8	15	8	5	13	20	4	3	93

Experiment 2

After completion of the first experiment, a second experiment was started to study the possibilities of optimizing the pheromone. Plant Research International, Netherlands, developed ten pheromone blends on the basis of the results of the S3 pheromone. Pheromone dispensers were mounted to transparent delta traps with sticky boards.

Objectives

Objective of the experiment was to test the biological activity of blends of suitable pheromones that would possibly be more attractive to the wood boring moth than the S3 pheromone.

Method

Ten different insect pheromones (S3A, S3B, S3C, S3D, S3E, S3F, S3G, S3H, S3I, S3J) with S3 (control) were tested at two locations (Ahungalle, Cinnamon Research Station) with 5 replicates. The number of wood boring moths captured in the traps were counted weekly during a period of 6 weeks. The results are given in Tables 3 and 4.

Results

The results of this experiment were confusing and difficult to interpret. The numbers of captured insects were very low and not significantly different among the treatments. This second experiment was conducted during the rainy season which probably had a large influence on the results. Further understanding of the attractivity of the optimised pheromone blends requires repetition of the experiment.

Table 3. Result of the 2nd experiment at Cinnamon Research Station in Matara.

Weeks after placement of Pheromone	Number of insects captured by the each of the pheromone blends											
	S3	S3A	S3B	S3C	S3D	S3E	S3F	S3G	S3H	S3I	S3J	Total
1	-	1	-	-	-	1	-	1	1	-	-	4
02	1	-	-	-	-	1	1	1	1	1	1	7
03	-	-	-	1	-	-	-	1	-	-	-	2
04	1	-	-	-	-	-	-	-	-	-	-	1
05	-	-	-	1	-	-	-	-	-	-	-	1
06	1	-	-	-	-	1	-	-	-	-	-	2
Total	3	1	-	2	-	3	1	3	2	1	1	17

Table 4. Result of the 2nd experiment at Ahungalle in Galle.

Weeks after placement of pheromone	Number of insects captured by each of the pheromone blends											
	S3	S3A	S3B	S3C	S3D	S3E	S3F	S3G	S3H	S3I	S3J	Total
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	1	-	-	1
3	-	-	-	1	-	-	-	-	-	-	-	1
4	-	-	-	-	-	-	-	-	-	-	1	1
5	-	-	-	-	-	-	-	-	1	-	-	1
6	-	-	-	-	-	-	-	-	-	-	1	1
Total	-	-	-	1	-	-	-	-	2	-	2	5

Experiment 3

A third experiment was set up to find the most suitable trap types and trap heights for the control of the wood boring moth.

Method

Three trap types were suggested by Plant Research International and three other local types were designed and made by Cinnamon Research Station using locally available material. Traps were loaded with S3 pheromone lures and placed in a severely infested cinnamon field in Yatiyana, Matara.

This experiment was carried out as a split block design with three replicates.

Main treatment: trap type

T_1 - Unitrap (Green)

T_2 – Unitrap (Yellow)

T_3 – Delta trap (Transparent)

T_4 – Local trap (Small)

T_5 – Local trap (Large)

T_6 – Local trap with green colour net (Large)

Sub-treatment: trap height

t_1 30 cm above ground level

t_2 150 cm above ground level

Table 5 shows the number of wood boring moths captured per week during a period of two months.

Figure 5. Unitrap (green).

Figure 6. Unitrap (yellow).

Figure 7. Delta trap.

Figure 8. Local trap (small).

Figure 9. Local trap (large).

Figure 10. Local trap with net.

Results

The numbers of captured insects in the traps were relatively low (Table 5). One of the main causes most probably was the heavy rain period experienced during this part of the season which (probably) caused the low insect population density during the experimental period. Although final conclusions cannot be drawn, the most suitable height is 150 cm above ground level and the most suitable trap type is the yellow Unitrap. This field experiment will be repeated at a different location to confirm these results.

