CROP PROTECTION PROGRAMME

Sustainable control of the cotton bollworm *Helicoverpa armigera* in small-scale cotton production systems

R7813 ZA0416

FINAL TECHNICAL REPORT

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Executive Summary

Project purpose: To capitalize on early DFID CPP projects addressing the problems of excessive insecticide use on cotton in Asia by supporting contributions of UK-based scientists to the much larger Common Fund for Commodities project with the same name and goals (2001-2005).

Research Activities: The project worked with scientists from India, Pakistan and China to establish the genetics and biochemistry underlying the biological mechanisms by which the key caterpillar pest of cotton in Asia, cotton bollworm (*Helicoverpa armigera*), resists the insecticide groups which are commonly used against it. Initial development of insecticide/protein conjugate molecules was undertaken to support the development of insecticide quality rapid detection kits in India. The global experience with the efficacy and biological impacts of the use of cotton insecticides was summarized as an aid to pest management decision making. Support was provided to the CFC project component ascertaining the minimum effective spray application methods required for adequate control of cotton caterpillar pests. Recommendations based on these results of all the activities were then provided for incorporation into field recommendations in China, India and Pakistan through the CFC project collaborators.

Outputs: The nature, genetics and cross resistance patterns for resistance in *H.armigera* across Asia are now understood. Resistance to all commonly used insecticides except spinosad is unfortunately inherited in a dominant fashion. Metabolic detoxification of insecticides is important: by specific enhanced oxidases (esp important for Pyrethroids) esterases (esp important for organophosphates and carbamates and probably endosulfan). Genetic alterations to the target sites of those insecticides (the sodium channel for pyrethroids and the structure of the target acetylcholine esterase molecule for organophosphates and carbamates) are important. Changes to the permeability of the insect cuticle and increasing rates of excretion have been demonstrated as important resistance mechanisms to pyrethroid insecticides.

Project contributions formed a basis for the production of insecticide quality kits for the pyrethroids and endosulfan (later extended to organophosphates) in India. The methods have been submitted for patenting (by the Indian Council for Agricultural Research) in India and manufacture and distribution of the kits is commencing now.

The world literature on insecticide efficacy and impact on beneficial organisms was reviewed, tabulated and summarized. A queriable data-base '*Helibase*' was produced and disseminated enabling the rapid abstraction of relevant information from that literature.

An initial report on cotton pest spraying was produced and technical support was provided to the CFC project which went on to produce highly detailed guidelines as to the spray droplet, spray districution and equipment parameters necessary to deliver effective insecticide-based control of *H.armigera* in Asia.

Contributions to development goals: The project has provided for the first time a comprehensive understanding of the mechanisms by which the key cotton pest in Asia has been resisting insecticide applications made against it, in addition to the first key to effective and environmentally less damaging chemicals and how to apply them most appropriately. As a consequence it has been possible to make recommendations as to the principles by which insecticides should be used in Asia (materials, rates, rotations, equipment). These have been directly incorporated into national insecticide use recommendations in China Pakistan and India though the project partners. In India alone, the Indian Government is directly applying the developed principles in an expanding nation-wide project (11 cotton states, 280 cotton producing districts and >45,000 cotton farmers in 2005). This has resulted in a significant decline in insecticide use (by an average of 47% across the 11 states in 2005), a significant yield increase (by 11% in 2005) and increased profit for farmers (by $US175/ha in 2005). This has confirmed Indian government continuing support for expansion of this programme into the future. As a result of the project, farmers are now able to cheaply assess insecticide quality for the first time. The insecticide quality kits will now empower farmers to challenge vendors of sub-standard insecticides (25% of all insecticides in N.India for example).

Background

This project aimed to extend and expand the knowledge generated in the 1993-2001, successful, DFID-funded work on insecticide resistance management in India in order to advance the ability to use insecticides appropriately for the control of the cotton bollworm *Helicoverpa armigera* in India, Pakistan and China. Within these countries, which account for over half the world's cotton production, cotton is singularly important as a source of livelihood for many millions of rural people. These livelihoods are threatened by the increasing difficulty in controlling bollworms because the pest has developed resistance and become more difficult to manage. The work aimed to improve the understanding of the efficacy of insecticides against this key pest of cotton and of the impact of these materials on beneficial insects and to support the development of insecticide quality detection kits. When allied with work focused on gaining a deeper understanding of insecticide cross resistance patterns, this allows greatly improved insecticide use recommendations to be made across Asia.

