CROP PROTECTION PROGRAMME

Technical support for SMEs supplying pheromone-based pest control technologies in South Asia

R8413 (ZA0645)

FINAL TECHNICAL REPORT

1 February 2005 – 31 December 2005

Project Leader: Professor Alan Cork

Project Leader's institution Natural Resources Institute, University of Greenwich, UK.

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1 – Summary Sheet

Title of project:	Technical support for SME supplying pheromone-based pest control technologies in South Asia
R Number:	R8413 (ZA0645)
Project leader:	Dr Alan Cork
Institution:	Natural Resources Institute
CPP Production System:	Land-Water Production System
CPP Purpose:	Purpose 2: Productivity and productive potential in production systems increased through removal or amelioration of constraints by crop pre-harvest pests. Output: Promotion of environmentally benign ICM strategies for major insect pests of rice appropriate for use by poor farmers.
Commodity base:	Cereals and vegetables
Beneficiaries:	Resource-poor farmers in South Asia and commercial producers of benign semiochemicals-based pest control technologies.
Target Institutions:	SMEs and policy-makers involved in producing and promoting IPM component technologies and pheromones in particular.
Geographic focus:	South Asia

	Planned	***Actual***
Start date:	1 February 2005	31 December 2005
Finish date:	1 February 2005	31 December 2005
Total cost:	£73,865	£73,865

2 - Contents

3 - Abbreviations

AGM	Annual General Meeting
AHRF	Asthagiri Herbal Research Foundation
ANOVA	Analysis of Variance
A.P.	Andhra Pradesh
APEDA	Agricultural Produce Export Development Agency
BCRL	Bio-Control Research Laboratories Limited, Bangalore, India
CPP	Crop Protection Programme of DFID
DBT	Department of Biotechnology
DFID	UK, Department for International Development
GC	Gas chromatography
IABT	Institute of Agricultural Biotechnology
IPM	Integrated Pest Management
NGO	Non-Governmental Organisation
NPM	Non-pesticide Management
NRI	Natural Resources Institute
Rs	Indian rupee (£1 = 80 Rs)
SBI	Sugarcane Breeding Institute
SE	Standard Error
SME	Small and Medium Enterprise
SASAPT	South Asia Society for Advancement of Pheromone Technology
ToR	Terms of Reference

4 - Executive Summary

The purpose of the project was to contribute to the promotion of pro-poor strategies that reduced the impact of key pests and diseases, improved yield and reduced pesticide hazards in a range of production systems. This was to be achieved by working with small and medium enterprises in South Asia to improve their ability to deliver good quality, bio-rational pest management tools for use by resource-poor farmers.

Project outputs were identified as the establishment of a Biocontrol Producers' Society for South Asia to provide a common platform for commercial exploitation of pheromone and related products in the region, training for analytical personnel from ten SMEs on quality assurance of pheromone products to comply with Government legislation, assistance to companies to develop and commercialise pheromone monitoring and control systems for key rice stem borer and sugarcane borers and an investigation into concerns over the pheromone blend composition for the economically important polyphagous pod borer and bollworm *Helicoverpa armigera* in South Asia.

The project was designed to address directly and indirectly a number of Millennium Development Goals adopted by DFID, notably Target 2 by acting to improve the quantity and quality of rice and vegetables produced and consumed by rural and urban poor alike. Such actions also impact on Targets 5 and 6 and, by acting to reduce pesticide consumption and improve the environmental sustainability of crop production systems, the project will impact on Target 9 and work towards Target 18 by building capacity in the Private Sector to make the benefits of new technology available to resource-poor farming communities. Indeed outputs from the project are being feed into a new Government of Bangladesh National IPM project to promote sustainable alternatives to pesticides in vegetable production and there is good evidence to suggest that initiatives by State Governments in India, notably Andhra Pradesh, are acting to support the NGO sector to promote non-pesticide management of crops and that the NGOs themselves are purchasing pheromone trap systems directly from SMEs assisted by the project.

In particular the project has facilitated the creation of a professional Society, known as the South Asian Society for the Advancement of Pheromone Technology, that will act to improve the quality of pheromone products, promote an awareness of the technology to farmer groups and act as an advocate to advance the interests of the industry in its dealings with policymakers. A training course on quality assurance was given to representatives from eleven companies and technical assistance provided to companies to assist in new product development, as required. A number of companies are interested to release new pheromone products for control of rice and sugarcane pests and assistance provided although in the latter case the science underlying the technology is not fully established. Importantly the regional field trial to optimize the pheromone blend of *H. armigera* not only provided evidence of pheromone polymorphism but also confirmed that the companies could work together to undertake field studies that would enable them to develop standards that would benefit the industry and, most importantly, farmers.

5 - Background

DFID has consistently supported pheromone-related research in South Asia for a number of years. The work has covered a wide range of agricultural pest species in a number of commodities and agro-climatic zones, notably cotton, sugarcane, rice, brinjal, coffee and groundnut (Cork and Hall 1998, Srivastava and Satpathy 2001). Many of the pheromones have been commercialised for use in pest monitoring but have not achieved a similar level of impact for control of economically important pests (Wightman and Rao, 1993). This is in part due to economic reasons (Cork, 1998) although such problems can be overcome by adapting technology to meet farmers' needs and developing methods of sustainable production and knowledge transfer. DFID funded the research on the assumption that the technology developed would have a significant impact on the livelihoods of resource poor farmers in South Asia. Small and medium enterprises involved in the commercialisation of pheromone products were identified as key players to enable this technology to reach farmers through their capacity to manufacture, distribute and promote technologies. Government too has a role to play in promoting such environmentally friendly sustainable alternatives to currently used synthetic insecticides.

The reasons for the poor adoption of pheromone technology in South Asia compared to other regions of the world were studied in project R8304. That project undertook a survey of SMEs to assess need and address constraints and opportunities identified through a workshop for SMEs and production of a technical 'Pheromone Manual'. The project highlighted the dependence of SME's on contracts for State Government procurement of pheromone products. This process is driven by price and not quality with the result that farmers are provided with low quality products and inadequate support for their use. The main products promoted by Government are traps for key cotton pests, *Spodoptera litura* and *Helicoverpa armigera,* that can not be controlled using available pheromone technologies. The situation has been exacerbated by the recent finding of pheromone polymorphism in *H. armigera*. The Chief Guest, Dr C D Mayee, Indian Agriculture Commissioner, to the project (R8304) workshop highlighted the need for a consistent voice from SMEs to articulate the needs of the industry and to seek commercial opportunities in crops where they do not compete directly with pesticide producers, such as rice, sugarcane and vegetables.

References cited

Cork, A. (1998) Pheromones for control of yellow stem borer in India: Does mating disruption meet the needs of the rice the cultivator? Proceedings of the Sixth Australasian Applied Entomological Research Conference, 29 September - 2 October 1998, Brisbane. Pp. 304-313.

Cork, A. and Hall, D. R. (1998) Application of pheromones for crop pest management in the Indian sub-continent. *Journal of Asia-Pacific Entomology*, 35-49.

Srivastava, C. P. & Satpathy, A. (2001) Insect sex pheromone researches and its application in India. *Entomological Sinica*, **8**, 31-47.

Wightman, J. A. and Rao, G. V. R. (1993) Pheromone trapping in South Asia: review and requirements. IOBC/WPRS Working Group on the use of pheromones and other semiochemicals in integrated control, Chatham, UK, 11-14 May, Bulletin OILB/SROP, **16**, 149-156.

7- Project Purpose

Promotion of pro-poor strategies to reduce the impact of key pests and diseases, improve yield and reduce pesticide hazards in a range of production systems.

8 - Research Activities & Outputs

To facilitate project activities Dr K. P. Jayanth, Bio-Control Research Laboratories Ltd. (BCRL) undertook to oversee activities associated with the formation of the Industry Society, Dr S. Narasimhan, Asthagiri Herbal Research Foundation (AHRF), led the organisation of the workshop on quality assurance and Dr K. Krishnaiah, consultant, led project activities associated with the regional field trial to investigate the assertion of polymorphism in the pheromone of *Helicoverpa armigera*.

During initial discussions with project partners following approval of the project it was recognised that the regional pheromone trial would benefit from molecular biology studies to determine whether any differences in response to pheromone blends could be correlated with differences in the genetic base of the species. Given the potential complexity of the issue and the fact that Dr. B. Fakrudin, Institute of Agricultural Biotechnology (IABT), University of Agricultural Sciences, Dharwad, had already published the results of some studies on the genetic variability of *H. armigera* in India a request was made and accepted by the CPP for additional funds to support a small molecular biology study designed to link in with the field programme.

Output 1.0 Biocontrol Producers' Society for South Asia established to provide a common platform for commercial exploitation of pheromone and related products in the region.

Activity 1.1 Organise meeting with SMEs to draw up ToR, elect officers and put together an agenda of activities and goals.

At the Bangalore workshop funded and reported on under R8304 company representatives recognised the importance of comments made by Dr C. D. Mayee, Indian Agriculture Commissioner, on the need for a consistent voice from SMEs to articulate the needs of the industry to Government. This could be best achieved by establishing a society to represent industry needs and provide a vehicle for publicizing pheromone and related bio-rational pest management products and advocating policy change at State and higher Government level to ensure that sustainable alternatives to pesticides are duly recognised and promoted.

Interest in the Society was gauged through a questionnaire sent to SMEs in the first announcement (Annex 1). Based on their responses the first draft of rules and regulations was developed in collaboration with Dr Jayanth and a meeting held at the AHRF, Chennai in May 2005, with all project partners to discuss. The meeting was extremely important for the development of a framework that would appeal to SMEs while encouraging researchers to join but not dominate the Society. This was achieved by creating a number of membership classes and restricting opportunities for office holders to representatives of SMEs. Associate membership was open to interested researchers at nominal cost.

Having developed a draft of the rules and regulations legal advice was sought in order to turn the society into a legal entity and the draft circulated by Dr Jayanth for comment. In order to register the Society a meeting was set up at which 12 signatories had to be present to act as witnesses. Indeed because of tax issues the society was registered as a Charitable Foundation and the rules and regulations

changed to accommodate this change (Annex 3). In the event 12 signatories came forward and the Society was created at the Office of the Sub-Register, Yelahanka, Bangalore on 15 September 2005.

In order to minimise travel costs and encourage participation an inaugural meeting of the Society, to be known as the South Asian Society for Advancement of Pheromone Technologies (SASAPT), was held in Chennai on the day before the proposed pheromone quality assurance workshop. The inauguration of the Society was held at the Residency Towers, Chennai, presided over by Shri K. S. Money, Chairman of Agricultural Produce Export Development Agency (APEDA) in the presence of Dr Seema Wahad, Adviser, Department of Biotechnology. APEDA are advocates of organic farming and Shri Money was particularly supportive of the initiative. Among the attendees were Dr B. Vasantharaj David and Prof. T. N. Ananthakrishnan.

Lighting the lamp at the Inauguration

Sri K S Money, Chairman APEDA

Dr K P Jayanth, President of SASAPT

Dr Seema Wahab, Advisor, DBT

Dr K. Krishnaiah, Ex-Project Director, DRR

Releasing Trust Documents to Founder Members

The meeting attracted sixty participants and the proceedings were reported on by four newspapers (two local and two national) interviews were also given to a local TV network who produced a video that was later broadcast. Following high tea the Society held its first AGM at which twelve companies agreed to join and nominations were made and enthusiastically accepted for all council positions. The Society nominated Professor Alan Cork as the first honorary member and editor of the Societies Newsletter, PheroNews. Membership fees were agreed providing an initial fund of £5,000 towards Society expenses, £200 of which will be used to develop the Societies website.

Nevertheless, in order for the Society to fully function there is a need for the production of a logo, printing of stationery and opening of a bank account to enable funds to be processed. With the election of council members responsibilities for these activities were passed to individuals outside the remit of the project thereby ensuring sustainability but also incurring delay. Issues over stationery and log have been resolved but the bank account has yet to be opened which is a major impediment to progress, but is being addressed by the President, Dr Jayanth.

Output 2.0 Analytical personnel from ten SMEs trained to undertake quality assurance of pheromone products to comply with Government legislation.

Activity 2.1 Liaise with Chairman of Indian Chromatography Society, AHRF and SMEs to develop GC course for SMEs.

The outline of the training course was presented to SMEs in a second announcement sent on 5 May 2005 (Annex 2). This announcement recognised that there were no Government recommended procedures for pheromone product analysis and so the course would be designed to present 'best practice' with the intention of advocating adoption of these procedures as a 'standard' through dialogue with Government. The course structure was later changed to accommodate preferences raised in feedback from potential participants and participation restricted to SME personnel.

Four lectures were developed that covered:

- Gas chromatography: The basics
- Pheromone analysis
- Detectors for analysis of pheromone and related semiochemicals
- Controlled release formulations

A three-day programme was developed in which participants were divided into three teams so that they could participate in one of the three practical sessions held between lecture periods. The practical sessions covered analysis of samples by gas chromatograph (GC), air-entrainment of pheromone sources such as lures, solvent extraction of pheromone sources for quantitation by GC.

Activity 2.2 Hold training course at AHRF.

The 'Quality control of Pheromones' workshop was held at the AHRF, Chennai, 10-12 October 2005. Twenty participants from 14 organizations attended the workshop conducted over three days (Annex 4).

Before leaving copies of the presentations were provided to participants and their feedback on the workshop requested. The responses (Annex 5) were generally very favourable with participants indicating their pleasure at the opportunity to learn more about how to manage the quality of their pheromone products and a number suggesting topics for future workshops and meetings that could be held by the SASAPT.

Workshop on Quality Control of pheromone products

Output 3.0 Pheromone monitoring and control systems for key rice stem borer and sugarcane borers commercially developed and promoted by at least two SMEs.

Activity 3.1 Identify at least four SMEs developing new pheromone products and provide technical assistance

As part of the first project announcement representatives of the SMEs were asked about whether they were planning to produce new products and whether they required technical assistance. The response from ten SMEs was mixed although most companies indicated their interest to increase their product portfolio (Table 1).

Table 1 Target pests identified by SMEs for future commercial exploitation

Scientific name	Common name	Number of companies
Spodoptera	Species not specified	1
Chilo	Species not specified	1
Helicoverpa armigera	Pod borer	1
Pectinophora gossypiella	Pink bollworm	1
Plutella xylostella	Diamondback moth	1
Species not specified	Cotton stem weevil	1
Leucinodes orbonalis	Brinjal shoot and fruit borer	2
Species not specified	Banana stem weevil	1
Species not specified	Sugarcane borers	1
Conogethes punctiferalis	Cardoman capsule borer	2
Plodia interpunctella	Indian meal moth	2
Ephestia	Species not specified	1
Lasioderma serricorne	Cigarette beetle	2
Stegobium paniceum	Drug store beetle	1
Trogoderma granarium	Kharpa beetle	1
Rhynchophorus ferrugineus	Red palm weevil	2
Oryctes rhinoceros	Rhinoceros beetle	1

The pheromones of most of these species are well described and companies were primarily interested to know where to source these blends rather than needing specific technical information. Most of the company responses did not specify which new pheromone products they were considering for development. The reasons why some of the companies selected these species appeared more to do with a desire to present as wide a choice of products as possible to potential customers rather than a desire to market these trap systems in a co-ordinated manner. Nevertheless, some of the SMEs are developing their product base in a more rational manner and three of these companies were visited to follow up on their comments, Company 1, Company 2 and Company 3.