Table 5. Number of wood boring moths captured at location of Yatiyana, Matara district.

Trap Type Weeks	T_1t_1	T_1t_2	T_2t_1	T_2t_2	T_3t_1	T_3t_2	T_4t_1	T_4t_2	T_5t_1	T_5t_2	T_6t_1	T_6t_2	Total
01	-	1	-	-	-	1	-	-	-	1	-	2	5
02	-	3	2	1	1	2	-	1	-	5	-	-	15
03	-	1	-	1	2	1	-	-	-	2	-	-	7
04	-	-	-	2	-	-	-	-	1	2	-	-	5
05	-	-	-	1	-	-	-	-	1	-	-	2	4
06	-	-	-	-	-	1	-	-	2	-	-	1	4
07	-	1	1	10	-	-	-	-	-	-	-	-	12
08	-	-	1	3	-	2	-	-	1	1	-	1	9
Total	-	6	4	18	3	7	-	1	5	11	-	6	61

4.2 Pheromone identification

The pheromone identification study was conducted by Plant Research International. Early September 2005 a package of cinnamon wood with fresh signs of wood boring moth damage were prepared by Cinnamon Research Station and were sent to the Netherlands. Emerging male and female wood boring moths were prepared for pheromone identification using Gas Chromatography coupled Electro Antenno Graphy measurements (GC-EAD).

Electro Antenno Graphy (EAG) is a technique that relies upon the specificity and sensitivity of the olfactory system of the insect, the set of olfactory receptors on the antenna. In moths, the antenna is covered with thousands of sensory sensilla, each of which contains two or more sensory neurones, sensitive to particular compounds or to a group of chemically related compounds. A neurone recognises a particular molecule through its binding with a receptor protein in the dendritic membrane. The subsequent depolarisation, the receptor potential, causes the neurone to fire action potentials, which are transmitted to the brain. Part of the receptor potential leaks into the haemolymph of the antenna and it is thought that the sum of these leaking receptor potentials is measured with EAG. EAG is restricted to the observation whether or not an insect is able to detect a particular compound and in what intensity. The effect of a perceived compound on the behaviour of the insect has to be determined by other methods. For an electro-antennogram, the antenna from a male moth is cut off and usually connected to glass electrodes filled with electrolyte. The electrodes are connected to an amplifier and recording equipment. A continuous air-flow is blown over the antenna to which a sample of an extract or a reference compound is added for a short moment. When the EAG technique is used as the detector of a GC, the retention times (or retention time intervals) are measured of the compounds that are EAG-active. These compounds are physiologically perceived by the insect and thus, are sex pheromone candidates.

GC-EAD is a technique where the antenna of the insect (its nose) is used as a detector. Pheromone compounds in an extract are most often present in very low concentrations. Sometimes even below the threshold of a standard GC detector, the Flame Ionization Detector or FID. By using the antenna of the insect we are able to detect the pheromones in extremely low quantities (< 10 pg) but we are also able to distinguish easily between pheromones, which are detected by the antenna and all other disturbing impurities that you always get in an extract. By duplicating the experimental conditions of the GC-EAD to the GC-MS, mass spectra are acquired of the compounds that have proven to be perceived by the insect. In this way, the molecular mass and elemental composition of the sex pheromone candidate are obtained.