Although this CPP project was a discrete piece of work, it was also the DFID-funded component of a larger multi-funded international project (Sept 2000 to Dec 2006) with the same name, which aimed to improve the sustainability of cotton production, via the key constraint of reducing damage caused by the cotton bollworm. This complex $US4 million project was supported by the Common Fund for Commodities (50%), through the International Cotton Advisory Committee, and the International Committee of the Insecticide Resistance Action Committee (IRAC) the industry body charged with reducing the impact of evolved resistance to insecticides. The other agencies funding the complementary activities were India: Indian Council for Agricultural Research (through the Central Institute for Cotton Research, Punjab Agricultural University and Tamil Nadu Agricultural University); Pakistan: Pakistan Central Cotton Committee (through the Central Institute for Cotton Research) and China (Ministry of Science and Technology through Nanjing Agricultural University and the National Agro Technical Extension Service Centre). The International Cotton Advisory Committee, Washington, USA, the commodity board for cotton, acted as the promoter and supervisor of the project for the Common Fund for Commodities (CFC). As a matter of policy, CFC does not expect to fund the direct costs of developed world collaborators on its development projects. Capitalising on the investment of the DFID Crop Protection programme in developing an understanding and management of insecticide resistance in cotton in India in particular (projects from 1993- 2001), CPP committed to a relatively modest funding of the UK technical base in the wider Asian CFC project. The proportion of the overall funding provided by this DFID project was approximately 11%, but it crucially included support for a key central components, namely understanding and overcoming the impact of the mechanisms of cross resistance between insecticide products and the development of diagnostic kits for the determination of insecticide resistance and quality. The DFID project covered UK-based technical work only, with some short visits to Asia to attend meetings of the larger programme staff. The overall project management costs and all overseas collaborator costs were borne by the umbrella CFC project. A short extension to the CPP project – March to Sept 2004, enabled input to the Indian programme of field implementation of the project's results and an assessment of the impact of the DFID funded work in the overall project.

Project Purpose

The project lay within the Semi-arid Production system whose overall purpose was:
"Benefits for poor people generated by application of new knowledge of crop protection in semi-arid cotton production systems". Within the Semi-arid production system the programme purpose was *"Strategies developed to reduce the impact of pests and stabilise yields in semi-arid cotton-based cropping systems."* The geographic focus of support covered almost half the world's cotton production, in India, Pakistan and China. Strongly based agricultural economies are important for development in these three countries. Each has significant reliance on cotton production (cotton provides 60% of Pakistan's total export value and between them the three countries produce over 50% of the world's cotton), but trends showed reduced economic viability of the crop due to the difficulty and cost of controlling pests in a time of falling world prices for raw cotton. Crop protection costs were increasing and the bulk of this increase is in insecticide costs . Across the three countries, in the late 1990s, insecticides accounted for 44% of the variable costs of growing cotton, (over US$ 1,500m annually (ICAC, 1998). The majority of this expenditure was for the control of caterpillars of *Helicoverpa armigera* (cotton bollworm), which had become the region's No1. agricultural pest, causing an estimated US$ 855m of crop damage per year in the region, despite the enormous quantities of insecticides used against it. In the late 1990s cotton consumed some 80% of all insecticides in Pakistan, 50% in India and 38% in China and in all three countries insecticide resistance is resulting in escalating costs of pest control measures and causing significant environmental and human health problems. In 2000, the 43 member countries of intergovernmental International Cotton Advisory Committee (ICAC) gave the highest priority to work that address the loss in yield, net revenue and quality of cotton associated with insect pests. This provided the background rationale for funding of the parent project by the Common Fund for Commodities, for which ICAC is the cotton commodity board. The well documented increase in pest management costs throughout Asia is due in large part to evolved resistance to the major chemicals available to control insect pests, and particularly the American

bollworm. All three countries have national programmes focussed on reducing control problems with this species, but without clearly understanding the implications of common evolved defensive processes against diverse insecticide products, developing rational insecticide use strategies is difficult or impossible. Understanding how to minimise and overcome the impact of insecticide resistance in a way which could be applied by farmers, represents a very considerable and immediate contribution to the many millions of farming families which depend on the crop across Asia and Africa.