Company 1 was originally established by the parent company to develop a market for natural enemies and parasitoids in control of insect pests. Over the years their interests have diversified to include biopesticides and pheromones. Today they have terminated their production of natural enemies and parasitoids and have developed a synthesis capacity to produce pheromone compounds in kg quantities. Currently they synthesise compounds for their own lures and have begun discussions with other companies with the intention of supplying both pheromone concentrate and finished lures. Company 1 have recognised the importance of brinjal fruit and fruit borer, *Leucinodes orbonalis,* as a commercial market and have developed their own

lures with assistance from NRI. Negotiations facilitated by NRI have encouraged the supply of pheromone to Syngenta Bangladesh Ltd. A situation compromised two years ago because of faulty lures supplied by Company 1 that led to a loss of farmer confidence in the technology and Syngenta to seek other commercial partnerships. Company 1 are developing new products for control of coffee white stem borer, *Xylotrechus quadripes* with assistance from NRI and are interested to expand activity into control of red palm weevil, *Rhynchophorus ferrugineus,* sugarcane borers, and rice pests. The latter crop pest complexes are areas where NRI has, through DFID funding, developed a number of technologies that have relevance to Company 1's interests.

Company 2 is the only company in India to exist solely by the manufacture of pheromone trap systems. The success of the company derives from a personal commitment to product quality and a close working relationship with farmers, cutting out middle-men. The founder holds a database of 13,000 farmers in which he records product sales over the past 4 years. Repeat orders are received from at least 30% of farmers every year being dependent on farmers' crop rotations and competition from other pest control products. The market for pheromone products was poor this year due to excessive rains resulting in high crop failure rates and low pest problems. As a consequence the company is looking to expand the business into the area of storage pests where insect populations are more predictable providing a more stable market place for products. The company is interested to produce pheromone products for rice stem borers but is unable to meet likely demand because peak populations coincide with that of cotton pests which is their main market. Stocks of new lures are kept to a minimum (5 to 6,000) to avoid premature aging of product. The manufacture of lures is undertaken personally by the owner and he employs worksers to produce traps. To expand capacity the company has invested 2,500 pounds in a machine to cut and pack lures and new tooling to produce plastic funnel traps, but continues to manufacture metal funnel traps because of farmer demand. Interestingly, although pest populations had been low throughout the year NGO's participating in promotion of non-pesticide management (NPM) of cotton pests, funded by the Government of A.P. have purchased trap systems for 20,000 farmers and that market is expected to increase to 1000,000 farmers next year.

Company 2. Semi-automated bagging machine for pheromone lures.

Company 2. Delivery van with display of pheromone traps in crop.

Pheromone products are a relatively small market for Company 3. Recent company initiatives have been into the development of bio-tech products for the agriculture and pharmaceutical industries led by the Director Research and represent a long-term

investment in technology development where Company 3 expect to add-value and sell on to a producer. Company 3 supply pheromone traps and lures to the State Government procurement system but it is widely recognised that the tendering process lacks transparency and is subject to corrupt practice. Company 3 is keen to see SASAPT act to address the issue of minimum product standards and provide advice to Government on acceptable costings for products in an effort to ensure farmers receive good quality products and the Indian tax payer receives value for money.

All three companies are keen to encourage farmer adoption of pheromone products but in reality only Company 1 and 2 are likely to invest to achieve this objective. Company 3 is more focused on the export market through a sister company in the UK.

Company 1 have undertaken a number of trials on sugarcane borers with apparently promising results (good trap catches). However, the General Manager confirmed that their new pheromone trap design has caused considerable controversy. He was unclear about the reasons for the controversy but suggested that may be associated with IPR claims. A visit was paid to the Sugarcane Breeding Institute (SBI) to assess interest in pheromone technology for control of sugarcane borers. The meeting confirmed that there is considerable interest in the technology and recognition that Company 1 has begun trials with a number of pheromone products synthesised in-house using blend compositions developed by NRI, funded by DFID. Nevertheless, SBI researchers confirmed that the trap design promoted by Company 1 was not appropriate because of the high evaporation rate of water and inability to change trap height as the crop matures. They were also concerned that the field work undertaken made no effort to link trap catches with damage and yield.

Sugarcane is typically produced by small and marginal farmers with acreages of up to one acre. Farmers get an assured income for yield from sugarcane factories and have little incentive to produce more. Acreage is fixed at 4 million ha in India producing an average of 100 tonnes/ha. However, by improving cultivation techniques and control of stem borer species, in particular, yields can be increased to up to 200 tonnes/ha. Because sugarcane is an annual crop growing up to 3 m in height it is difficult to control insect pests with conventional insecticides and so farmers take little interest in optimising yields but accept whatever yields they obtain. However, sugarcane is the most efficient converter of carbon dioxide into biomass and ideally suited for production of bio-fuels (gasohol), an area of increasing Government and international interest. Because the sector is relatively organised there is considerable potential to achieve impact and while the efforts of Company 1 were assisted there is a clear need to work with public and private stakeholders in order to facilitate the development and adoption of sustainable pest management technologies of value to the farmer. Researchers at the SBI highlighted concern over new pests becoming more prevalent notably root borers such as *Emmalocera depresscella* (Pyralidae) and in particular the Plassey borer, *Chilo tumidicostalis* that occurs in the highly fertile regions of Bihar and West Bengal where it causes severe loss with up to 100 larvae per stem.

Given the interests of Company's 1 and 2 in the commercialisation of rice stem borer pheromones a visit was made to the Directorate of Rice Research (DRR) to assess progress with the adoption of pheromone-based control systems for rice pests and notably yellow stem borer, *Scirpophaga incertulas* in India, based on research conducted by NRI in collaboration with the DRR, funded by DFID. The researchers confirmed that there was considerable farmer interest in the technology, in part because of recognition that there is an increasing market for pesticide-free rice.

However, in order to be adopted on a wide-scale the researchers felt that pheromones for rice leaffolder, *Cnaphalocrocis medinalis* and gall midge, *Orseolia oryzae,* should also be included in the package of technologies because *C. medinalis* is now attacking later in the season in Northern India and causing economic damage to basmati producers who are having to apply insecticide and with the identification of six biotypes of the rice gall midge there is increasing concern about the potential for resistance to current varieties. The sex pheromone has been tentatively identified by researchers in China and preliminary field trials were conducted by NRI in Bangladesh in 2005 in collaboration with researchers from Sweden and Germany. However, it was recognised that access to the cultures held by the DRR would greatly facilitate optimisation of the pheromone and enable researchers to confirm whether the pheromone would have applicability across the region or whether each biotype produced a different pheromone.

Given the range of Government initiatives to promote pheromone and other NPM technologies increasingly through the NGO sector (e.g. Centre Sustainable Agriculture are involved in adoption of the System of Rice Intensification (SRI) in 4 districts of A.P. as part of an initiative to reduce water consumption advocated by Mr Y. S. Rajasekhara Reddy, Chief Minister, Government of A.P. This project will involve promoting crop diversification and in particular a reduction in rabi rice crop production for which a subsidy of 2.25 million pounds has been set aside together with free access to electricity for irrigation pumps for the next four years) and interest in the private sector the prospects for widespread adoption of pheromone technologies is high but still in need of considerable assistance to bring stakeholders together, build capacity and overcome technical constraints to production and utilisation of these environmentally acceptable pest management tools.

Output 4.0 Crop management role of *Helicoverpa armigera* pheromone in South Asia, defined and blend composition resolved.

Activity 4.1 Develop and co-ordinate SME field trials of *H. armigera* pheromone

Background information and a field trial protocol for this activity was sent out to potential participants in the first announcement (Annex 1) with specific questions seeking information on companies willingness to participate in the proposed field trials and an indication of which crops and numbers of replicates they were prepared to handle. Feedback from this exercise suggested that eight companies were prepared to undertake trials at 22 locations from Gujarat to Sri Lanka with a total of 75 replicates in five crops (cotton, chickpea, tomato, chilli, red gram).

Table 2. Pheromone blend compositions used in regional field trials

	Blend ratio		Loading (μg)	
Treatment	Z9-16:Ald	Z11-16:Ald	Z9-16:Ald	Z11-16:Ald
1	0	100	0	2000
2	0.3	100	6	2000
3	1	100	20	2000
4	3	100	60	2000
5	5	100	100	2000
6	6	100	120	2000
7	7	100	140	2000
8	10	100	200	2000
9	15	100	300	2000
10	30	100	600	2000

Based on this high level of interest 10 blend combinations were developed that both reflected and extended the work of previous workers notably Tamhankar et al. (2003) with the intention of determining whether there is pheromone polymorphism or whether a range of Heliothine species were responding to the different blends tested.

Notably, pheromone blends were based on a single dose of Z11-16:Ald (2,000 μg) with the amount of Z9-16:Ald being the only variable unlike the trials conducted by Tamhankar et al. (2003) where the quantity of both components, Z9-16:Ald and Z11-16:Ald were both varied in the blends tested. In order to ensure that participants received identical lures, stock solutions were prepared for each blend at NRI and lures were prepared only from these solutions. Lures were dispatched according to requests either directly by courier or via Dr Krishnaiah who acted to co-ordinate field activities and visited field trials to ensure that the experimental protocol was followed by all participants.

The trials got off to a slow start with unprecedented rainfall (3 cyclones) over much of the sub-continent and excessive crop damage. *H. armigera* populations remained very low in most areas until late September but with falling temperatures high trap catches could only be expected in chickpea and red gram crops in the spring months and therefore outside the short project timeframe.

Nevertheless, trap catch data was received from four companies with trials conducted in tomato and cotton although because of the poor weather conditions much of the data received consisted of zero catches. Thus, data was selected for

analysis on the basis of catches per replicate exceeding 3 per sampling period (between 2 and 7 days) per replicate (one trap of each of the 10 blends). Data were converted to catch per trap per night and analysed by analysis of variance (ANOVA). Significant differences in treatment means at the 1% level or lower were compared by Newman-Keuls multiple range test (Statgraphics, Version 2.00).

Moth catch from traps in the cotton crop were obtained from Gujarat (Anand), Andhra Pradesh (Warangal) and Karnataka (Belgaum, Sangareddy, Jogipet). Even though trap catches per night were low overall trends were similar at each location and summarised in Table 1. Blends containing between 1 and 13% of Z9-16:Ald caught significant numbers of male moths with blends containing 6.5% Z9-16:Ald catching significantly more male moths than other blends except the blend containing 4.8% Z9-16:Ald.

The significant reduction in catch observed with the blend containing 5.7% Z9-16:Ald was observed in trap catch data for each location. The reasons for two apparent peaks in trap catch are unknown however, this effect was not observed in trap catch data collected from tomato where a single peak in trap catch was observed with the 4.8% Z9-16:Ald blend although differences in mean catches were not statistically significant.

Table 3 Catch of *H. armigera* to pheromone blends tested in cotton and tomato

Percent composition		Catch/trap/night	
Z9-16:Ald	Z11-16:Ald	Cotton	±SE [1]
0	100.00	0.17	0.04 a
0.29	99.71	0.20	0.04 a
0.99	99.01	0.47	0.12 ab
2.91	97.08	0.63	0.09 ab
4.76	95.24	0.82	0.14 bc
5.66	94.34	0.52	0.13 ab
6.54	93.46	1.04	0.19 c
9.09	90.91	0.54	0.09 ab
13.04	86.96	0.57	0.17 ab
23.08	76.92	0.23	0.05 a
		Tomato	±SE
0	100.00	0	0 a
0.29	99.71	0.04	0.04 a
0.99	99.01	0.25	0.16 a
2.91	97.08	0.22	0.09 a
4.76	95.24	0.53	0.22 a
5.66	94.34	0.50	0.24 a
6.54	93.46	0.18	0.13 a
9.09	90.91	0.29	0.13 a
13.04	86.96	0.36	0.20 a
23.08	76.92	0.14	0.59 a

[1] Means followed by the same letter in a group are not significantly different *P* = 0.01 by Newman-Keuls multiple range test.

The data are best represented in graphical form where the differences in relative catch are most apparent (Figures 1a and 1b).

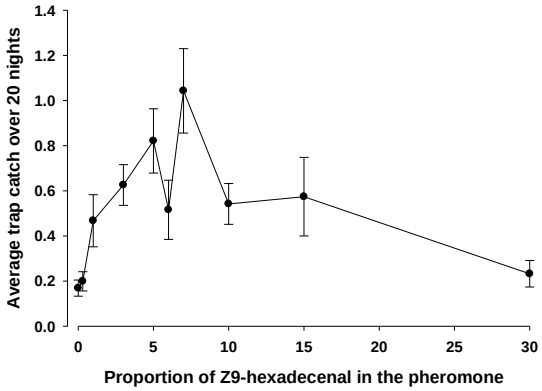

Figure 1a Trap catch from cotton

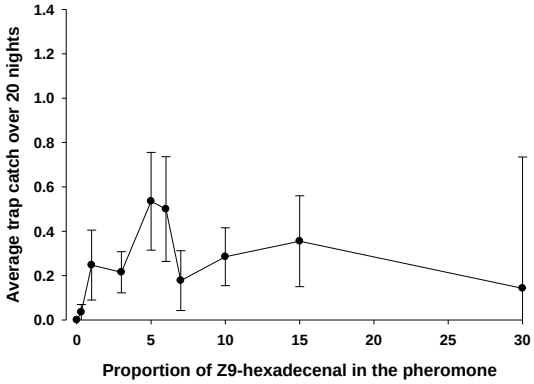

Figure 1b. Trap catch from tomato

The range of attractive blends is unusual for a single species and the presence of two peaks in the trap catch from cotton suggests pheromone polymorphism. Interestingly data from tomato suggests a single peak at 5% Z9-16:Ald. Assuming two populations are present in the cotton then the data from traps in the tomato crop would suggest the presence of one of these two populations! However, even though the summary is derived from almost 500 trap catches (50 per treatment) trap catches were low this season and any conclusion based on a single data set and crop should not be taken as definitive.

Tamhankar et al. (2003) did not undertake any studies to understand the biology behind their ascertain that pheromone polymorphs existed and indeed did not undertake any taxonomic studies to determine whether the insects caught were a single species or not. They suggested that farmers should be provided with a range of blends to maximise catch in any crop. The current data suggests that at least two populations of *H. Armigera* are present in areas of India where trials were conducted and these may be separated on the basis of host preference. Further research is now needed to confirm and extend these findings but if correct then producers could provide farmers with lures for *H. armigera* based on crop rather than lures containing a mix of blends that would be problematic both to manufacture and sustain as a product.