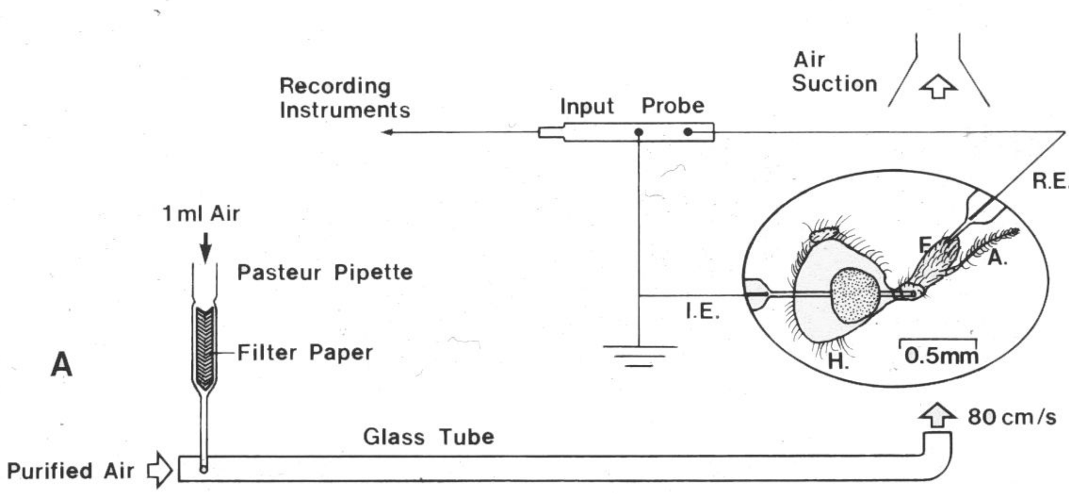

Figure 11. Schematic set-up of an EAD system. Purified air is blown over an antenna of an insect. To this air-flow a test compound can be added by Pasteur Pipette or by the GC.

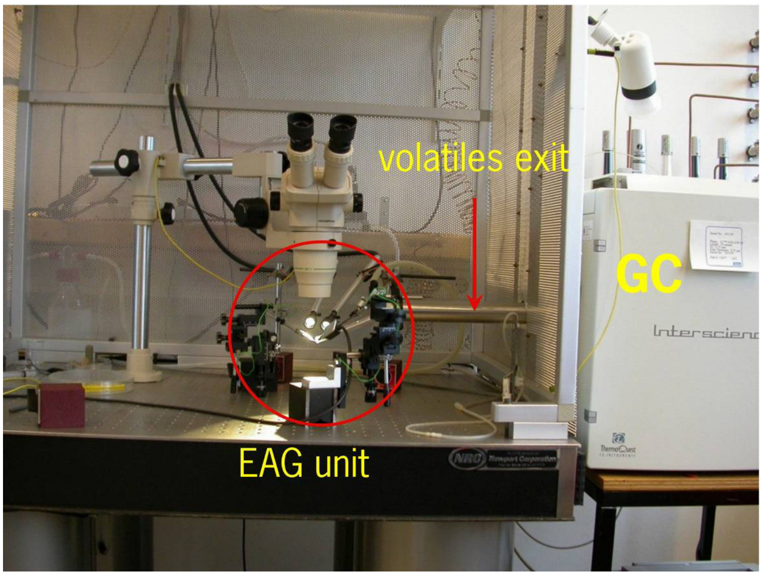

Figure 12. *Experimental set-up of GC-EAD. A complex extract mixture is separated into single compounds by the GC and then through the volatiles exit added to the air-flow over an antenna.*