Within the larger CFC project (which included a major component on the national implementation of the results of the programme) the project set out to support the clarification of the patterns of insecticide resistance and cross resistance in relation to the key cotton pest. This aimed to provide essential information on chemistries which may be effective and of others which will soon be rendered useless by the evolved resistance to particular materials by particular evolved (mainly metabolic) mechanisms. Recommendations based on the work were to be incorporated into pest management strategies which will reduce and reverse the trend towards applying ever increasing dose rates and spraying frequencies in the cotton crop, thereby maintaining the crop as a supporter, directly and indirectly, of many rural livelihoods in the region.

Work focused on 5 major areas.

1) Understanding the biochemical and genetic mechanisms by which cotton bollworm *(H.armigera*) is resistant to the major classes of 'conventional' insecticides used in the three countries. This was based at Rothamsted Research, Harpenden, UK with long-term scientific inputs from visitors from China (Nanjing Agricultural University), India (Central Institute for Cotton Research) and Pakistan (Central Cotton Research Institute) funded under the CFC project. (This component took c. two thirds of the project funding)
2) Development of insecticide resistance management strategies using the above information.
3) Supporting the production of cheap, rapid, diagnostic kits which can be used by farmers to identify pesticide quality directly, rather than waiting for expensive, cumbersome and ineffective redress of grievances through the formal court system.
4) Analysis of the efficacy of insecticides used against *H.armigera* and their impact on non-target organisms, esp beneficial insects, with a view to enabling improved decision making by farmers of optimal materials to use in particular circumstances.
5) Support for the identification of minimum insecticide spray application practices which are necessary for effective control of cotton bollworm (practical work by Punjab Agricultural University under CFC funding.)

The target organizations benefiting from and implementing the results of this work were:

- The Agricultural Universities and research institutes of the Indian Council for Agricultural Research (ICAR), in particular the Central Institute for Cotton Research, Tamil Nadu Agricultural University and the Punjab Agricultural University. *In particular the project worked with the technical back-stopping team of the Indian Government Cotton Technology Mission project (2002-2007), an ambitious national programme to promote the effective assimilation of insecticide use in hundreds of demonstration villages across the country (already commenced based the useful knowledge generated by DFID CPP projects R6760 and R6734).*

- The Pakistan Central Cotton Committee of the Ministry of Agriculture *(its cotton centre, the Central Cotton Research Institute, provides the pest management advice to the Pakistan*

Government extension services and is directly involved in the national and Asia Development Bank funded projects on farmer field schools (FFS) in cotton).

- The National Agricultural Technology Extension Service Centre (NATESC) of the Chinese Ministry of Agriculture and Nanjing Agricultural University. *(NAU is the Chinese 'key' laboratory on insecticide resistance, providing resistance management advice to NATESC which then implements it nationally.)*

Asian partners in EU/FAO programme "*IPM in Cotton in Asia*" (2000-2004*) (EU funded , FAO implemented, farmer field school project in Asia (China, India, Pakistan and others including Bangladesh, Philippines, Vietnam, Thailand with overall funding of some $12 million over four years from 2000).*

The project-funded work was carried out almost entirely in the UK in collaboration with (CFC funded) visitors from overseas target institutions. The separate funding by Common Fund for Commodities (CFC) also covers a range of complementary activities within the target countries.

Structure of the project

The project ran from August 2000 (official start date in March but funding was delayed) to March 2004 with a brief extension in the summer of 2004, allowing the PI to assist the Central Institute for Cotton Research to organise and run the 2004-5 season planning meeting for the state, district and village level co-ordinators associated with the Government of India village insecticide resistance management (IRM) programme held at CICR Nagpur in late June 2004.