In addition to the need to determine the most attractive blend for attracting *H. armigera* and determining whether pheromone polymorphism is present in the species the regional field trial was devised to provide an opportunity to increase the SMEs capacity to undertake field trials for assessing new products and also to work together to generate data that would be acceptable industry wide and provide evidence-based knowledge that can be used by the Society to influence Government and other stakeholders. In this regard the trial was a success with companies following the trial protocols very closely in order to deliver useful data. So that even if trap catches were essentially zero, as was the case in Sri Lanka the reasons for this were understood (high wind speeds and low adult populations) and efforts were made to overcome these drawbacks by undertaking further trials in different locations. Nevertheless, not all companies took part in the trials which may have been expected. The reasons for this were manifold and included lack of access to suitable field sites, lack of resources to pay for trials and in the case of one company on-going trials to optimise their own lures with different pheromone blends. Inevitably there will be companies that are not prepared to shoulder responsibility for such

activities however this can be overcome by the Society itself conducting trials on behalf of the membership. Importantly, such activities would provide manufacturers with confidence in the results and ensure that field activities were relevant to the development of effective technology unlike trials conducted by researchers who are not necessarily motivated by the need to develop more efficient products.

Molecular biology study

A sample of male moths caught in pheromone traps baited with the different pheromone blends were stored in 95% ethanol and transported to IABT, Dharwad for molecular biology studies to determine whether there was a correlation genetic relatedness and behavioural response to blends, as defined by trap catch.

These studies are on-going (see Annex 7) for full description of the methods to be used) with preliminary results given below.

Results

A set of 13 SSR markers amplified unambiguously across individual DNA samples tested in this experiment. A total of 144 amplicon levels of microsatellite amplicons resulted from a set of 13 markers (Table 4). Some of SSR primers amplified more than one allele and further varied across individual moths caught in different treatments of pheromone composition.

Table 4 Summary of the statistics of SSR analysis

Total no. of amplicons levels	: 144
Average no. of amplicon per primer	:11.07
Maximum no. of alleles amplified by a primer	: 37.0
Number of monomorphic allele	: 0
Number of polymorphic level	: 144

The dendrogram drawn from the similarity matrix derived from the binary data of the SSR markers is presented in Figure 1. A genetic heterogeneity of 0.25 was evident among individuals sampled from all pheromone treatments, which is in agreement with reports on *H. armigera* populations from India, but somewhat higher than those reported from Australian and Israeli populations. Moths branched into different clusters at a similarity coefficient of 0.17 and grouped sharing various degrees of similarity with a range of 0.17 to 0.75 similarity coefficient. It is clear from the tree that clad X comprised of moths that were caught by traps baited with treatment 9 only and that they too differed from the rest by a very low (0.17) similarity coefficient. Similarly, at a different similarity coefficient, moths from treatments 3 and 5 clustered together with a few inter-mixings from other treatments. These observations clearly point to the genetic distinctiveness of populations responding to different lure compositions. However, the bearing of geographical origin was also evident, because all those caught at Kolar tended to cluster more strongly than those caught in Sangareddy within a treatment.

Migrants caught in a trap from a different geographical locations would confuse the clustering pattern but despite crop and geographical differences, clustering patterns of moths vis-à-vis pheromone treatments at least in treatments 9, 3 and 7 strongly suggested that there was genetic distinctiveness between moths responding to different lure compositions, although further research is needed to determine the basis of this segregation.
Only two moths were available from treatment 1, one each from Kolar (klr) and Sangareddy (srd). The one from Sangareddy (Srd1-1) is distinct from the rest of the sample and also from Klr 1-1 possibly indicating a geographical effect.

Research is still ongoing to determine whether a clearer view can be obtained from insects caught during the early part of the season from Sangareddy because these insects are more likely to have originated from that region. Preliminary results of ITS have indicated a drastic difference, at least between a few treatment results tested but are being reconfirmed and not presented here. Data on COI has not been obtained yet because of a need to sequence the gene from each treatment.

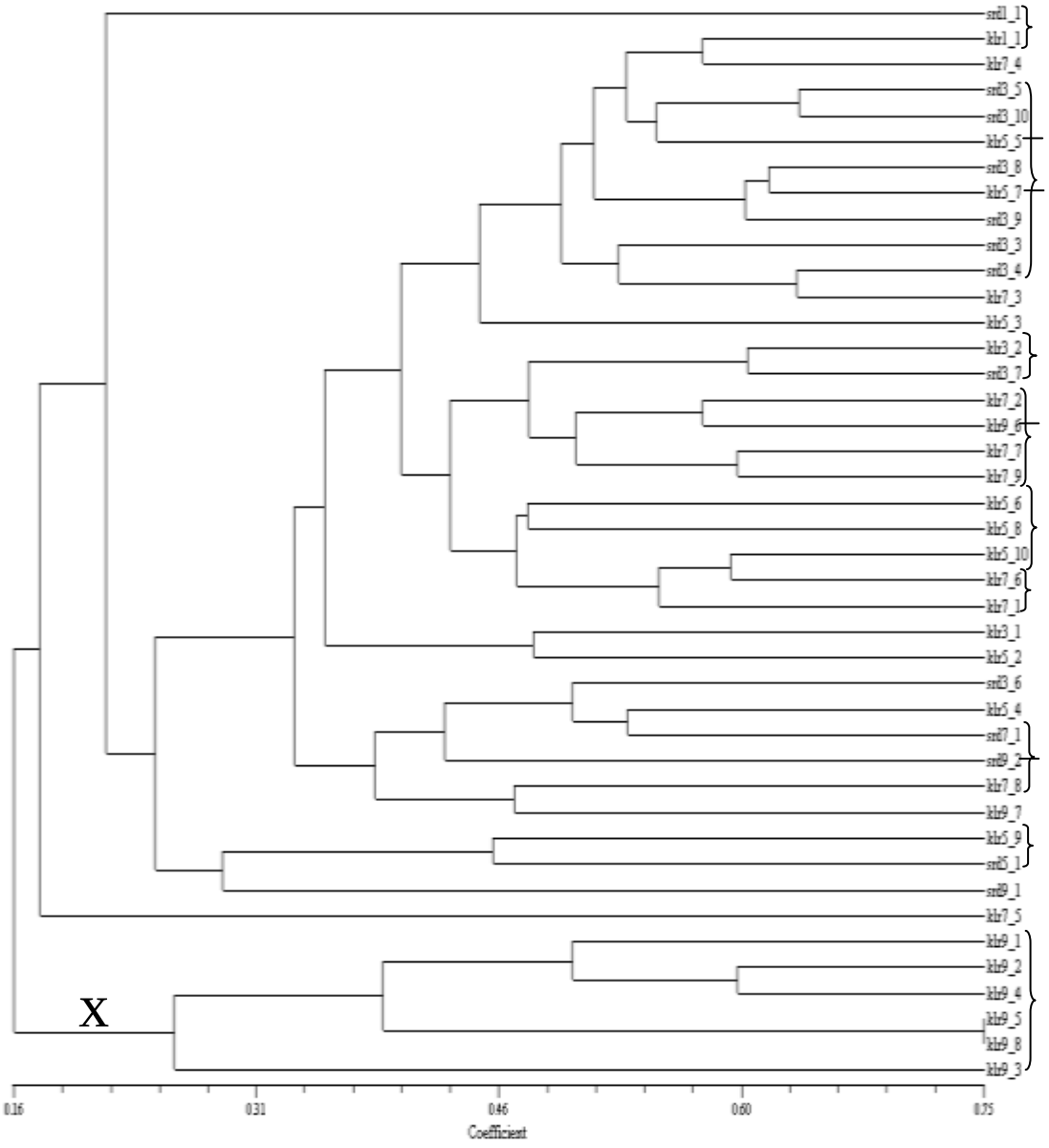

Fig 1 Dendrogram showing genetic relatedness among *H. armigera* that differentially responded to a range of pheromone lure compositions. Letters klr and srd refer to

the areas the insects were caught, Kolar and Sangareddy respectively. Klr9 refers to insects from Kolar collected from traps baited with pheromone blend nine, while klr9-2 identified the individual insect (number 2).

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9 - Contribution of Outputs to developmental impact

Pheromone-based pest control technologies are species-specific, low dose, non-toxic, environmentally acceptable and cost-competitive with conventional insecticides. Despite these advantages over conventional insect pest management practices they have not been widely adopted in South Asia, although they are an accepted means of pest control throughout the developed world, with a global market in excess of 120 million dollars per annum. Part of the reason why they have not been so readily adopted in South Asia is because farmers need to see the impact of their interventions. Pheromones are traditionally used for control by mating disruption which can not be assessed by means of the number of insects killed. More recently technologies have been developed that utilise mass trapping as the basis for control, notably for control of the economically devastating brinjal fruit and shoot borer, *L. orbonalis*, rice stem borer, *S. incertulas*, fruitflies (funded by CPP) and palm weevils. This technology allows farmers to assess the direct impact of their interventions on adult populations and later crop yield.

The current project was undertaken in an effort to increase the adoption of pheromone-related pest management strategies by working with the commercial sector in South Asia that has taken a lead in manufacturing and marketing these tools.

Central to the activities of the project has been the creation of a professional Society to represent the producers in negotiation with Government bodies and provide a forum for promoting the technology to other stakeholders. This output was fully achieved and under the banner of the Society a workshop organised and held to increase the capacity of SMEs to manage the quality of their products. While these activities have no short-term direct impact on the poor the impact will be considerable in the longer term. Indeed the eight SMEs who have recently begun to produce and market pheromone products for control of brinjal borer as a result of promotional activities associated with R7465D received technical assistance under this project that will enable them to provide farmers with high quality products that will ensure the technology is presented to farmers in a cost-effective manner that will sustain the market and enable poor farmers to benefit from increased yields and reduced pesticide inputs. This approach contrasts with that taken by State Governments where trap systems are procured on the basis of a tendering process in which the winner provides the lowest price. This process results the quality of the product being compromised leading to farmer dissatisfaction with the technology and lack of sustainable adoption.

Similarly, the Government of India promotes pheromone products for use in cotton pest control through state subsidised procurement schemes. This activity is being extended in states such as Andhra Pradesh (A.P.) where non-pesticide management (NPM) of cotton and rice pests has been found to provide farmers with more sustainable yields than other approaches. Indeed the widespread failure of GM varieties this year, due to susceptibility to bacterial wilt, has led to Mahyco-Monsanto seed being banned in A.P. The project has assisted the NPM technology promoted in cotton by developing a more-efficacious pheromone lure for the main insect pest of cotton, *H. armigera.* The improved product will also benefit poor farmers who produce tomato and legume crops such as pigeonpea and chickpea. Nevertheless, because of the heavy rains experienced throughout India in the past year the field trials were only completed on cotton and further work is required on other crops to fully understand how these biotypes are distributed across the sub-continent. Such information is essential if effective control strategies are to be developed to combat this pest in a sustainable manner without recourse to pesticides or transgenic crops which have proved such a mixed blessing to farmers' to-date.

The project has created a framework within the commercial sector for extending the range and impact of pheromone and related bio-rational pest control products in South Asia particularly for use in eggplant and cotton pest management. The companies in India are already beginning to address opportunities for control of coffee stem borer, palm weevil and fruitflies with pheromones, however further assistance is needed to enable the commercial sector to work with in-country researchers to tackle rice and sugarcane pests that will benefit a broader sector of resource-poor farmers in the sub-continent, particularly sugarcane given that the crop employs 280,000 farmers with holdings of less than 2ha and has considerable potential to act as a carbon neutral bio-fuel.

10 - Complete the logframe:

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions
Goal			
Productivity and productive potential in production systems increased through removal or amelioration of constraints by crop pre-harvest pest.	To be completed by CPP Programme Manager	To be completed by CPP Programme Manager	To be completed by CPP Programme Manager
Purpose			
Promotion of pro-poor strategies to reduce the impact of key pests and diseases, improve yield and reduce pesticide hazards in production systems	To be completed by CPP Programme Manager	To be completed by CPP Programme Manager	To be completed by CPP Programme Manager
Outputs			
1.0 Biocontrol Producers' Society established.	Society formerly established	Project reports to CPP	Sufficient interest among SMEs.
2.0 Analytical personnel from ten SMEs trained to undertake quality assurance of pheromone products.	At least 10 representatives from SMEs trained in GC analysis and application for use with pheromone products.	Project reports to CPP	Sufficient interest among SMEs
3.0 Pheromone products for key rice stem borer and sugarcane borers commercially developed.	At least two new pheromone products in development.	Project reports to CPP	Sufficient interest among SMEs
4.0 *Helicoverpa armigera* pheromone defined and blend composition resolved.	Blend optimised in at least 4 regions and 3 crops in India Status of pheromone polymorphism understood.	Project reports to CPP Peer-reviewed publications	Sufficient interest among SMEs and populations of *H. armigera* sufficient to provide meaningful analysis of data
Activities	**Inputs**	**Means of Verification**	**Important Assumptions**
1.1 Organise meeting with SMEs to draw up ToR, elect officers and put together agenda of activities and goals.			
2.1 Liaise with Chairman of Indian Chromatography Society, AHRF and SMEs to develop GC course for SMEs. 2.2 Hold training course at AHRF.			
3.1 Identify at least four SMEs developing new pheromone products and provide technical assistance	.		
4.1 Develop and co-ordinate SME field trials of *H. armigera* pheromone.			

Note: Outputs should be numbered 1, 2, 3, *etc.* Activities should relate to these outputs and be numbered 1.1, 1.2, ...2.1, 2.2, *etc.*
It is expected that most projects will achieve only one or two outputs and a small number of activities.

Biometricians Signature

The projects named biometrician must sign off the Final Technical Report before it is submitted to CPP. This can either be done by the projects named biometrician signing in the space provided below, or by a letter or email from the named biometrician accompanying the Final Technical Report submitted to CPP. (Please note that NR International reserves the right to retain the final quarter's payment pending NR International's receipt and approval of the Final Technical Report, duly signed by the project's biometrician)

I confirm that the biometric issues have been adequately addressed in the Final Technical Report:

Signature:

Name (typed):

Position:

Date:

Annex 1. First Project announcement to SMEs regarding project activities

5 May 2005

Dear Colleague,

First Announcement
'ENABLING SMES TO PROMOTE PHEROMONE PEST CONTROL TECHNOLOGIES IN SOUTH ASIA',
Phase II

You will recall that with funding from the UK, Department for International Development we were able to undertake a survey of SMEs involved in the production and marketing of pheromone trap systems in south Asia. This survey led to a successful workshop in May 2004 hosted by BCRL in Bangalore from which arose a proceedings and pheromone manual. The proceedings highlighted a number of actions that the participants were keen to pursue and we are pleased to say that we have managed to secure further funding to carry some of these issues forward. The planned outputs of the second phase are:

1. Biocontrol Producers' Society for South Asia established to provide a common platform for commercial exploitation of pheromone and related products in the region.

2. Analytical personnel from ten SMEs trained to undertake quality assurance of pheromone products to comply with Government legislation.

3. Pheromone monitoring and control systems for key rice stem borer and sugarcane borers commercially developed and promoted by at least two SMEs.