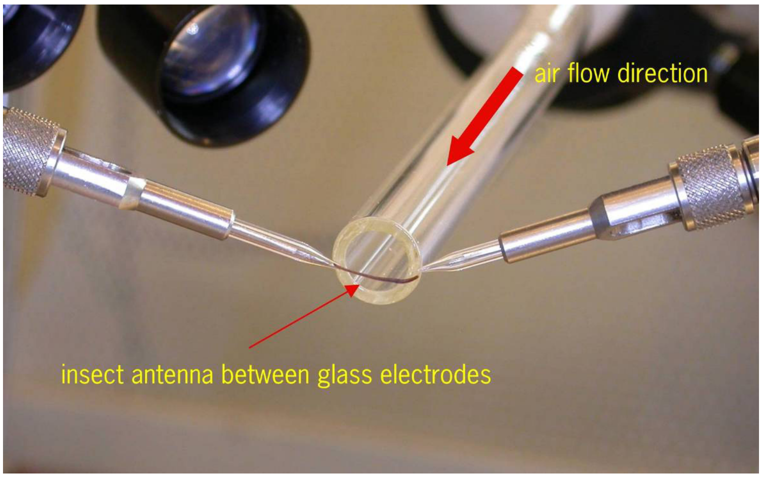

Figure 13. *GC-EAD detail. Antenna between glass electrodes.*

All GC-EAD measurements were carried out on a Chrompack 9000 gas chromatograph equipped with a split/splitless injection system. Injections were done in splitless mode only. The column was an Altech 30 m EC-5 (5% phenyl methyl polysiloxane), 0.25 mm id and 0.25 µm film thickness. The sample was equally split between a flame ionisation detector (FID) and the EAG detector. Conditions were: carrier gas, helium; continuous flow, 1.7 ml/min.; temperature programming, 80°C (0.8 min hold) to 260°C (10 min hold) at 20°C/min; on column injector; detector temperature, 250°C. The EAG recorder, software, IDAC (Intelligent Data Acquisition Controller) interface board and other peripheral equipment were manufactured by Syntech Laboratories.

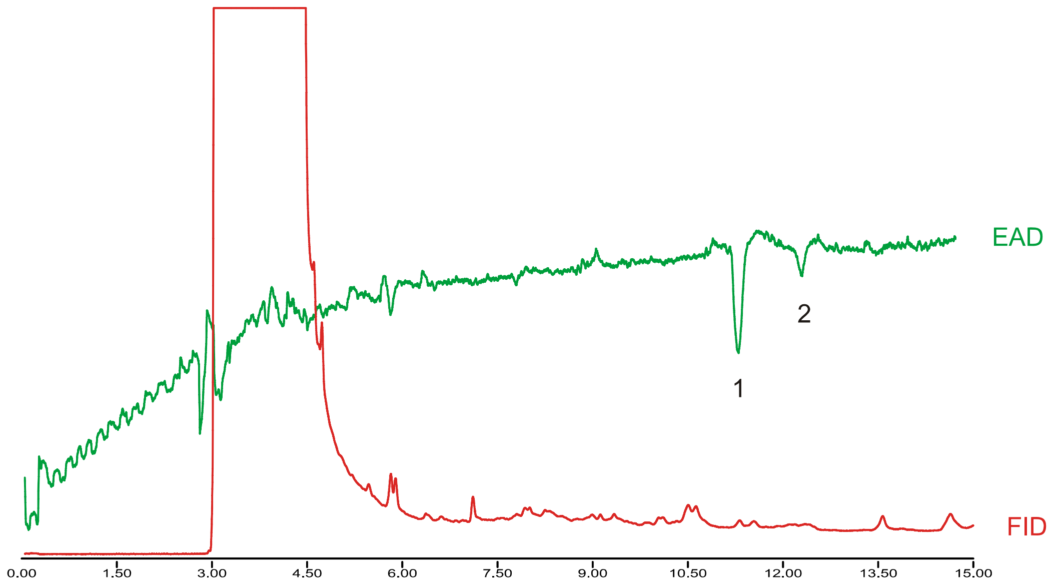

Figure 14. GC and EAD trace of 1 ul pheromone extract of cinnamon borer pheromone glands. Green line: EAD trace. Red line: FID trace.

Figure 14 shows that there are two peaks in the extract that can be perceived by the insect's antenna. The larger one has been identified by GC-MS as (E,Z)-3,13-octadecadien-1ol. The smaller peak could not be identified other than that it has to be a related acetate of the main compound.