Work on components 1 and 2, on resistance and cross resistance patterns was mainly undertaken at the Rothamsted Research Laboratories in Harpenden, UK. Rothamsted provided the support of entomologists (Ian Denholm and Greg Devine), molecular biologist (Martin Williamson) and biochemist (Grahame Moores). The project provided technical assistance at Rothamsted to 2003, which was continued under CFC funding from the under utilised Pakistan budget in 2003-4. Dr Kranthi from the Central Institute for Cotton Research was the Indian visiting scientist, Dr Yidong Wu, Ms Yehua Yang and Mr Song Chen from Nanjing Agricultural University were the Chinese visitors and Dr Mushtaq Ahmad from the Central Institute for Cotton Research was the Pakistan visitor.

The laboratories at Rothamsted had all the specialized equipment required for these studies but a small electrophoresis kit (Mini-PROTEAN II) was purchased on this project to enable rapid processing of the large number of samples on a dedicated unit.

The development of insecticide quality detection kits was undertaken by the Central Institute for Cotton Research under the CFC budget. However, under the current project, insecticide/ protein conjugates (output 3) were produced in the Chemistry Department of the Univ of Greenwich and forwarded to CICR for use in raising anti-sera for development of these immunodiagnostic kits.

Summarising, tabulating and drawing conclusions from the global literature on cotton insecticide efficacy and impact on natural enemies (output 4) was undertaken by Hans Dobson of NRI, working at Imperial College at Silwood Park.

The development of minimum acceptable spray application practices for control of cotton bollworm *Helicoverpa armigera* was led from Punjab Agricultural University under CFC funding. Mr Jerry Cooper of NRI actively supported this work with advice, experimental protocols and technical visits. Training of PAU staff was undertaken by NRI in collaboration with the International Presticide Applications Centre of Imperial College at Silwood Park.

The project was managed for NRI by Dr Derek Russell, who was also the manager of the eponymous CFC 'parent' project (2000-2005).

Insecticide Resistance in Cotton Insects in Asia

Resistance to insecticides across Asia:

A global history of insecticide resistance is given in Kranthi et al (2005). China, India and Pakistan have been monitoring resistance of *H.armigera* to the common insecticides since the late 1980s (Armes *et al* (1996) for India, Tan (1999) for China and Ahmad et al. (1999) for Pakistan) using discriminating dose assays for the chemicals common in their regions. Cypermethrin was used as an example pyrethroid, quinalphos (phoxim in China) as an example organophosphate, endosulfan as the only widely used cyclodiene and methomyl as an example carbamate. India has the most comprehensive set of data, collected from at least four sites (and often more) across the country since 1992. India and China have used topical assays on 3rd instar larvae while Pakistan has used the leaf dip method (IRAC Method No 7). The data is voluminous. Results naturally vary with the area of the country and the history of insecticide use in each area (Kranthi *et al*. 2001). As a preliminary to the CFC project, ICAC held a Regional Consultation on Insecticide Resistance Management in Cotton, at CCRI, Multan in 1999 (ICAC-CCRI 1999) to which all the current team contributed. Resistance survey results since then have largely re-enforced the earlier findings with only relatively minor changes. Details for Pakistan are in Arif *et al.* (2004); for India in Kranthi *et al*. (2002 and 2005). A regional summary results of the recent work were presented at the 3rd World Cotton Conference (Regupathy *et al.* 2004). In very brief summary, the regional resistance to the commonly used chemicals is shown in Table 1.

In summary we could say that the synthetic pyrethroids are highly resisted, and that cypermethrin and fenvalerate in particular have lost most of their usefulness (with resistance frequencies (RFs) frequently in the hundreds and often in the thousands). Resistance to organophosphates and carbamates have remained moderate (RF <30 generally), with endosulfan resistance generally low to moderate, especially early in the cotton season. Full or almost full susceptibility is limited to the newer and less used materials (often more expensive). There is no reason to think that they will not be resisted in their turn as they are more widely used. These levels of resistance are maintained by selection with the insecticides. Where, as in Pakistan, certain insecticides have been strongly discouraged for use on cotton, resistance to these materials has fallen quickly.