4. Crop management role of *Helicoverpa armigera* pheromone in South Asia, defined and blend composition resolved.

Biocontrol Producers' Society for South Asia established to provide a common platform for commercial exploitation of pheromone and related products in the region

We believe that in many ways this will be the most difficult output to achieve and yet could prove to be the most important. A Society, Association, Club, call it what you will, that can act to represent and promote the interests of the industry at local, State, Government and perhaps international level will produce undoubted benefits. Nevertheless, to create such a body would require affirmation of need, participant of SMEs both in the organisation and running of such a body and an active dialogue on the organisations scope and constitution. With your input we will gauge interest and, if appropriate, endeavour to develop the foundations of an industry society through the co-ordinating efforts of **Dr K P Jayanth.**

Analytical personnel from ten SMEs trained to undertake quality assurance of pheromone products to comply with Government legislation

Dr S Narasimhan has kindly offered to provide facilities at the Asthagiri Herbal Research Foundation to conduct a 2-day training course in *quality assurance of pheromone products*. The intention is to hold two 2-day courses back-to-back with up to 10 participants per course. However, this will be reviewed depending on response from industry. Dates for the course have not been decided though it is likely to take place in September. The course will focus on the use of GC as the primary analytical tool although other techniques such as GC-MS will be discussed.

As far as we are aware there are no Government recommended procedures for pheromone product analysis and so the course will discuss 'best practice' and perhaps, through dialogue, the Industry Society could advocate suggested 'standard' procedures to Government.

The project provides no funding for accommodation during the workshop or travel costs although light refreshments and lunch will be provided during study periods. However, in order to make local travel arrangements more straightforward we would be happy to assist with locating accommodation and transport to AHRF.

Pheromone monitoring and control systems for key rice stem borer and sugarcane borers commercially developed and promoted by at least two SMEs

The purpose of this output is to provide technical assistance to any SMEs whose companies are actively in the process of developing new pheromone-based products perhaps, but not necessarily, in rice or sugarcane. The assistance is restricted to technical knowledge and advice. However, should you be interested in extending your product range and feel that we could assist you to achieve this goal please contact **Dr A. Cork** in the first instance.

Crop management role of Helicoverpa armigera *pheromone in South Asia, defined and blend composition resolved.*

As many of you are aware the pheromone of *H. armigera* has been a problem for many years. Stories of the ineffectiveness of lures have been a common theme. One solution to this conundrum articulated at the Bangalore Workshop was the possibility of pheromone polymorphism, that is a complex of sub-species that respond to different pheromone blends. A recent publication by Dr Tamhankar and colleagues provides good evidence to support this argument. However, from the point of view of biocontrol producer companies this scenario is hard to deal with because you will be obliged to provide farmers with lures containing different ratios of compounds not knowing which pheromone morph is locally present. An alternative view is that the trap catches to different blends were due to the capture of other species because the taxonomy of the insects caught was not checked.

Irrespective of the view taken, the issue will cause confusion in the minds of extension agencies and other state functionaries concerned with crop protection. If the biocontrol industry worked together to provide scientifically-based data to resolve this issue it would increase the standing and credibility of the industry to Government policy makers and farmers alike.

We propose to undertake a national 'pheromone trial' with the assistance of biocontrol company partners to clarify the situation. The trial will be co-ordinated by **Dr K. Krishnaiah,** well known to you for his pioneering research on pheromones and a keen proponent of the technology.

We have attached a short questionnaire on the next page that we would be obliged if you could complete and return to inform us of your interest. We have also included a detailed protocol and background information on the field trial to enable you to judge the level of commitment required to participate in the trial. Of course any constructive comments on the project and our approach to the issues raised would be gratefully received and we will endeavour to incorporate them into our thinking.

With our best regards,

Dr Alan Cork

On behalf of the organising Committee:
Dr A. Cork, Reader in Chemical Ecology, University of Greenwich, UK
Dr K. P. Jayanth, General Manager, Bio-Control Laboratories Ltd., Bangalore
Dr K. Krishnaiah, Consultant, Hyderabad
Dr S. Narasimhan, Director, Asthagiri Herbal Research Foundation, Chennai

Company name:	

Preliminary Questions on Biocontrol Producers' Society

Would you join a society that represented your industry?	Yes		No	
Should the society be restricted to pheromones	Yes		No	
If not, then suggest scope of society				
How many officers in Society				
Suggested functions of officers				
Would you be interested to stand for election				
Terms of office? 1 Year, 2 Year, or more				
Comments of scope and function of Society				

GC course on analysis of pheromone products

Interested to attend GC course?	Yes		No	
How many personnel would attend?				
Comments on content / timing etc.				

Technical assistance with new pheromone products

Are you planning to produce new pheromone products?	Yes		No	
Are you interested to obtain technical assistance?	Yes		No	
Target pests envisaged				

Field optimisation of H. armigera *pheromone lure*

Would you like to participate in the trial?	
How many crops will you work in – specify?	
How many replicates per crop?	

HELICOVERPA ARMIGERA PHEROMONE BLEND TRIAL

Background

A recent publication by Tamhankar et al. (2003) suggests the presence of pheromone polymorphism in *H. armigera*. Academically this is a very interesting issue and will no doubt generate considerable new research effort to promote the idea. From a commercial point of view it is potentially quite damaging. Which blend or blends should be sold to farmers and does the proportion of each morph in a population vary in time and space? Do different morphs have different preferences for host crops? SMEs could each decide to sell lures containing different blends just to add to the plethora of dispensers and loadings currently available. The result will be additional confusion for farmers.

As you know we believe this provides an opportunity to bring SMEs involved in producing and marketing pheromone lures together to develop a unified policy on the issue. The best way to do this is for a number of volunteer companies to undertake field trials with the same blends at different locations, times and crops throughout the country. These data can then be collated centrally and the results used to define the 'best blend'. This information could then be used as the basis for an industry-wide standard and by presenting the data to Government the possibility exists for assisting them to identify the best parameters for the most effective attractant.

We can only think of two ways of interpreting the data published to-date. One is that there is pheromone polymorphism and the second is that the insects caught were actually different *Helicoverpa* species. The latter can be established by examination of insects caught in traps. This was not undertaken in published data to-date. The authors assumed that all the insects caught were *H. armigera*.

By working as a 'team' we hope that SMEs can produce a much more comprehensive data set than the previous study. This will allow informed discussion on this important issue and confirm, one way or the other, whether pheromone polymorphism exist in *H. armigera*, and if so, how many and where they exist.

We suggest that NRI provide the lures. A list of suggested blends is given below. The main differences between the blends we have suggested and those used by Tamhankar et al. are that the amount of the major component is fixed so that we only have one variable and we have widened the range of ratios slightly.

Taxonomy

Dr Jayanth has kindly offered to assist with the identification of samples of insects caught. In particular this will be undertaken using molecular biology tools although this will be backed up by classical taxonomy.

Crops

Because *H. armigera* is polyphagous and related species may have preferences for particular crop plants we need to undertake this work in a number of crops. We suggest that the number of crops is limited to four and for each crop the trial will be replicated at least three times per SME. Suggested crops include cotton, tomato, chickpea and pigeonpea.

SUGGESTED FIELD PROTOCOL

Pheromone traps	Plastic funnel trap (provided by SMEs)
Pheromone lures	Natural rubber septa with 2 mg of pheromone blends supplied by NRI
Number of blends	10
Number of replicates per crop	Minimum of 3, ideally 5.
Spacing between traps in replicate	Minimum 20 m.
Spacing between replicates	Minimum of 100 m.
Trap height	1 m above crop canopy (adjusted weekly)
Record trap catch	Every day
Trap rotation (clockwise movement of treatments in a replicate)	One position clockwise every day
Lure field life	20 days

Preliminary work

Identify potential crops for field trials. Contact farmers and obtain permission to work in their crops. You will require enough land for at least three replicates of the trial in a single crop (not necessarily a single field per replicate but keep age of crop uniform). Choice of crop depends on local availability and likelihood of attack by *H. armigera*. Suggested crops include cotton, tomato, chickpea and pigeonpea. *H. armigera* does not normally enter a crop before the flowering stage. Thus, place a single standard pheromone trap in each field selected to assess *H. armigera* adult population before committing to a full scale trial. When trap catch typically reaches more than 2 moths per trap per night and the crop is flowering then can make a final decision on which fields to use for the main trial.

Main trial

Plastic funnel traps should be attached to 3 m wooden supports to restrict movement. Trap height should be periodically adjusted to keep them 1m above crop height. A replicate should consist of ten traps, one for each of the ten treatment blends to be tested. Traps in a replicate should be placed in a circle with a minimum of 20 m between traps. The order of the treatments in a replicate should be randomised (note position of each treatment in each replicate). Three replicates should be placed in a single crop (cotton, tomato, chickpea, pigeonpea) with a minimum of 100 m between replicates.

Moth catches should be recorded from traps every day (or every two days) for a period of up to one month (two months if data collected every two days). Lures should be changed every 20 days (three lures will be provided for each treatment for each replicate – total field life of 60 days). Remove trapped insects after each data set is collected and ensure traps are in good working order. Replace damaged traps or lures that are lost and keep notes of lure replacements. The position of treatments within a replicate should be rotated one position in a clockwise direction after each trap catch is recorded. Thus for example, after ten days all the treatments will have occupied each position once.

Collate trap data (using forms provided below) and send by email to project field program coordinator, **Dr K. Krishnaiah**, once a week for data collected daily and once every two weeks for data collected every two days. Contact details will be provided once you have made a firm commitment to the trial. For each rotation, trap

catch data will be combined, averaged and statistically analysed by analysis of variance (ANOVA) after transforming to log (x + 1). Significant differences in treatment means at the 5% level or lower will be compared by LSD or Newman-Keuls multiple range tests.

Where possible provide maximum and minimum temperatures, averaged daily wind speed, and data on crop status (date of planting, age when trial started, start of flowering and fruit setting, information on impact of other pests and diseases might also be useful together with farmers views on impact of *H. armigera*).

Table 1. Treatments for field testing

	Ratio		Loading (ug)	
Treatment	Z9-16:Ald	Z11-16:Ald	Z9-16:Ald	Z11-16:Ald
1	0	100	0	2000
2	0.3	100	6	2000
3	1	100	20	2000
4	3	100	60	2000
5	5	100	100	2000
6	6	100	120	2000
7	7	100	140	2000
8	10	100	200	2000
9	15	100	300	2000
10	30	100	600	2000

***Helicoverpa armigera* pheromone blend optimization trial**

Researchers name:		Replicate number:	
Organisation:		Crop:	
Date started:		Crop stage:	
Location of trial:		Comments:	

Pheromone trap catch data sheet 1.

Date	Day	Treatment									
		1	2	3	4	5	6	7	8	9	10

Pheromone trap catch data sheet 2.

Date	Day	Treatment									
		1	2	3	4	5	6	7	8	9	10

Annex 2. Second Project announcement to SMEs

23 June 2005

Dear Colleagues,

Second Announcement
'ENABLING SMES TO PROMOTE PHEROMONE PEST CONTROL TECHNOLOGIES IN SOUTH ASIA',
Phase II

You will recall from our first announcement (5 May 2005) that we are now actively engaged on a second phase of the UK, Department for International Development funded project to provide technical assistance to SMEs engaged in the manufacture and sale of pheromone related technologies in South Asia.

We would like to thank you for completing and returning your questionnaires on the proposed activities outlined in the first announcement. The feed-back has provided us with valuable insight into your current needs and at a recent meeting at the Asthagiri Herbal Research Foundation (AHRF) we have endeavoured to reconcile these needs to refine our actions. The purpose of this second announcement is to up-date you on these actions and provide you with an opportunity to comment on progress.

Biocontrol Producers' Society for South Asia established to provide a common platform for commercial exploitation of pheromone and related products in the region
Based on comments we have received from you we have decided to restrict the scope of the Society's activities to pheromone and allied industries. By allied we are referring primarily to companies engaged in activities associated with the production of materials that are relevant to pheromone producers such a plastic and polymer producers (for traps and lures), pheromone components, GIS and IT companies with an interest in developing technologies for use in monitoring networks, organised crop production systems where pheromones would add value for organic production (tea, coffee, sugarcane, palm products), quarantine and storage companies where pheromones would have application for pest insects, animal and human health related industries (food producers, hospitals, hoteliers). To reflect these diverse linkages we propose the Society should be named the 'South Asian Society for Advancement of Pheromone Technology'. The Society recognises that pheromones are only one class of semiochemicals but that in order to reach a wider audience we have used the term pheromone, while the term technology has been included to encourage individual researchers to join in order to enrich the Society's access to technical expertise, although we have taken steps to restrict voting rights to the primary beneficiaries of the Society's activities, namely the industrial members.

We believe that we have now got sufficient support from industry to carry this process forward for the creation of a Society and have attached a copy of the draft rules and regulations for your perusal and comment. Please send comments in the first instance to **Dr K. P. Jayanth** for onward transmission to other members of the organising committee. It is our intention to launch the Society at a one-day meeting that will be called in early September. If we are able to achieve this milestone then we will be in a

position to recognise the pheromone analysis workshop that will be held immediately afterwards at AHRF, Chennai, as Society's first workshop.

Many respondents have indicated that they wish the Society to conduct research. It should be remembered that such activities are costly and time consuming. Should members wish the Society to undertake such activities then membership fees would have to be increased substantially and not all potential members would wish to contribute to such endeavours! We are only in a position to undertake research on the *Helicoverpa armigera* pheromone for this season because of the generous sponsorship received from the UK, DFID. Nevertheless, the Society will endeavour to conduct appropriate research where members indicate both a significant interest to see the research undertaken (e.g. market research) and can provide ad hoc contributions to support such activities. Moreover, the Society will lobby researchers and donors, such as DBT, enable research to be undertaken that has been identified by Society members as being critically important to the industry and their customer base. In particular the Society will encourage an integrated crop management approach and seek to promote pheromones in organic, high value crop production systems, animal and human health and related industries as highlighted in the rules and regulations.

Analytical personnel from ten SMEs trained to undertake quality assurance of pheromone products to comply with Government legislation
Plans for the training workshop are now well advanced. We have decided to hold a single workshop and extend the period of training to 3-days in order to provide more depth and allow time for practical sessions. While we believe that gas chromatography is a critical technique for analysing pheromones and related products we accept the need to put this in the context of other techniques and have increased the scope of training to accommodate issues raised by respondents to the questionnaire.

Proposed course structure

Introduction		
Session 1	Preparation of dispensers	Monitoring
		Mass trapping
		Mating disruption
Session II	Controlled release formulations	Polymer matrices used
		Effect of molecular structure on release
		Effect of polymer shape on release
		Effect of temperature on release
		Storage of formulations
Session III	Pheromone analysis	Gas chromatography
		Detectors
		FID
		EAG
		MS
		GC phases

		Kovat's indices
		Pheromone identification
		Quality assurance
		Case studies
Session IV	Demonstrations	Analysis of pheromones
		Determination of release rates from dispensers
		Residue analysis
		Air entrainment
		Quantitation
		Use of internal and external standards

As previously mentioned the project can not provide funding for accommodation or travel costs for attendees at the workshop, although light refreshments and lunch will be provided during study periods. However, in order to make local travel arrangements more straightforward we would be happy to assist with locating accommodation and transport to AHRF. As mentioned earlier we hope to conduct the workshop immediately after the inauguration ceremony of the Society in early September. The dates will be announced by **Dr S. Narasimhan** once we have established a venue for the ceremony and received confirmation of attendance by invited dignities.