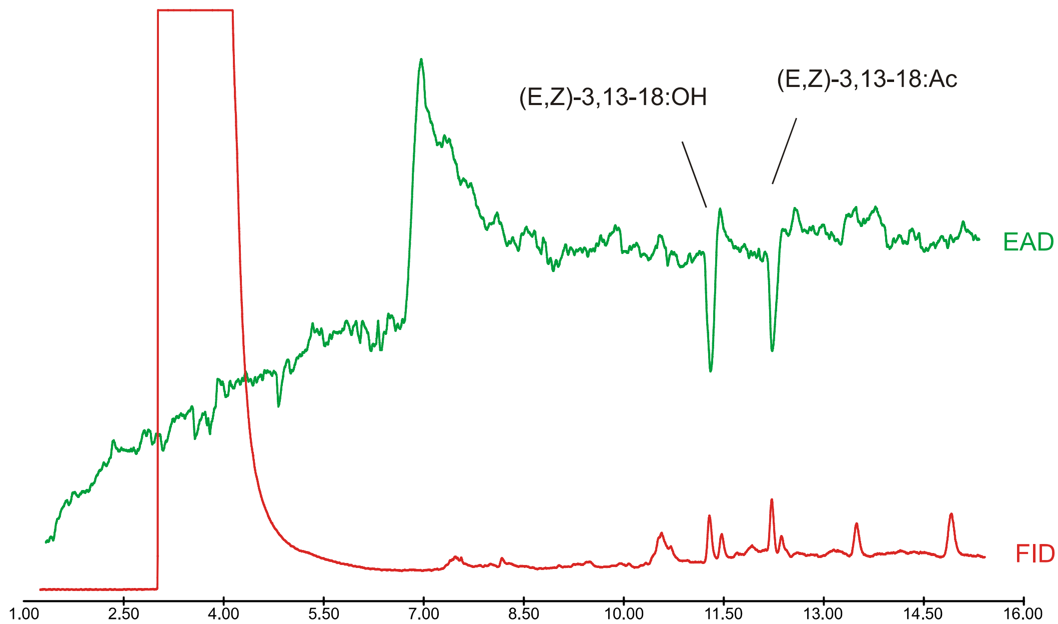

Figure 15. GC and EAD trace of 1 ul reference pheromone compound solution.

Because (E,Z)-3,13-octadecadienyl acetate gave the highest response of all possible acetates, this compound is suspected to be the minor compound.

4.3 Taxonomical study

A taxonomical study on wood boring moth of cinnamon was done by Dr. I. Tosevski, researcher at CABI *Bioscience*, Switzerland. CABI *Bioscience* forms a dynamic group of highly qualified scientists with an international remit dedicated to tackling some of the world's most challenging problems in agricultural sustainability and biological diversity. Dr. Tosevski is an international recognized expert in the taxonomy of *Lepidoptera* species. The British Museum of Natural History in London was contacted for the verification of different related species. In October, 2005, a total of 10 insects (5 males and 5 females) were selected from the wood boring moth rearing programme in the laboratories of Plant Research International for this study.

The taxonomical study was conducted in November and December 2005. Dr. Tosevski contacted colleagues in the field of taxonomy of *Lepidoptera* sop. as reference. After initial research it turned out that the identification of specimens of the cinnamon wood boring moth was complicated. According to the identification study the cinnamon wood boring moth belongs to the genus of *Ichneumoniptera.* Pictures from type specimens of *Ichneumoniptera cf. flavicincta* (Hampson, 1893) and *Ichneumoniptera cf. xanthosoma* (Hampson, 1893) were requested from the British Museum of Natural History in London to study similarities and differences with the cinnamon wood boring moth in detail. Dr. Tosevski determined these two species as the most closely related species to the wood boring moth from Sri Lanka.

According to his observations it seems that *Ichneumoniptera cf. xanthosoma* is closer related to the cinnamon wood boring moth than *Ichneumoniptera cf. flavicincta*, but according to Hampson's original description both species are quite different. With such distinct differences between the wood boring moth and the two most closely related reference species it became clear that the cinnamon wood boring moth has never before been described. The abovementioned species have been described by Hampson from Myanmar/Burma, Tenasserim (*I. flavicincta*) and from Myanmar/Burma, Maulmein (*I. xanthosoma*). The host plant of both species is unknown. The third species that belongs to the same group *Ichneumoniptera flavipalpus* (Hampson, 1893) is described from Barackpore, West Bengal, India, but according to the description (complete yellow palpi and legs) this species is very unlike the Sri Lanka specimens.