Table 1: Typical resistance levels to widely used chemicals in India (I), China (C), and Pakistan (P)

	Resistance level*				
Chemical	**Susceptible**	**Low**	**Medium**	**High**	**Very high**
Pyrethroids					
Cypermethrin					**I, C, P**
Fenvalerate				**I**	**I, C, P**
Deltamethrin			**P**	**I**	**I**
Lambda cyhalothrin			**P**	**I**	**I,P**
Bifenthrin			**P**	**P**	
Beta Cyfluthrin				**I**	
Organophosphates					
Quinalphos			**I**		
Phoxim		**P, C**	**C**		
Chlorpyrifos		**P**	**I**		
Profenophos		**I, P**			
Monocrotophos		**P**	**I**		
Cyclodiene					
Endosulfan		**P**	**I**		
Carbamate					
Methomyl	**C**	**I,P**			
Thiodicarb	**P**		**I**		
Organotin					
Indoxacarb	**P**				
Fungally derived					
Spinosad	**I, C, P**				

* *Susceptible* – RF<3; *Low* – no field effects; *Medium* – some reduction of field efficiency but chemical still useful; *High* – chemical compromised for field use; *Very High* – high larval survival at the field rate, chemical not useful

Research Activities

Activities for outputs:

Output 1. Clarify the patterns of cross resistance of *Helicoverpa armigera* to different insecticides

Output 2: Apply the underlying principles in the development of sustainable control strategies.

Aim: To provide a schedule of insecticide use that can ameliorate current resistance problems and which do not select for further insecticide resistance mechanisms.

Activities:

1. Establish cross resistance patterns in Asian *H. armigera* (supported by CICR Nagpur and other collaborating institutes).

a) Establish reference susceptible strain and one colony of H.armigera showing multi-resistance to a range of chemistries from the participating Asian countries
b) Bioassay the response of both strains to chemicals representative of the major resistance groupings. Obtain dose-response relationships. Establish which insecticide classes are resisted

2. Develop insect lines with single mechanisms of resistance (supported by collaborating institutions)

a) If necessary cross susceptible and resistant strains to 'dilute' the resistance genes present.
b) Select original or derived strain with relevant indicator compounds, e.g. endosulfan, two contrasting organophosphates and a pyrethroid.
c) Bioassay selected strains for resistance to a range of insecticides to investigate resulting cross-resistance patterns.

3 Identify resistance mechanisms for each of the main cross resistance groupings (supported by CICR).

a) Assay selected strains for biochemical or molecular markers indicating possible resistance mechanisms (monoxygenases and hydrolases, insensitive acetylcholinesterase and knockdown resistance).
b) Determine, on the basis of the above, whether selected strains appear to possess a high frequency of (i) just one mechanism or (ii) more than one mechanism.
c) If (ii) above, back-cross strains to susceptible insects in an attempt to uncouple coexisting mechanisms and obtain better resolved lines.
d) If (i) above, further bioassays with a wider range of insecticides to establish cross-resistance patterns in more detail.

The Approaches used in activities 1-3 were (see attached Rothamsted report for details of methods and analyses):

- Correlations in insect mortality in response to different insecticides
- Reciprocal selection experiments (does selection with compound A also increase resistance to compound B?

Metabolic resistance

- Use of synergists to block enzymes potentially involved in insecticide detoxification
- Direct comparisons of the amounts of detoxifying enzymes (esterases, oxidases and glutathione-s-transferases present
- Comparisons of metabolism rates

Target site alterations

- Looking for DNA mutations in target-site proteins known to occur in other species (sodium channel for pyrethroids, acetlycholinesterase structure for OPs and carbamates).
- Neurophysiology to detect direct evidence for nerve insensitivity

Cuticular penetration resistance

- Comparisons of insect cuticle penetration and insecticide excretion rates using radio-labelled deltamethrin

4. Understanding of cross resistance patterns confirmed experimentally in the field in Asia (in support of all collaborating laboratories)

a) Use results to date to support the design of field experiments in Asia comparing the effectiveness of control regimes based on a knowledge of cross-resistance patterns with standard control practices.
b) Investigate the consistency of cross-resistance patterns within and between countries by comparing the genetic composition and resistance spectra of field strains. Laboratory assays of field material and biochemical assay for resistance markers for a range of national populations.