Pheromone monitoring and control systems for key rice stem borer and sugarcane borers commercially developed and promoted by at least two SMEs

This activity is designed to provide those SMEs who are thinking of commercialising pheromone products in these crops with technical assistance. However, it was apparent from the responses we received to the short survey form that companies are thinking of a much wider range of products, or at least, species of insects. Issues that will be important for SMEs wishing to extend their product range will relate not only to sourcing pheromone blends and identifying potential customers but also how the technology will benefit the customer and the importantly the mode of action of the product, simply demonstrating an ability to catch insects in traps may not be sufficient to sustain sales. Can the semiochemicals be used for control and would the cost of mating disruption, the preferred method of choice for controlling insects with pheromones, be justified and more sustainable in a high value export crop for example.

Crop management role of Helicoverpa armigera *pheromone in South Asia, defined and blend composition resolved.*

We were delighted with the positive response we have received from companies keen to engage in this research effort and we are now actively in the process of disseminating lures for the trial. Currently we expect there will be almost 70 times replications of the trial in five crops (cotton, tomato, chickpea, red gram and chilli). The trials will be conducted in a range of agro-climatic zones across the region and by continuing the trials over at least 2-months data will reflect periods of high and low moth populations. **Dr K. Krishnaiah** will contact participants soon to confirm trial activities and provide a detailed field protocol. We realise that given limited time and resources we will not be able to provide comprehensive coverage of the entire region but that was never our intention. The trial will serve three objectives:

a) To provide SMEs with experience of conducting field trials to optimise lures. This knowledge will be useful for validating new company products,

b) Results from the trial will provide individual SMEs with scientifically rigorous data that they can show to customers to demonstrate that the pheromone blend they are offering is optimised for use in their region,

c) To demonstrate that by collaborating the industry can produce outputs that have regional significance to policy-makers and will act to give credibility to the aims and objectives of the Society and the companies that it will represent.

The results of the trial will provide a valuable insight into the optimal *H. armigera* pheromone blend in different crops and regions but will nevertheless not provide a basis for understanding why the blend should vary. Such an understanding can only be achieved through parallel research into the variability in the population of insects present. Such insight is traditionally achieved through related taxonomic studies with samples of the insects caught. However, I am pleased to inform you that through the efforts of Dr K. P. Jayanth, Dr. B. Fakrudin, a talented molecular biologist from UAS, who has studied the genetic variability of *H. armigera* in Southern India has agreed to undertake genetic studies of selected samples from the field trial in an effort to ascertain the molecular basis for any variability found in the response of *H. armigera* to the pheromone blends tested.

It is the intention of the organizing Committee to make available the results from the *H. armigera* trial initially to those who have actively contributed and subsequently to a wider audience at the second Society workshop.

As always we appreciate any constructive comments you may have on the issues that we have raised and actions we have initiated.

With best regards,

Dr Alan Cork

On behalf of the organizing Committee:
Dr A. Cork, Reader in Chemical Ecology, University of Greenwich, UK
Dr K. P. Jayanth, General Manager, Bio-Control Laboratories Ltd., Bangalore
Dr K. Krishnaiah, Consultant, Hyderabad
Dr S. Narasimhan, Director, Asthagiri Herbal Research Foundation, Chennai

Annex 3. Declaration of Trust for South Asia Society for Advancement of Pheromone Technology

DECLARATION OF TRUST

This Deed of trust executed on the 15th day of September 2005 at Bangalore between:-

1. Dr. K. P. Jayanth, representing Bio-Control Research Laboratories, A division of Pest Control (India) Pvt. Ltd., 36/2, Sriramanahalli, Near Rajankunte, Arakere Post, Bangalore – 561 203 aged about 54 years, S/o. M. P. Mukundan and presently residing at Block 2, 202, Heritage Estate, Yelahanka, Bangalore 560 064.

Hereinafter be called as "Managing Trustee"

2. Dr. M. S. M. Christopher, representing Margo Biocontrols Ltd., 344/8, IV Main, Sadashivanagar, Bangalore – 560 080, aged about 48 years and S/o Mr. M. Moses Sundara Raj and presently residing at 3655, II Floor, I Main, 5th Cross, Gayathrinagar, Bangalore – 560 021.

3. Mr. Saurabh Singhal, representing Biotech International Ltd., VIPPS Centre, 2, Local Shopping Centre, Block EFGH, Masjid Moth, Greater Kailash-II, New Delhi – 110 048, aged about 37 years and S/o Mr. Vivek Singhal and presently residing at 24, Palam Marg, Vasant Vihar, New Delhi – 110 057.

4. Mr. Kaja Raghunath, representing Pheromone Chemicals, 404, Arpitha Heights, HMT Nagar, Nacharam, Hyderabad 500 076 aged about 32 years and S/o Kaja Narasimha Rao and presently residing at 404, Arpitha Heights, HMT Nagar, Nacharam, Hyderabad 500 076.

5. Mr. D. N. Nagaraj, representing Bio-Pest Management Pvt. Ltd., 59/1, 8th Cross, 5th Main, Radhakrishna Layout, Padmanabhanagar, Bangalore 560 070, aged about 41 years and S/o Mr. D. Narayan and presently residing at No. 597, Taliru, Bank Officers & Officials House Building Co-op Soc. Ltd., Doddakallasandra, Kanakapura Road, Bangalore – 560 062.

6. Mr. O. R. Murugadas, representing Basarass Biocon (India) Pvt. Ltd., 24/7, Dr. Radhakrishnan Salai, Palaniappan Nagar, Valasaravakkam, Chennai – 600 087, aged about 39 years, S/o Mr. O. Ramadass and presently residing at 4/1, II Cross street. I Main Road, Natesan Nagar, Virugambakkam, Chennai – 600 092

Hereinafter be called as "Founder Trustees"

Whereas we are all engaged in the development of Pheromone Technology, and representing our respective organizations, are desirous of forming a common platform for the development of and Research in Pheromone Technology and with that object have decided to Form a Trust and have consented to be the First Board of Trustees for the above purpose.

NOW THEREFORE this indenture of Trust witnesses as follows: -

1**. Creation of Trust: -** The trust created by these presents shall be known as **"South Asian Society for Advancement of Pheromone Technology"**. The Trustees do declare that they shall hold and stand possessed of all sums and monies, securities, properties both movable and immovable which the trustees may hereinafter receive or

acquire for this trust and all additions and accretions thereto and Incomes therefrom shall stand vested in the Trustees in office upon Trust for the promotion of the objects of the trust in accordance with these presents.

2. **Office of the Trust: -** The Principal Office of the Trust shall be at Bangalore. Presently its office shall be located at the premises of Bio-Control Research Laboratories, A division of Pest Control (India) Pvt. Ltd., 36/2, Sriramanahalli Village, Hessaraghatta Hobli, Bangalore North Taluk (Present postal address: 36/2, Sriramanahalli, Near Rajankunte, Arakere Post, Bangalore – 561 203). It shall be open for the Trust to change the location of the Office, to open and close Branch Offices/ Offices at such other places as may be decided by the trustees.

3. **Nature of the Trust: -** This trust shall be a Public Charitable Trust. All properties funds and Income therefrom shall be applied to and solely for the purpose of this Trust without any distinction as to Caste, Creed, Language, Gender, Region or Religion.

4. **Funds of the Trust: -** The trustees herein have together contributed Rs.5000/- (Rupees Five Thousand Only) as Trust Funds.

5. **Number of Trustees: -** The property and its management shall vest in the Board of Trustees. The first in the Board of Trustees comprises of 6 Trustees as hereinbefore stated. The total strength of the board of trustees shall stand restricted to 15 at any point of time.

6. **Management Committee Members: -**

a) As already stated the persons who have executed these presents shall be the members of the First Executive Committee i.e., Governing Body to whom the management of the Trust is entrusted under the Rules & Regulations of the Trust for the First 2 years. The governing body shall elect one among them as the Managing Trustee who shall preside at all meetings of the Trust. In the absence of the Managing Trustee, the majority of the Trustees who are present at the meeting shall elect a Chairman for the meeting. Dr. K. P. Jayanth will be the first Managing Trustee.

b) Quorum: - The quorum required for any meeting shall be 60% of the total number of trustees. All decisions of the trust shall be taken by a simple majority of the trustees present and voting at a meeting unless otherwise stated elsewhere. In the event of a tie the Chairman will have a casting vote.

c) The Managing Trustee, presently Dr. K. P. Jayanth, shall be Ex-officio and shall hold office initially for 2 years, and shall be eligible to be re-appointed.

d) One third of the other members of the governing body shall retire from Office every year but will be eligible to be re-elected.

e) **Construction of the Trust Deed: -** In matters wherein the Trustees have discretionary powers or wherein there is a difference of opinion regarding the construction of these presents or the management of the Trust fund or any part thereof or the execution of any of the terms or powers of these presents, or as regards any act or things to be done by the Trustees, the votes of the majority of the trustees for the time being, voting in the matter, shall prevail and binding on all the trustees including those who may not have been present or voted and if the trustees be equally divided in opinion, the Chairman shall have a casting vote.

f) **Powers of the President/ Managing Trustee:** - Without prejudice to the generality of the powers of the Managing Trustee, (also known as President) he

shall have the powers detailed in Article 22 of the Rules & Regulations of the Trust.

7. The affairs of the Trust shall be carried out in accordance with Rules and Regulations of the Trust annexed hereto which shall from part of this Trust Deed.

8. The trust formed hereby shall be irrevocable.

RULES AND REGULATIONS

Article 1: – Name
The name of the Trust shall be **"South Asian Society for Advancement of Pheromone Technology ".** Hereafter referred to as the 'Trust'

Article 2: – Address for correspondence
C/o. Bio-Control Research Laboratories, A division of Pest Control (India) Pvt. Ltd., 36/2, Sriramanahalli Village, Hessaraghatta Hobli, Bangalore North Taluk (Present postal address: 36/2, Sriramanahalli, Near Rajankunte, Arakere Post, Bangalore – 561 203), unless and until changed by the Trustees.

Article 3: – Founding Date
The Trust shall be considered founded on 15th September 2005.

Article 4: – Founder Members
The Founder Members of the Trust shall be those actually present on the date of founding of the Trust and whose signatures are taken in the Trust Deed and herein below.

Article 5: – Area of Operation
South Asia, for the time being.

Article 6: – Objectives
1. To provide a common platform for commercial exploitation and promotion of pheromones, other semiochemicals and related products in South Asia, and to carry out research in the development and application of Pheromone Technology and Allied Science.
2. To promote close relationships and better understanding among manufacturers engaged in organic farming, post harvest, animal and human health activities.
3. To provide a medium for exchange, discussions and dissemination of current developments by holding meetings, workshops, training programmes, etc., for Scientists and for the benefit of Farmers in the field of latest Pest Control Measures.
4. To publish an electronic newsletter "Phero-News, South Asia", containing articles, information, etc, for circulation among Members, including vernacular printed publications and for this purpose develop its own website and other facilities.
5. To develop standards for quality assurance of pheromones and other semiochemical products.

Article 7: – Membership
1. Membership of the Trust shall be open to all registered companies, Firms, Concerns and Individuals engaged in production, manufacture, selling and distribution of pheromone products and related technologies. Membership is also open to individuals interested in the above fields. Any such eligible company/ individual may apply for

Membership in the prescribed proforma to the Secretary by paying the prescribed Admission and Membership fee. Membership shall be subject to acceptance of the application by the Managing Committee.

2. There shall be three classes of Members, viz, Member, Associate, Honorary.

a. Membership is open to companies and concerns engaged in production of pheromone products or other allied activities who pay the prescribed Membership fee.
b. Associate Members are those Researchers and individuals who have an abiding interest in the science of semiochemicals.
c. Honorary Members are those who have contributed substantially to the cause of development and the promotion of pheromones. No Membership fee will be collected from such Members {Honorary Members will be invited on recommendation to the Trust Managing Committee}.

3. Any Member who defaults on payment of the dues for one year shall cease to be a member of the Trust at the end of that year. Defaulted Members shall forfeit Membership privileges. By payment of arrears any defaulted member may apply to the Trust for reinstatement. Membership fee shall be paid in advance.

4. A Member may withdraw from the Trust by expressing his desire to do so in a letter addressed to the Secretary. The Trust, however, shall not be liable to return any fee that may have been paid by the Members in advance

Article 8: – Patrons
Any person or Institution, donating a sum of not less than Rs.100,000/- to further the objectives of the Trust, shall be considered a Patron of the Trust. The Patron or a representative of the Patron shall enjoy all the privileges of a Member.

Article 9: – Privileges of Members
1. Members have the right to vote and hold office and shall further be entitled to issues of the Newsletter and any other publications of the Trust as the Executive Committee may determine. They shall also have the privilege of introducing visitors or guests to the meetings of the Trust.

2. Associate and Honorary Members shall enjoy all privileges of Members, except the right to vote.

Article10: – Country Representatives
Country representatives are nominated members appointed by the President to work for the development and welfare of producers of pheromones in their respective regions and shall work in close association with the President (Managing Trustee).

Article11: – Membership Fee
1. The Membership fee, to be paid annually, shall be as fixed by the Board of Trustees from time to time.

2. Payment in foreign currency shall be in the form of International Bank Draft.

Article12: – The Financial Year
The financial year of the Trust will begin on the first of April and end on the 31st of March in the following year.

Article13: – Functions and power of the Executive Committee

1. There shall be an Executive Committee, which shall carry on all the affairs of the Trust. The Executive Committee shall not act contrary to the general policy of the Trust and sanctions of the Executive Committee shall be subject to review at the next meeting of the Trust. The Executive Committee shall meet at least once in six months.

2. In case the Executive Committee meeting is not possible, decisions may be taken by correspondence which shall be notified at the next meeting of the Executive Committee. The period of the Executive Committee, unless extended, shall be for two years.

3. The Executive Committee shall consist of the following:

a. President	1
b. Vice-President	2
c. Secretary	1
d. Treasurer	1
e. Members	2

4. All Executive Committee Members shall be elected by the General Body by secret ballot.

Article14: – Duties and functions of the office bearers

1. President (Managing Trustee): - Shall preside over meetings of the Executive Committee and General Body. He/ she shall have the power of taking decisions on matters by email correspondence with the Executive Committee Members. He/ she shall be the Chief Executive Officer of the Trust. He/ she shall execute decisions taken by the Executive Committee and call for meetings of the Executive Committee. His/ her prime duty shall be to work for the promotion of the Objectives of the Trust by lobbying and liaising with policy makers, growers and input producers and associated stakeholders. He shall have the Powers stated in Clause 6 (f) of the Trust Deed/ Article 22 hereinafter contained. Subject to the approval of the Executive Committee in office for the time being the President shall have power to co-opt additional trustees to the Executive Committee, within the limits laid down by clause 5 of the Trust Deed provided however that such co-opted trustees shall hold office for the remainder of the term in the relevant financial year, provided further that they will be eligible for reappointment subject to Article 13.