After communication with Dr. Tosevski we decided to use *Ichneumoniptera cf. xanthosoma* (Hampson, 1893) as a working name for wood boring moth. Dr. Tosevski is collecting more information on the species and the ecology of the insect to prepare a scientific publication on the species identification. For this publication more information is needed about the ecology of the Sri Lanka specimens, for example: host plant (primary and alternative host), swarming period in accordance to dry and rainy session, type of damage on host plant (branches, trunk, root), possible induction of galls or tumours. It is expected that the cinnamon clearwing moth is definitely a new species, which is not yet been described according to current knowledge.

Figure 16. *Digital photo of* ***I****chneumoniptera cf. flavincta (copyright: British Museum of Natural History London).*

Figure 17. *Digital photo of Ichneumoniptera cf. xanthosoma (copyright: British Museum of Natural History London).*

5. Options for control strategies

The results from the laboratory and field studies open new options for environmentally sound insect control of the cinnamon wood boring moth in order to reduce the adverse effect on crop production.

Monitoring
Until now, little or no information was available about the seasonal abundance and distribution of the wood boring moth between districts, between plantations, and within cinnamon plantations. Application of the pheromone of the wood boring moth in insect traps can yield systematic information on the presence in time and the spatial distribution of the moths. Such information can guide growers to understand where and when plantations are susceptible to infestations. In addition, the efficacy of agronomic measures on the presence of the wood boring moth can be monitored and evaluated. Another possibility of monitoring could be the detection of 'hot spots' within plantations infested with wood boring moth. During the field visits such areas were observed in infested fields. The infestations apparently start from one cinnamon plant, which will die off after a couple of years, while infecting neighbouring plants over time. This suggests that spreading of the pest from infected to non-infected plants within a plantation is systematic and relatively slow, and that there are distinct differences in local infestation density. This could offer possibilities for local crop measures, e.g. mass trapping at hot spots in combination with other environmentally sound methods to reduce wood boring moth populations. The costs of systematic monitoring at the district or at the plantation level to understand and get control over the problem are relatively low when local traps are loaded with pheromones.

Mass trapping
Mass trapping of male wood boring moths is another possible control strategy that could be applied to reduce the population of wood boring moth in cinnamon. This method is effectively applied in commercial farming in North America, South America, Europe and Asia. The method consists of the application of larger numbers of insect traps loaded with female insect pheromone with the aim to reduce the number of male insects. This will significantly lower the number of mating and reduces the insect population to levels that cause only small or moderate yield loss. Good results have been achieved with this method in e.g. potato, tomato and vegetable crops. The effect of mass trapping of the insect population at field level and cost-effectiveness should be subject of further research. Effective mass trapping could possibly reduce the damage to a cinnamon crop, which is 40% at most, by e.g. 70 to 80%. On an average net return from cinnamon of 1200 US$ per hectare, such a reduction of yield loss could generate a gain in net return of 320 US$ against expected costs of 40-80 US$ per hectare, which may be lower when concentrating on hot spots only. Keeping up to 10 to 15 insect traps per hectare in place for about 4 to 6 months with pheromones the method can be applied cost-effective.

Lure and Kill / Attract and Infect
Lure and kill and attract and infect are other methods that can be applied to control insect populations. With 'Lure and Kill' and 'Attract and Infect' insecticides or fungi are used to control male insects that are attracted to specific locations with female insect pheromones. Agrochemicals, however, are unwanted inputs in sustainable cropping systems. The 'Attract and Infect' option could also be effective but is less robust (more dependent on environmental conditions) and needs high numbers of insects to obtain efficient control. The effectiveness, maintenance and robustness of this method in cinnamon are questionable.