As part of the CFC project, NAU in China, CICR in India and CCRI in Pakistan empirically examined cross resistance patterns of local strains using topical assays. The results support the conclusions presented below in Table 5.

5. Incorporation of the understanding of cross resistance patterns into the strategy for control

a) Condensing of findings into application sequence recommendations based on local measurement of mechanisms and resistance present (using the kits developed elsewhere).

The evolving understanding of resistance mechanisms and consequent cross resistance was reported to the CFC project co-ordinating committee at its annual meetings. The information was used to help design the national extension recommendations as detailed in the Outputs section below.

Output 3: Development of insecticide quality testing kit components for use in Asia and Africa

Aim: Develop and provide conjugate molecules (haptens) comprised of a protein moity recognizable by vertebrate immune systems and an appropriate part of example insecticide molecules in order for the Central Institute for Cotton Research in Nagpur to produce immunodiagnostic kits for insecticide quality.

Activities:

1. **Synthesis of insecticide-specifc haptens for use in the development of insecticide quantity and quality kits**
2. **Purification of haptens for cross-reactivity testing.**

This work was carried out in the first year of the project by the University of Greenwich's Department of Chemistry. A range of protein/insecticide conjugates for pyrethroids and endosulfan were produced using Bovine Serum Albumen and Keyhole Limpet Haemocyanin. These were purified and sent to CICR Nagpur. Methodologies were later improved on at CICR and the 'difficult' organophosphate haptens created there. Cross reactivity to both other insecticides in the same groupings (not necessarily undersirable) and to insecticide breakdown products proved to be a problem requiring refinements in production.

Output 4: Synthesis of existing knowledge of the efficacy of particular insecticides against bollworm in cotton and of how insecticides used against *H.armigera* affect natural enemies.

Aim: To provide a sound background on insecticide efficacy and impacts to support the development of appropriate insecticide rotation strategies for Asia (which would also incorporate the resistance management finding of the project.).

Activities:

1. **Review the existing literature on efficacy and non-target (beneficial insect) impact**
2. **Synthesise the data into a report.**
3. **Formulate guidelines on rational decision making between 'competing' chemicals.**

All published literature on the efficacy and impact of insecticides on *Helicoverpa armigera* was collected (557 papers), by Hans Dobson of NRI and the information tabulated as to insecticide efficacy against the target pest, the impact on beneficial insects and the mammalian toxicity of the material as used. The data was used to construct a pesticide suitability index and to create the Visual Basic software programme "Helibase'. This is a queriable database of all relevant papers allowing the user to extract summarized information by insecticidal material, insect affected, date of publication etc. The results were presented in the four CFC final project workshops (India, Pakistan, China and Africa) and written up as a chapter for the CFC project *Handbook of Control of Helicoverpa armigera.*

Output 5: Definition of minimum effective spray application equipment and practices for applying pesticides to cotton in Asia.

Aim: To provide a 'current' status document, early in the CFC project life, on which the practical field and lab work on this component could be developed (assisted by this project) for work led from Punjab Agricultural University, India with support from Nanjing Agricultural University and the Central Institute for Cotton Research, Pakistan.

Activities:

1. **Review existing work on cotton spraying methods**
2. **Synthesise the data into a report.**

This study was undertaken collaboratively with Punjab Agricultural University. Jerry Coooper and Derek Russell of NRI produced an initial report in Dec 2001. This identified the literature work required from PAU who worked with NRI to produce a full literature review in 2002. This informed the practical work of the CFC project on this issue, which led to the recommendations summarized in the CFC Handbook and produced by PAU as a farmer spray recommendation booklet.