2. Vice-President: - The duties shall include assisting the President to promote the Objectives of the Trust, organizing workshops and production of Trust publications, and officiating for the President during his absence from Headquarter.

3. Secretary: - The Secretary shall organize all Trust Meetings, prepare and file annual reports of the Trust, handle day-to-day correspondence, make reports of activities to the President and execute the orders of the President. He shall be responsible for maintaining the Minutes of the meetings of the Executive Committee and the General Body. He shall also be responsible to submit all Statutory returns, if any, in time to the Statutory Authorities, Taxation authorities, etc.

4. Treasurer: - The Treasurer shall be responsible for the financial affairs of the Trust. He/ she shall keep accounts, receipts, collect and deposit and disburse money on behalf of the Trust. By the beginning of January he/ she shall send notices to every member for the Membership fee for the next year.

A Bank account in the name of the Trust shall be opened in a Scheduled Bank and shall be operated jointly by any two of the three office bearers, viz., President,

Secretary and Treasurer. Honoraria and salaries to office bearers shall not be permissible or allowed.

Article15: – Meeting of the Executive Committee

1. Executive Committee meetings shall be held periodically at the discretion of the President or on the request of at least three Members of the Committee.

2. The date, venue and time of meetings shall be notified by the Secretary to each of the Committee Members, at least three weeks before the date on which such meeting are due to be held. Non-receipt of such notice or postal delay in the notice reaching any member shall not invalidate the proceedings of such meetings. A minimum of three Members or 60% of the strength of the Executive Committee whichever is high are required to complete the quorum.

3. All decisions will be by consensus, in the absence of which voting will be resorted to.

4. Draft Proceedings of Meetings shall be circulated to Executive Committee Members immediately after the meeting, to get their concurrence on the matter reported in the minutes.

Article16: – The Editorial Board

The Executive Committee shall constitute an editorial board with an Editor and Two Associate Editors for bringing out the newsletter of the Trust. The Editor shall be responsible for the timely publication of the Official Newsletter. The Committee will amend the editorial policies of the Newsletter at their discretion. The period of a Board Member's appointment to the Committee, unless extended, shall be two years.

Article 17: – Accounts and Audit

The Accounts of the Trust shall be maintained day-to-day so as to reflect the true and correct nature of the transaction. All monies received shall be kept deposited in a Banking Account in the name of the Trust immediately on collection. The Accounts shall be maintained in accordance with Applicable Accounting Standards prescribed by Law, if any, and generally accepted Accounting Principles followed in India.

Article 18: – Functions of the General Body

1. The General Body Meeting of the Trust shall be held annually before the expiry of six months from close of each financial year and is open to all Members with voting rights.

2. To present the report of the action taken on the decision of the previous meeting, initiate discussions for the current year and audit report of the Trust.

3. To appoint auditors and fix their remuneration.

4. To elect office bearers once in two years.

Article 19: – General Body Meetings of the Trust

1. The time, date and venue of the Annual General Body Meeting shall be arranged by the Executive Committee. The venue shall be fixed by the Committee according to facilities and convenience of the Members.

2. The Agenda for the General Body Meeting shall be circulated at least one month in advance.

3. Extraordinary Meetings of the Trust shall be convened if one third of the Members of the trust send a signed requisition to the Secretary for such a meeting. An emergent or extraordinary meeting of the General Body may be called by the Executive Committee by giving Members 21 days notice.

4. Meetings of the Trust shall be presided over by the President or in his absence one of the Vice-Presidents/ or any member of the Executive Committee elected at the Meeting.

5. All decisions will be by consensus, in the absence of which a secret ballot vote will be conducted but restricted to those members in attendance.

Article 20: – Headquarters of the Trust

The Headquarters of the Trust shall be at the Premises of Bio Control Research Laboratories, A division of Pest Control (India) Pvt. Ltd., 36/2, Sriramanahalli Village, Hessaraghatta Hobli, Bangalore North Taluk (Present postal address: 36/2, Sriramanahalli, Near Rajankunte, Arakere Post, Bangalore – 561 203), until otherwise decided by the Trustees.

Article 21: – Election of Office Bearers

1. All elections of Office Bearers shall take place at the General Body Meeting of the trust. The Executive Committee shall co-opt Members to fill interim vacancies.
2. The following procedure shall be adopted for all the elections to the Office:-
    a) The Secretary shall circulate a list of Members three months before the Annual General Body Meeting. He/ she shall invite nominations, duly seconded by another member and shall ascertain the willingness of the nominee for the office two months before the election. The list of Members' nominees shall also be circulated 30 day prior to election. The presiding officer should not contest for any office of the Council.
    b) All voting shall be by secret ballot. The ballot paper will be supplied by the Secretary and returned as advised by the Secretary.
    c) Votes shall be scrutinized by persons nominated for the purpose by the Executive Committee.
    d) A majority shall elect. In the event of a tie the outcome shall be decided by drawing lots.
3. All retiring office bearers shall be eligible for re-election.

Article 22: – Powers of the Managing Trustees, (President)

1. To delegate any powers as he can lawfully delegate to any other person and to execute such power as he may think fit for the purpose, nevertheless retaining the ultimate control and supervision.
2. To convert, to call in, to sell or otherwise dispose of any of the investments comprised in the Trust property and reinvest or change the nature of any other investment contained therein but only with the prior approval of the Board of Trustees.
3. To make investments from the funds of the Trust in the modes specified under the provisions of Sec.13(1)(d) read with Sec.11(5) of the Income Tax Act, 1961, as approved by the Board of Trustees.
4. To withdraw any power or revoke any appointment of any employee or attorney.
5. To appoint and to terminate the services of or impose punishment on any employee of the Trust and fix remuneration of staff, consultants, etc. However, the Board may rescind the order or action of the Managing Trustee, after due deliberation and for reasons to be recorded.

6. To let out any portion of any immovable property acquired out of the Trust Funds at such rent and for such period and on such terms and conditions as he may think fit and accept or surrender of any lease with the written consent of the Board of Trustees.
7. To appoint proxy or proxies for voting at any meeting of creditors, contributors, shareholders or otherwise.
8. To borrow funds for the purposes of the Trust with or without security of the property and for this purpose to mortgage, charge and encumber any asset, both movable and immovable of the Trust with the written consent of the board of Trustees.
9. To open and operate a Banking Account in accordance with the provisions in Article 14 herein above.

Article 23: – Funds

The funds of the Trust shall consist of (a) Membership fee, (b) Patron fee (c) Donations, (d) Grants in aid from Govt. Institution, Societies or individuals interested in the activities of the Trust, (e) Charges for publishing advertisements in the publications of the Trust and (f) any other sources determined by the Executive Committee.

Article 24: – Investments

The Trustees shall make investments of the funds of the Trust in accordance with and in the modes specified under the provisions of Section 16(1)(d) read with Section 11(5) of the Income Tax Act, 1961.

Article 25: – Exemptions from Taxes

The Powers of the Trustees shall be so exercised as to ensure that the Income of the Trust would be exempt Under Section 11 of the Income Tax Act, 1961 read with Sections 12 and 12A as amended from time to time. The Power of the Investment of the Company of the Trust funds shall be exercised in accordance with provisions of Section 13 of the Income Tax Act, 1961, in general and in particular shall not violate the provisions of Section 13(1)(d) read with Section 11(5) of the Income Tax Act.

Article 26: – Utilization of the Trust Funds

The funds and Income of the Trust shall be utilized solely in pursuance of the objects of the Trust and no portion of it shall be utilized for payments to trustees by way of Profits, Dividends, Interest, etc.

Article 27: – Indemnity

The Trustees shall at all times be kept fully indemnified and harmless by the Trust against any action, claim demand or liability arising to them for anything done by them in good faith pursuant to the power and authority vested in them under these presents.

Article 28: – Amendments

1. The above rules may be amended at any Special General Body Meeting of the Trust by voting for the amendment. Any amendment proposed by a member should be forwarded to the Secretary two months in advance of the Special General Body Meeting for circulation among the Members.
2. No amendments to any of the clauses of the Trust Deed and/ or Rules and Regulations shall be made which may prove to be repugnant to the provisions of Section 2(5), 11, 12, 13 and 80G of the Income Tax Act, 1961 as amended from

time to time. Further no amendments shall be carried out without the prior approval of the Commissioner of Income Tax.

Article 29: – Financial Status, Dissolution and Amalgamation

1. The Trust is a non-profit body. No member of the Trust shall be entitled to any distributive share of its assets and profits. In the event of dissolution, its assets, remaining after payment of its just debts, shall be given to public purpose or to another Trust whose objectives are similar to those of this Trust and which enjoys recognition under Section 80 G of the Income Tax Act, 1961 as amended from time to time.
2. Dissolution or amalgamation of the Trust shall be decided by a consensus of Members.

Article 30: – The benefit of the Trust shall be open to all irrespective of caste, creed or religion or region.

In witness whereof the parties here to have set and subscribed their respective hands and seals hereunto on this 15th day of September 2005 in the presence of witnesses.

Witnesses:-	Trustees: -
1.	1.
2.	2.
	3.
	4.
	5.
	6.

Annex 4 List of workshop participants

'Quality control of Pheromones' workshop, Chennai, 10-12 October 2005

No.	Name	Company
1	Mr K. N. Anand	AgBiosystems Pvt. Ltd., Secunderabad
2	Mr Ramchandra Awalekar	Bio-Control Research Laboratories Ltd., Bangalore
3	Dr (Mrs) K. P. M. Bhanu	Bio-Control Research Laboratories Ltd., Bangalore
4	Mr T. Boopathi	SunAgro Biotech Research Centre, Chennai
5	Ms Samadara Dissanayaka	Serendib Natural Products Ltd., Sri Lanka
6	Mr Jaisankar	Basarass Biocontrol Pvt Ltd., Chennai
7	Mr P. Laxminarayan Rau	AgBiosystems Pvt. Ltd., Secunderabad
8	Mr Paraq Jhaveri	Yasho Industries Pvt Ltd., Mumbai
9	Dr K. Krishnaiah	Consultant
10	Mr P. S. R. Murthy	S.V. Agricultural College, Tirupathi.
11	Mr O. R. Murugadass	Basarass Biocon Pvt. Ltd.
12	Mr D. N. Nagaraj	BioPest Management Pvt. Ltd., Bangalore
13	Mr Ashvin Patel	Ganesh Bio Control Systems, Gondal
14	Mr Mukesh Patel	Agriland Biotech Ltd, Baroda
15	Mr Prabhu	Basarass Biocontrol Pvt Ltd., Chennai
16	Mr Raghavendra	Margo Biocontrol Ltd., Bangalore
17	Mr K. Raghunath	Pheromone Chemicals, Hyderabad
18	Mr Mandar R Shedge	Bio-Control Research Laboratories Ltd., Bangalore
19	Mr S. Singaravelu	SunAgro Biotech Research Centre, Chennai
20	Prof. S. Srinivasan	S.V. Agricultural College, Tirupathi.

Annex 5 Feedback from workshop participants

'Quality control of Pheromones' workshop, Chennai, 10-12 October 2005

- The workshop on 'Quality control of Pheromones' was very useful. Whatever information was told by Dr Alan Cork was important and absolutely beneficial. Particularly selection of columns, GC analysis, comparative studies of lures from various companies was very useful.
- Also the how much loading of pheromones, election of dispenser is important. This information was very important.
- The main is storage of pheromones at -20^{o}C and what is the effect of temperature on the half life of pheromones, this information was very important.
- Such type of programme should be arranged. I would like to attend such type of programmes and I am thankful to give me opportunity to attend the programme. I am also thankful to Dr Cork and Dr Narasimhan.

Yours sincerely,

Mr. Ramchandra Awalekar
BCRL, Bangalore

- Session on column selection was very knowledgeable.
- I would like to attend such workshop in future on pheromones.
- If you could explain more about antioxidants that will be better for lure preparation.
- I would also like to know about the various techniques for impregnation of lures.
- But all together Dr A Cork has really increased our knowledge about how can we control quality of our product.

Mr. Mandar R. Shedge,
BCRL, Banalore

- All the theoretical aspects of the GLC were covered but we could not do the practicals much.
- The lab facilities here are very good. All the members of the Institute are very co-operative and their response has been commendable.
- Dr Alan Cork is a nice, highly talented person and more pragmatic. Our interaction with him has been highly informative.
- Dr Narasimhan and his team have been really doing a good job towards R&D work on the pheromone.

Mr. Raghavendra
Margo Biocontrol Pvt Ltd., Bangalore

- The workshop is very useful to know detection of volatile compounds (i.e.) pheromone lures and also the other instrument for purity the chemicals with solvent (hexane).
- Teaching is good, deliver the points, explanation and point out the examples.
- The pheromone release rate is composition with field and lab conditions. It is very good data for known the field condition and lab conditions.
- Isomers ZZ, ZE, EZ, EE. These isomers useful to trap the species specific and also similar genus eg *E. vittella* and *E. insulana*. According to the isomers the catching is there.
- Arrangement is good
- Share the experience among the participants it is good me.

Unsigned.

Workshop was comprehensive and knowledgeable. Appreciate Dr Cork effort.
Like to have this kind of workshop twice a year.

Mr. Pavag Jhaveri
Yaso Industries Pvt Ltd., Mumbai

- It's very fine to have some good answers on quality of pheromones. In fact a lot of technical knowledge which may not be understood well by non-chemists like us. But we got a good understanding of quality control aspects of pheromones, handling, storage and preparation of lures.
- It would be most helpful for us if we can have a detailed methodology in printed form on how carry on certain quality control aspects (like rubber septum quality, structure and any standards for quality) with release formulations with pheromones. And information in particular like in case of *Leucinodes* where release rate and performance of lure is most related to mode of release septum vs plastic vial.
- We would like our society to organize more workshops in future to help us have some understanding on latest technology, development, set-up and follow certain industry standards to enable industry gain its reputation from end customers – Government and farmers.

Mr. K. Raghunath
Pheromone Chemicals, Hyderabad

- Lectures are very good and informative
- One workshop for every alternative year/every year would be useful with the latest developments for pheromone technology

Dr (Mrs) K. R. M. Bhanu,
BCRL, Bangalore

- Right from the launch of the Society to training was really well conducted and with hospitality is most appreciated.
- Dr Alan's lectures were really a mind opening session and the interaction with the heterogeneous group (mixture of chemists and entomologists) was really beneficial.
- The practical session though organize very well due to power-cut was a slight setback.
- Standby in future in such meetings will be helpful for fulfilment of training.
- The study material if circulated as CD it will be very nice with footnote.
- Frequent at least two such workshops in a year will be a welcoming one for updating as I have learnt out of this workshop a new concept on 'Quality control of pheromones' – That is the release rate of the pheromones is the key factor for benefit to the Society.
- From the quantifying method now we are forced to look into the quality methods.
- The opening up of mating disruption technology in India will be possible is clear. Through this workshop and the avenue for pheromones in India is explorable now.
- I welcome such training and workshops.

Mr. O. R. Murugadass
Bassarrass Biotech pvt Ltd., Chennai

- Sessions were organized very well.
- It has covered so much detail to overcome the difficulties that we meet in the fields as well as in the laboratory.
- I suggest that we should have meetings once a year.
- In the meantime we should have up-date our outcomes among the members in the society.
- Next workshop will be included more details from the instrument side.