Mating disruption
With mating disruption, the mating between male and female insects is disrupted by means of realizing a constant, low female pheromone concentration in a plantation. With mating disruption male insects are disturbed and can no longer find and mate females. Although cost-effective in high-intensive fruit production in the western world, high costs of larger volumes of synthetic insect pheromones are undesired and are in the case of the cinnamon crop not cost-effective.

6. Future development and implementation

Increasing acreage and increasing crop intensity tends to result in increased spreading and potential crop damage by the cinnamon wood boring moth. The effectiveness of the application of highly toxic organic insecticides to reduce the population and subsequent damage is limited because larvae are very difficult to kill as result of their protected habitat at the base of the cinnamon plant. Moreover, residues of organic insecticides are unwanted in cinnamon quills.

The application of the insect pheromones discovered in this study makes it possible to show growers the abundance of the insect (specimens of which are very rarely seen at daytime) in cinnamon plantations. More quantitative information on abundance and insect ecology enable the further development of possibilities for preventive and corrective measures as well as evaluation of their effectiveness. Preliminary research 'beyond the original terms of the project' on the application of cost-effective (locally produced) insect traps showed that the method could be further developed locally with sufficient supply of expert knowledge. The precise method combining effective insect control with the best cost-effectiveness is not known yet and needs further development and testing under field conditions. If monitoring or mass trapping is only needed during part of the season (4-6 months) or could be applied locally to reduce insect incidence at hot spots the costs of pheromone dispensers and local traps are affordable. If the yield reduction by wood boring moths is accounting for a large part to the difference between actual and potential yields of cinnamon there is a good economic return for the cinnamon grower. Further research to validate the current findings should be focussed on: cost-effective trap types, placement (in time and in space) of traps.

The relation between a possible application method, cost-effectiveness and control efficacy, and environmental impact needs further study. Positive results of such research would enable the development of an application protocol for cinnamon wood boring moth control. This protocol should first be tested and if successful the technology should be transferred to the extension wing of DEA, cinnamon growers, traders and exporters. A reasonable reduction of insect damage by means of such a biological solution is profitable for production levels as well as for the quality traits of the cinnamon crop (increased uniformity) while the negative impact of agrochemicals to the environment will be strongly reduced.

If the new method is successful in a pilot project, e.g. in one district, the district pilot can be scaled up to regional or national scale. Before up scaling, cost-effectiveness and business approaches need careful analysis, covering aspects such as the production of traps using local materials and imported pheromones (this could be done by DEA or private partners).

To support the growing demand for and export of (organically grown) cinnamon world-wide, the introduction of methods for biological control using non-toxic compounds could well be combined with crop measures that support quality production of true (Sri Lankan) cinnamon. If efficient control of the wood boring moth can be combined with guidelines for the production of quality cinnamon, actual yields, product quality and net return for the cinnamon grower can be substantially improved.

A possible future project on environmentally sound quality production of cinnamon should cover organic production of cinnamon, added value to the product plus quality development. Such a project should not be implemented only at the farm level but should cover the whole production chain from grower, to extension, peeler, trader, packer and exporter.

7. Literature

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Clear wing moth *(Synanthedon sp.),* a new pest damage in Cinnamon, *(Cinnamomum verum Presl.)* cultivation and its damage severity in Sri Lanka. Sri Lankan Association for the Advancement of Science, 56th Annual session, Proceedings Part 1- Abstracts.

Jayasinghe, G.G. & P.J. Wickramasinghe, 2001.
Control measure of wood boring moth *(Synanthedon sp.)* in Cinnamon *(Cinnamomum verum* Presl.). Sri Lankan Association for the Advancement of Science, 57th Annual session, Proceedings Part 1- Abstracts.