Ms. S. A. Samadara Dissanayaka
Serendib Natural Products Ltd, Sri Lanka

- It's really good beginning made by forming a Society or advancement of pheromone technology. I take this opportunity to wish the efforts of Dr Alan Cork, Dr S. Narasimhan and Dr K. P. Jayanth for their efforts to bring together the manufacturers, researchers of sex pheromone traps and lures, and also Asthagiri Herbal Research Foundation for providing their facilities for practical training on analysis of pheromones.
- A small suggestion is that, when the website of the Society is created, any work carried out by individuals, companies or any research papers (published) if someone comes across, can be shared among members by putting them on the website, by doing so one can come to know what's happening around and the members can share their thoughts and doubts and get them clarified.
- The technical session by Dr Alan Cork was really informative and I wish him for his efforts and sharing his works carried out. His suggestions are really useful to us.

Mr. D. N. Nagaraj
Bio-Pest Management Pvt., Ltd.
Bangalore

- PowerPoint presentation of course Director, Dr Alan Cork, on 'Quality control of pheromones' is excellent.
- Literature, infrastructure i.e. chairs, room etc. is satisfactory.
- Coffee, lunch and snacks is very excellent.
- Practical training is also very good.
- I received good support from M.R.M.K, J. Joseph, J.P. and others.
- As I am, this is the first workshop attending in my career. I gained lot from Dr Alan Cork on pheromones.
- Overall, I am very thankful to Dr Narasimhan for giving this opportunity.

Thank you once again.
Unsigned.

- The workshop is very useful. Needs some more pre-planning.
- Excellent lecturing by Dr Alan.
- Some more details on septa quality and loading studies for *Spodoptera, Pectinophora* and *Earias* and pheromone trap design needed.
- Practical operation of GC by every member should be made possible.
- The Society can meet at prefixed interval.
- We can list the suppliers and producers name in the website.
- In the FAQ section common doubts about usage can be explained.

Mr. Prabhu,
Basarass, Chennai

- I am very happy to participate in ceremony function of 'South Asian Society for Advancement of Pheromone Technology' and workshop on 'Quality control of Pheromones' held at Asthagiri Herbal /research Foundation.
- Course is conducted by Dr Alan Cork is quite informative and useful for day-to-day production, research, development and marketing of pheromone traps and lures. We learnt first time chemistry and analytical part of the pheromones and that will very much useful for our further pheromone development programme.
- I am very thankful to Dr Narasimhan, Director, AHRF providing their small but excellent laboratory for demonstration of analysis of pheromone lures.
- I hope that society will conduct another program particularly focused on pheromone technology on sugarcane pest management in future so sugarcane farmer gets some useful tool for management of pests. Otherwise spraying is very difficult to sugarcane crops.

Again thanks to Dr Alan Cork and Dr Narasimhan

Mr. Mukesh Patel
Director, Agriland Biotech Ltd., Baroda, Gujarat

Annex 6 Minutes of first AGM of Society

Minutes of the Ist General Body meeting of South Asia Society for advancement of Pheromone Technology held on Sunday, the October 9, 2005 at Hotel Residency Towers, Chennai.

The following members have been elected by the members present in the meeting.

	Position	Name of the Company	Represented by
1)	President	Biocontrol Research Laboratory	Dr. K.P. Jayanth
2)	Secretary	Margo Biocontrols Pvt. Ltd	Dr. M.S.M. Christopher
3)	Vice President	Biotech International Pvt. Ltd.	Mr. Saurav Singal
3)	Vice President	A.G. Biosystems Pvt. Ltd.	Mr. Laxminarayan Rao
4)	Treasurer	Basarass Biocon (I) Pvt. Ltd.	Mr. Murugadas
5)	Member	Agriland Biotech Limited	Mr. Mukesh Patel
6)	Member	Yasho Industries Pvt. Ltd.	Mr. Parag Javeri
7)	Member	Sun Agro Industries Pvt Ltd	Mr Siddanandam

The following points were discussed in the meeting.

1) Membership fees

The members proposed a fee of Rs. 25000/- towards admission and membership fees for the society. The executive committee will decide an Annual membership fees during their next meeting.

2) Bank Account

A resolution has been passed by the members to open a running account with HDFC Bank at Bangalore which will be operated by either of two authorised persons mentioned.

a) President
b) Treasurer
c) Any one member

3) Editorial Committee

The members also elected Dr. Alan Cork, National Research Institute, UK as a head of the editorial committee of the society's magazine/ newsletter

1) Corpus fund

A corpus fund of Rs. 5000/- will be deposited in the Bank Account.

2) Appointment of Chartered Account

Mr. Devi Lal & Associates will be appointed as Chartered Accountant to look after the accounting activities of the society and a service fees of Rs. 2000/- is also been approved by the members present.

6) Income Tax Rebate

The society will avail Income tax rebate under Section 80G as per rules prevailing of Income Tax Act.

7) PAN No.

The society will also apply for PAN No. registration based on the requirements.

8) Day to Day activities

The executive committee will decide on the accessories and inputs required to carry out the day to day activities of the society. The activities would take care of the printing of regular stationeries, letter heads, stamps and others based on the need.

9) The members also proposed to invite and appoint Dr. Alan Cork as the Honorary member of the society.

10) Website

The members also proposed to create a website and will be looked after by Mr. Murugadas of Basarass Biocon. The estimated cost will be Rs. 15000/- to create a website. He would take the help of Dr. Alan Cork and other members in creating the website.

The members also proposed to invite talks on relevant subjects by the experts at the places convenient once in a year.

The committee will send circular of the next meeting to be held.

Annex 7 Methodology used for molecular biology study

Collection of *Helicoverpa armigera* from different treatments:

The moths that were differentially attracted to different lure compositions at Bangalore, Kolar, Belgaum, Sangareddy, Jogipet and Karnool in different crops formed the source. Details on moth received for DNA analysis is presented in Table 1.

Genomic DNA isolation

Genomic DNA was isolated from individual moths following the procedure of CTAB (Cetyl Trimethyl Ammonium Bromide) method, described by Murray and Thompson, (1980) with some modification. The moths were wrapped with tissue paper to drain the alcohol. The wings were removed retaining the whole body. Three fourth of the abdomen was cut and kept in separate eppendorf tubes for further taxonomic studies and the resulting portion of the insect was used for preparation of genomic DNA.

Genomic DNA extraction Protocol

1. Quickly freeze the moth in liquid nitrogen and grind into fine powder in liquid nitrogen, using autoclaved mortar and pestle.
2. Transfer the fine powder to a 2 ml sterile tube containing 800 µl of extraction buffer and mixed gently.
3. The contents were mixed well and incubated at 65 ^{0}C for 45 min.
4. The contents were then cooled at room temperature.
5. Add an equal volume (750 µl) of chloroform: isoamyl alcohol (25:24:1) and mix well by inverting (do not vortex) by gently. The contents were spun for 15-20 min at 13000 rpm at 4 ^{0}C.
6. About 600 µl of supernatant was transferred to fresh tube and the remaining was discarded.
7. About 600 µl of isopropanol – ammonium acetate mixture was added to supernatant to precipitate nucleic acids.
8. The contents were mixed thoroughly and keep it for 2 hr or over night at –20^{o}C.
9. Centrifuge for 20 min at 13000 rpm to spool out pellet or nucleic acids.
10. The supernatant was discarded and the pellet was washed with 70 % alcohol.
11. Discard the alcohol and dry it completely for 1-2 hrs
12. Pellet was dissolved in 100-200µl of $T_{10}E_{1.}$
13. Add 1-2 µl of RNAase (1 mg /ml) and incubate at 37 ^{o}C for 30 minutes.
14. Store the DNA samples at –20 ^{o}C till further use.

Purification of genomic DNA

The genomic DNA isolated was purified according to the protocol described by Maniatis *et al*., (1982). To the DNA solution, RNase @ 100 µg/ml was added and this solution

was incubated for two hours at 37°C in an incubator. The suspension was treated with equal volume of buffered phenol (pH 8.0) and centrifuged. The upper aqueous layer was taken in a fresh tube and treated with equal volume of phenol: chloroform (1 : 1 v/v). The above suspension was centrifuged and upper aqueous layer was taken into fresh tube. About 1/10th volume of 3.0 M sodium acetate and 2 volumes of absolute ethanol were added and incubated at 4 ^{0}C for 2 h. The DNA was pelleted by centrifugation at 10,000 rpm for 10 min. The pellet was washed with 70 % ethanol, air dried and dissolved in 200-500 µl of $T_{10}E_1$ buffer and stored in refrigerator at –20 ^{o} C until further use.

Determination of quantity and quality of isolated DNA

The exact amount of DNA was quantified by taking the spectrophotometer readings at a wavelength of 260 nm, which allows the calculation of nucleic acids in the sample. Double stranded DNA at 50 µg/ml in aqueous solution has an absorbance (OD) of 1.0 (Sambrook *et al*., 2001). The procedure used for quantification of DNA is as follows.

1. Five µl of DNA sample was added to 995 µl of deionised distilled water or $T_{10}E_1$ mixed thoroughly and the absorption (OD) at 260 and 280 nm was read using spectrophotometer.
2. The concentration of DNA in the solution was calculated according to the following formula.

DNA conc. (µg/ml) = $OD_{260} \times$ 50 X dilution factor

The ratio between the readings at 260 and 280 nm (OD 260/OD 280) was used as an estimate of the purity of the DNA samples. Pure preparations of DNA have OD 260/OD 280 values ranged between 1.7 and 1.8 (Sambrook *et al*., 2001). Computed OD values were used to dilute the DNA samples to working concentrations.

Following procedure was used to accomplish the quality determination.

1. The DNA degradation and contamination with other substances were checked by electrophoresis of an aliquot of the sample in mini agarose gel of 0.8 %. It is assumed that large molecular weight DNA appears as a band with sharp stripes, whereas partially degraded material forms a smear of long to small fragments.
2. The ends of perspex tray was sealed with spacers and comb was inserted (Hoefer Supersub)
3. Agarose gel (0.8 %) was prepared by adding 0.42 g agarose to 60 ml of TAE (1×) buffer (EDTA 0.5 M at pH 8).
4. The solution was boiled by putting the flask in microwave oven and cooled to 60° C.
5. Ethidium bromide (3 µl of conc. 10 mg/ml) was added to the gel and mixed gently.
6. The gel was poured into the tray and air bubbles were removed by using pipette. After the gel was completely set, tape was removed and the gel was placed into the electrophoresis tank.
7. Approximately 500 ml of TAE (1×) buffer was poured into the electrophoresis tank enough to cover the gel to a depth of 5 mm.
8. Comb was removed carefully.
9. About 1/10th volume of loading buffer (6×) bromophenol blue dye was added to DNA samples and mixed by gentle tapping and spinning 2-3 sec in a microcentrifuge.

10. DNA samples were loaded into the wells and power supply of about 80 V was provided to run the gel.
11. The power supply was switched off when dye front was about 2 cm from the other end, and the gel was removed from the gel apparatus.
12. The gel was viewed and photographed by using gel documentation system (UVi Tech, England).

Selection of moths for experimental analysis

Available moths (max of 10) from each of 1,3,5,7 and 9th treatment were considered to check out the patterns of genetic variation. Across location and crops the individuals were pooled to make up 10 moths per treatment. In some treatments only a few were available, such as No. 1, only two insects were available from Kolar and one from Sangareddy. Catch time was not a criterion in this experiment.

Table 7.1 Individual moths from each treatment chosen for SSR analysis

Treatments				
1	3	5	7	9
kol-1-1	kol-3-1	kol-5-2	kol-7-1	kol-9-1
srd-1-1	kol-3-2	kol-5-3	kol-7-2	kol-9-2
	srd-3-3	kol-5-4	kol-7-3	kol-9-3
	srd- 3-4	kol-5-5	kol-7-4	kol-9-4
	srd-3-5	kol-5-6	kol-7-5	kol-9-5
	srd-3-6	kol-5-7	kol-7-6	kol-9-6
	srd-3-7	kol-5-8	kol-7-7	kol-9-7
	srd-3-8	kol-5-9	kol-7-8	kol-9-8
	srd-3-9	kol-5-10	kol-7-9	srd-9-1
	srd-3-10	srd-5-1	srd-7-1	srd-9-2

In an effect to overcome the compounding effect of geographical location, available moths from only one location *viz*., Sandareddy on cotton were considered.

Table: 7.2 Individual moths that were caught early in the season at Sangareddy

Treatment				
1	3	5	7	9
srd-1-1	srd-3-3	srd-5-1	srd-7-2	srd-9-2
	srd-3-4	srd-5-2	srd-7-3	srd-9-3
	srd-3-5	srd-5-3	srd-7-4	srd-9-4
	srd-3-6	srd-5-4	srd-7-5	srd-9-5
	srd-3-7	srd-5-5	srd-7-6	srd-9-6
	srd-3-8	srd-5-6		srd-9-7
	srd-3-9	srd-5-7		srd-9-8
	srd-3-10	srd-5-8		srd-9-9
		srd-5-9		srd-9-10
		srd-5-10		

DNA of individual moths was used for analysis.

Molecular markers

Microsatellites or simple sequence repeats (SSRs) are tandemly repeated motifs of varying length in all prokaryotic and eukaryotic genomes. They are present in both coding and non-coding regions and are usually characterized by a high degree of length polymorphism. A set of 20 SSR primer pairs available to date for *H. armigera* was used to assess the genetic variability among the moths (Ji et al., 2003; 2005; Jackson et al., 2003). These markers were synthesized from Sigma Aldrich USA. The sequence information of the primers used is presented in Table 7.3.

Table 7.3 SSR primer pairs available for *H. armigera*

Primer name	Sequence	Annealing temp (‘C)
1	F: 5’ CATAGGAAGTGGTGAAGGGT 3’ R: 5’ CACATTCGTCTTTCATCGAC 3’	47
2	F: 5’ ACGTCGATGAAAGACGAATGTGA 3’ R: 5’ AAGCTGGTCTTGCTGCCAT 3’	53
3	F: 5’ GCCGTAATGCCCTCAATTCTT 3’ R: 5’ TTCCCTCGGAGAGCCGT 3’	52
4	F: 5’ TAGTCTGGGATTTTGTCTGGTGT 3’ R: 5’ CGTGCCATTGAAATAGTAAGCCAT 3’	51
5	F: 5’ TAAGTATGCCCTCGACTGTCGT 3’ R: 5’ CACTTTCCAATTAGCCTCGATGCT 3’	52
6	F: 5’ TCCACACAGTTTGCATTATGA 3’ R: 5’ CGCCATAATCCTATTGATTC 3’	45
7	F: 5’ CACCACCTGACATAACGC 3’ R: 5’ AAGGAGCAGCAATTGCAAGC 3’	50
8	F: 5’ TCAAACACACATACTTGACTA 3’ R: 5’ TCCAGCAGTGGAATGCGA 3’	48
9	F: 5’ GCTGTGTATGGTAGACTTGT 3’ R: 5’ CGGATATAAATCTATACCTC 3’	43
10	F: 5’ ACGCGAGCACCAACTGTAA 3’ R: 5’ GAGACCAATAGCAGTAGTTC 3’	49
11	F: 5’TAGGTGATTGTGGCTCAGTTTT3’ R: 5’ CAAACCCATCAGCAAATGCAAC3’	60
12	F: 5’ AACACCCATTGAAGTCCCATGAA3’ R: 5’ TTCCTATGTTCACTGCTAGTT3’	57
13	F: 5’ATCTTTATGCTTTTAGCCGTTTA3’ R: 5’CAGTGGACTGCTATAGGCTGA3’	56
14	F: 5’TGTTACTTGGGTTTCCTGAATA3’ R: 5’ACCACCGACACGTGCCGACTTC3’	62
15	F: 5’GATAAGTTATTTCGGTTTAGTATT3’ R: 5’AAGTACCTAATCCGTTTTTATTC3’	50
16	F: 5’TGTTGTTGCAGAGCTGCC3’ R: 5’TTCAGCAACACAACCGTACA3’	58
17	F: 5’AAGCAATAATTACCAGAAACAG3’ R: 5’GTTTATTCGTGTATTCATTAAATAG3’	51
18	F: 5’TTAGGTGATTGTGGCTCAGT3’ R: 5’ATTTTAGCACATGCAGCAAAC3’	55
19	F: 5’AGCTCCACAACTCTTAACTAC3’ R: 5’GCAAACGATCACTGATATTAAC3’	51
20	F: 5’CAGGACATGCAATGATGAG3’ R: 5’TTTGATACTGAGTCTGATGTG3’	53

Individual PCR reaction mixture consisted of following components in a total volume of 25 µl;

Genomic DNA template (5 ng/µl)	2.0 µl
dNTP mix (2.5 mM) (Eppendorf Pvt. Ltd.)	1.0 µl
PCR assay buffer (10x):	2.5 µl
Deionised distilled water:	17.2 µl
Primer (5 pM/µl)	
Forward primer:	1.0 µl
Reverse primer:	1.0 µl
Taq polymerase (3U/µl):	0.5 µl

PCR reaction was carried out using Master Cycler gradient 5331-Eppendorf versions 2.30. 31-09, Germany. The cycler was programmed as follows,

Step	Temperature	Duration	No of cycles
Initial denaturation	94^0 C	: 5 min	1
Denaturation	94^0 C	: 1min	
Primer annealing	42-62^0 C	: 1 min	40
Primer extension	72^0 C	: 2 min	
Complete primer extension	72^0 C	: 5 min	1
Store	4^0 C	: Till end	

Annealing temperatures were adjusted depending on primer used each time.

Separation of PCR products for SSR

Separation and visualization of PCR products was done on both agarose (2 %) as well as polyacrylamide gels (6 %). Agarose gels were used only for visualization of amplification considering two limitations in their use: 1) each sizing of microsatellite alleles cannot be accomplished on agarose. 2) It is difficult to distinguish two, three and four base pair differences in DNA fragment length on agarose (Cregan and Qugley, 1998). Therefore, the exact allele sizing of PCR amplified microsatellite product was performed in denaturing poly acrylamide gels of 6 per cent.

Agarose gel electrophoresis

Agarose was casted in 2 per cent gels in TAE buffer (1×). Gels were casted in a horizontal gel frame (Hoefer HE99X 18 x 30 cm Amersham Bioscience Ltd.) and products were visualized by incorporating 1 µl (10 mg/ml) ethidium bromide per 10 ml of gel solution and viewed in a gel documentation system. These gels were used just to check the amplification status before embarking on to 6% PAGE.

Polyacrylamide gel electrophoresis

Six per cent polyacrylamide gels were used for better separation and visualization of PCR amplified microsatellite products. Since, polyacrylamide gels have better resolution for amplified products denaturing gels were casted in Sequi-Gen GT nucliec acid electrophoresis cell (Biorad Ltd) as per the protocol in manual published by MRF, Hyderabad manual.

Glass plates were prepared before making the gel solution. Both outer (large) glass plate (IPC unit) and inner (small) glass plate were cleaned thoroughly with warm water and detergent. Second wash was given with de-ionized water.

Fresh binding solution was prepared in chemical fume-hood by adding 4 µl of bindsilane (SIGMA) to 1 ml of 0.5 per cent acetic acid in 95 per cent ethanol in a 1.5 ml micro-centrifuge tube. The mixture was poured into a notched plate (inner glass plate) and spread using tissue paper over the entire surface of the plate. The treated side was demarcated to distinguish between untreated and treated sides.

Repel silane (SIGMA) of about 250 µl was added to 750 µl of 0.5 acetic acid in 95 per cent ethanol in a 1.5 ml microcentrifuge tube. The mixture was poured onto a large glass plate and spread using tissue paper.

Assembling and pouring the gel:

Spacers of 0.4 mm thickness were placed along the side edges of the bind silane–treated surface (small glass plates). The large plate was then put on small plate so that treated surfaces faced each other (in a sandwich – like fusion). Care was taken care to see that the spacers fitted well against each other so that there were no gaps or leakage. Clamps were then put on both sides and the assembly was put in a precision caste base for sealing both sides to ensure no leakage from the bottom or sides.

For casting each gel, 80 ml of 6 per cent acrylamide gel was prepared. Just prior to pouring, for each 80 ml of solution, 60 µl of N, N, N'N'-tetramethylethylenediamine (EDTA, Temed Inc.) and 600 µl of 10 per cent ammonium persulphate (APS) was added to initiate the polymerisation process. The contents were mixed gently by swirling and bubbles avoided. Before pouring the assembly was kept on the bench top so that it made a 45-degree angle with the bench top. Assembly was tilted so that one of the bottom corners is raised above the other (off the bench top) and then the solution was carefully poured into the space between the glass plate starting at the lower corner. As the acrylamide solution filled the space the gel assembly was lowered so that both bottom corners were on the bench, parallel to the bench top.

Shark toothcomb of 0.4 mm thickness (49 wells) was inserted with straight side facing the gel at the top of the gel. Comb was put straight across the top moving not more than 5 mm of notched plate. If bubbles formed during the pouring, they were dislodged by tapping. Gel was left for 20 to 40 minutes for complete polymerization.

Electrophoresis of sequencing gels

1. After the polymerization process the assembly was detached from the clamp and precision caste base and was placed in a universal base against the back wall. IPC was locked to the base in vertical position by fitting a stabilizer bar.
2. Tris-free base, boric acid and EDTA (TBE) (5x) was poured in upper tank with IPC unit and the rest poured into the bottom chamber (1.8 L of buffer was prepared fresh each time).
3. Comb was removed and then excess polyacrylamide gel was removed with a razor blade. Tissue paper was used to clean the glass plates with buffer.
4. Air bubbles and unpolymerised acrylamide on the top of the gel were removed by squirting with 5× TBE.
5. Pre-run was given to achieve gel surface temperature of approximately 45 to 50 ^{0}C with following conditions. Temperature 50 ^{0}C, power 2000 V, 50 mA, constant watt of 75 W.
6. SSR loading dye (3x STR dye) was added to PCR products to a final of 1× and samples were denatured by heating to 95^0 C for 4 min and immediately cooled on ice.
7. After the pre-run, urea was flushed from the well area using a transfer pipette and carefully inserted the shark tooth comb into the gel so that the teeth were just touching the surface of the gel. Care was taken to avoid piercing the gel too deeply.
8. Samples (6 µl) were loaded into the wells to facilitate the sizing of the various alleles. 100 bp marker was loaded after denaturing into the first or last well.
9. Gel was run using the same conditions as the pre-run step. The chromatogram was developed until the dye reached the bottom of the gel.

Visualization of SSR bands

After electrophoresis, clamps were loosened and buffer was removed. Glass plates were separated using a plastic wedge at the right corner. The gel was affixed to a small glass plate.

Separated DNA fragments were detected using the following silver staining protocol. The technique was followed using a solution prepared in-house. Each solution was prepared in separate containers. The same solutions were used four times over a period of 48 h except for the developer, which was freshly prepared during the staining process.

Silver staining

1. Gel was rinsed with distilled water for 3 to 5 min and placed in a shallow plastic tray.
2. Gel was soaked in 2 L of 2 per cent acetic acid (fix solution) for 20 min.
3. The gel was rinsed twice with water, each for 2 min.
4. The gel was kept for staining with 2 L of 1per cent silver nitrate for 20 min.
5. A quick water wash was given for 10-15 sec.
6. Developer solution was added to the tray and agitated until the bands appeared.
7. Developer was removed and the plate placed in fixer or the stop solution for 5 min.
8. Gel was placed in 2 L of impregnate solution for 15 min.
9. Lastly, the gel was washed with water for 5 min and dried overnight.

All steps were done with constant shaking conditions

Scoring of gels and analysis:

Individual gel pictures were acquired through a computer were processed and scored to get binary data. The presence/absence of data (1,0) matrix was analyzed using standard procedure in NTSYS Pc2 package. Similarity matrix was computed for each and genetic distance/similarity was determined by Jacquard similarity. The resulting similarity matrix was used to generate tree by UPGMA (unweighted pair group method with arithmetic average) in NTSYS Pc2 package.

ITS regions

This category of markers is highly useful in evolution and phylogenetic analysis as these regions are generally under no or little selection pressure. It includes introns of nuclear genes, internal transcribed spacer (ITS) regions of nuclear ribosomal genes and mitochondrial regions. These marker systems are good for differentiating individuals of the same species or very closely related ones. rDNA is a multi-gene family with nuclear DNA copies in higher eukaryotes. These are arranged in the nuclear organizer region in a tandem fashion, where each subunit consists of gene coding for 18S and 28S rRNA subunits. The primers that can amplify ITS regions have been designed to work well across taxa using the most conserved regions. The ITS region was amplified using the combination of primers specific to 5.8S and 18S genes.

Cloning of Cytochrome oxidase I (COI) gene:

Primers specific to the COI gene were designed and used for the amplification.

Cloning of PCR product:

The purified 99bp PCR fragments, from different treatments were ligated to pTZ57R/T cloning vector (2868 bp), separately, as described in InsT/A clone^{TM} PCR product cloning kit (K1214) from MBI, Fermentas, USA. For ligation an optimal 1 : 3 molar ratio of ends of vector : insert was calculated. The components of the ligation mix were mixed in 0.5 ml micro-centrifuge tubes and incubated at 16°C for 16 hrs.

Preparation of competent cells

The component cells of *E. coli* DH5α were prepared following the protocol described in Sambrook and Russell (2001) with minor modification as described below.

An isolated colony from *E. coli* DH5 α plate was inoculated into 5 ml Luria broth and incubated at 37°C overnight at 200 rpm. The next day, the culture was diluted to 1 : 100 using Luria broth *i.e*., 0.5 ml of culture was added to 50 ml of Luria broth. It was incubated for 2 to 3 hrs until it attained a OD of 0.3 to 0.4 at 600 nm. The culture was chilled in ice for 30 min and 25 ml of culture was dispensed into two 50 ml centrifuge tubes. The cells were pelleted at 6000 rpm for 5 min, the supernatant was discarded and pellet was suspended in 12.5 ml of ice-cold 0.1 M calcium chloride. The centrifuge tubes were again kept in ice for 45 min and later centrifuged at 4000 rpm for 10 min. The pellet was dispensed in 1 ml of 0.1 M calcium chloride and to this 88 μl of dimethylsulphoxide (DMSO) was added. About 200 μl of cell suspension was distributed to each pre-chilled 1.5 ml micro centrifuge tube and immediately used.

Transformation of *E. coli* DH5 α:

About 100 μl of freshly prepared competent cells were taken in a chilled centrifuge tube and 10 μl of ligation mixture added into the tube and mixed gently. The mixture was chilled in ice for 45 min and heat shock was given by shifting the chilled mixture into a water bath at 42°C for 2 min and then immediately transferred onto ice to chill for 5 minutes. To this, 800 μl of Luria broth was added and incubated at 37°C at 200 rpm for 45 minutes, to allow bacteria to recover and express the antibiotic marker encoded by the plasmid. The culture was centrifuged at 13,000 rpm for 1 min and about 700 μl of supernatant discarded and the pellet re-suspended in the remaining supernatant and spread on the plates having Luria agar with Amp_{50}, X-gal IPTG and incubated overnight at 37°C.

The recombinant clones were identified by blue/white assay. After incubation, only white colonies, having recombinant vectors were picked up and streaked on plates having Luria agar with Amp_{100}, X-gal, IPTG and incubated at 37°C overnight.

Confirmation of clones and sequencing:

The confirmation of the presence of cloned fragments was achieved by PCR amplification of clones with respective primers. The total DNA and cloning vector were used as positive and negative controls in the PCR.

The full length amplicons were cloned in pTZ257R/T is that they could be sequenced using M13 primers. The sequence of bases from each treatment will be compared and phylogeny tree would be drawn.

Table 7.4 Male moth samples from pheromone trap catches in Karnataka held at IABT

Station	Treatment	Number of moths received on			
		8/10/2005	5/11/2005	9/11/2005	Total
Bangalore	1	0	0	0	0
	2	0	0	1	1
	3	0	0	4	4
	4	0	0	3	3
	5	1	0	4	5
	6	0	0	8	8
	7	0	0	1	1
	8	1	0	4	5
	9	1	0	5	6
	10	0	0	2	2
				Total	**32**
Belgaum	1	0	0	2	2
	2	3	0	1	4
	3	0	0	3	3
	4	0	0	2	2
	5	0	0	4	4
	6	0	0	7	7
	7	16	0	8	24
	8	0	0	0	0
	9	8	0	0	8
	10	0	0	0	0
				Total	**27**
Kolar	1	0	0	1	1
	2	0	0	1	1
	3	0	0	2	2
	4	0	0	11	11
	5	0	0	11	11
	6	0	0	9	9
	7	0	0	9	9
	8	0	0	7	7
	9	0	0	8	8
	10	0	0	4	4
				Total	**63**

Table 7.5 Male moth samples from pheromone trap catches in A.P. held at IABT

Station	Treatment	Number of moths received on			
		8/10/2005	5/11/2005	9/11/2005	Total
Sangareddy	1	0	0	1	1
	2	0	0	1	1
	3	12	0	1	13
	4	0	0	3	3
	5	12	0	3	15
	6	0	0	7	7
	7	5	0	1	6
	8	0	0	2	2
	9	15	0	1	16
	10	0	0	2	2
				Total	**22**
Jogipet					
	1	0	0	0	0
	2	0	0	2	2
	3	0	0	1	1
	4	0	0	1	1
	5	0	0	1	1
	6	0	0	1	1
	7	0	0	1	1
	8	0	0	1	1
	9	0	0	1	1
	10	0	0	0	0
				Total	**9**
Karnool					
	1	0	0	0	0
	2	0	0	0	0
	3	0	2	0	2
	4	0	0	0	0
	5	0	3	0	3
	6	0	0	0	0
	7	0	10	0	10
	8	0	0	0	0
	9	0	2	0	2
	10	0	0	0	0
				Total	**17**
				Total	**244**

Genomic DNA samples were prepared from all moths received