Appendix I.
Workshop program

09.45 a.m.	Registration
10.00 a.m.	Lighting the Traditional Oil Lamp
10.05 a.m.	Welcome address Mr. W.D.L. Gunaratne Senior Dep. Director (Research)
10.10 a.m.	Opening Remarks Dr. P.J. Wickramasinghe, Director, Dep. of Export Agriculture
10.30 a.m.	The Economic Importance of Cinnamon Industry Dr. J. Weerasinghe, Assistant Director (Communication)
11.00 a.m.	Tea
11.15 a.m.	The Use of Pesticide in Cinnamon Production Mr. W.D.L. Gunaratne, Senior Dep. Director (Research)
11.45 a.m.	Cinnamon Wood Boring Moth (*Synanthedon* spp.): Overview of Ecology, Biology and assessment of Yield loss Mr. G.G. Jayasinghe (Research Officer- Entomology)
12.30 p.m.	The Demand for Sustainable Cinnamon Production Dr. I.M. Illangasinghe, Senior Dep. Director (Development)
01.00 p.m.	Environmentally Sound Insect Control in Cinnamon: Is there a possible role for Pheromones? Mr. Willem Stol, Plant Research International, The Netherlands
01.45 p.m.	Lunch
02.30 p.m.	Interactive Workshop Theme ‘How to implement Environmentally Sound Insect Control in Cinnamon’ What strategy should be taken for development of Sustainable Production system of Cinnamon
04.30 p.m.	Vote of thanks
04.35 p.m.	End

Appendix II.
Workshop participants

1. Dr. P.J. Wickramasinghe, Director, Dept. of Export Agriculture, Peradeniya
2. Mr. W.D.L. Gunaratne, SDD/R, Dept. of Export Agriculture, Peradeniya
3. Dr. R.S. Kularathne, DD/R, Research Station, DEA, Matale
4. Mr. A.P. Heenkenda, RO, Research Station, DEA, Matale
5. Mrs. I.C. Eresha, RO, Research Station, DEA, Matale
6. Dr. Keerthi Mohotti, RO, Tea Research Institute, Entomology division, St. Coombs Estate, Talawakale
7. Mr. Indrajith Wickramananda, RO, Coconut Research Institute, Bandirippuwa Estate, Lunuwila
8. Mrs. Samadara Dissanayake, Executive, Serendib Natural Products Ltd., 12/2A, Tricle road, Colombo 08
9. Dr. Nugaliyadda, S. Lecturer, Univ. of Rhuhuna, Faculty of Agriculture, Mapalana, Kambrupitiya
10. Mr. K.G.G. Wijesinghe, ROIC, Cinnamon Research Station, Palolpitiya, Thihagoda
11. Mr. D.N. Samaraweera, RO, Cinnamon Research Station, Palolpitiya, Thihagoda
12. Mr. G.G. Jayasinghe, RO, Cinnamon Research Station, Palolpitiya, Thihagoda
13. Mr. R.S. Munasinghe, RDA, Cinnamon Research Station, Palolpitiya, Thihagoda
14. Mrs. S.N. Weerasuriya, RDA, Cinnamon Research Station, Palolpitiya, Thihagoda
15. Dr. Illangasinghe, SDD/D, Dept. of Export Agriculture, Peradeniya
16. Dr. J. Weerasinghe, AD, Dept. of Export Agriculture, Peradeniya
17. Mr. A. Rupasinghe, AD, Dept. of Export Agriculture, Peradeniya
18. Mr. Lindara, AD, Dept. of Export Agriculture, Badi Para, Labuduwa, Galle
19. Mr. Amarasinghe, AD, Dept. of Export Agriculture, Rahula Road, Matara
20. Mrs. S. Senewirathne, AD, Dept. of Export Agriculture, Palaturu kammala Road, Narahenpita, Colombo
21. Mr. A.D.W.K. Jayathileke, Cultivator, Dasanayeke Walawwa, Nape, Kosgoda
22. Mr. Primal Wickramasinghe, Cultivator, Kaliponiya watta, Gonapenuwala
23. Prof. Jayasiri Lankage, Cultivator, No. 73/11, Sri Saranankara pedesa, Dehiwala
24. Mr. W. Stol, Plant Research International, Wageningen, The Netherlands