Outputs

Outputs committed to in the PMF

1. Understanding of the principles underlying cross-resistance patterns due to different metabolic resistance mechanisms, which will inform practical *H.armigera* and insecticide resistance management strategies for Asia and Africa..
2. Formulation of informed strategies for reducing insecticide use and combating resistance based on control failures in the field, based on interpretation of toxicological data and identification of the resistance mechanisms responsible.
3. Participation in the development of insecticide quality and quantity testing kits for commercialisation and use in India, Pakistan, China and elsewhere (in association with certain components of the CFC project).
4. Synthesis of existing knowledge of the efficacy of insecticides used against *H.armigera*, and how they affect natural enemies
5. Definition of minimum spray application equipment and practices for effective application of pesticides to cotton.

PROJECT LOGFRAME (first 4 columns from PMF)

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions Met?	Achievements
Goal				
Livelihoods for poor people improved through sustainably enhanced production and productivity of RNR systems	To be completed by Programme Manager	To be completed by Programme Manager	To be completed by Programme Manager	**To be completed by Programme Manager**
Purpose				
Strategies developed to reduce impact of pests and stabilise yields in semi-arid cotton-based cropping systems, for the benefit of poor people	To be completed by Programme Manager	To be completed by Programme Manager	To be completed by Programme Manager	**To be completed by Programme Manager**
Outputs				
1. Demonstration of an understanding of the pattern and mechanisms of cross-resistance within and between chemical classes as mediated by single resistance mechanisms and elaboration of typical cross-resistance patterns from Asia strains to a range of major conventional insecticide chemistries.	Reports on cross- resistance assessment of *H.armigera* brought from India, Pakistan and China. Y1, Y2, Y3 and Y4	Project reports on cross-resistance patterns in bollworms imported from the three countries	Successful implementation of the arrangements to import insects **Met**	**Patterns of resistance and cross resistance measured for the three countries for the four major insecticide classes (organophosphates, carbamates, cyclodienes and pyrethroids) and the known resistance mechanisms :1.metabolic detoxification via enhanced esterases, oxdases and glutathione-s-transferases; 2. Target-site via altered acetylcholone esterase; altered sodium channels) 3: Reduced cuticular penetration**
2. Formulation of informed strategies for combating resistance based on control failures in the field, based on interpretation of toxicological data and identification of resistance mechanisms responsible.	Insecticide resistance information incorporated into recommended strategies for managing the pest according to the region from which they derive. Project and CFC reports. Y3 Y4	Tactics for *H.armigera* management integrated in Handbook of Sustainable Management of *H.armigera* and promulgated via the CFC regional project.	The multilateral components proves successful and become a vehicle for incorporating the findings into national cotton pest management policy **Met**	**Strategies for the rotation of effective chemistries developed. Results promulgated as national recommendations for cotton pest management in all 3 countries. India recommendations embedded in national Insecticide Resistance Management for Cotton programme in all cotton states**
3. Development of insecticide testing kit components for use in Asia and Africa (in association with certain components of the CFC proposal).	Production of insecticide specific haptens (antigen test precursors) for the insecticide test kits Y1	Insecticide-specific haptens produced and provided for use in insecticide test kit development to CFC project Y1	Appropriately specific chemical bind sites on pesticide molecules **Met**	**Initial insecticide/ protein conjugates produced and passed to India where the kits were developed by the Central Institute for Cotton Research (patents applied for in the name of ICAR)**
4. Synthesis of existing knowledge of the efficacy of particular insecticides against bollworm in	Review of literature and available	Review produced and published. Results included in CFC	None anticipated	**Review undertaken and the results incorporated into a software decision support tool**

cotton and of how insecticides used against *H.armigera* affect natural enemies	unpublished material prepared for the efficacy if insecticides commonly used against *H.armigera*, and their effect on beneficial insects Y2	Handbook Y4		**'*Helibase*' made widely available and incorporated in the CFC Handbook**
5. Definition of minimum effective spray application equipment and practices for applying pesticides to cotton in Asia.	Report on application equipment requirements for cotton spraying Y1	Report produced	None anticipated	**Work undertaken in support of Punjab Agricultural University. Minimum effective practices demonstrated and report produced.**
Activities	**Inputs**	**Means of Verification**	**Important Assumptions**	**Achievements**
1a. Establish cross-resistance patterns in Asian *H.armigera*. Rearing techniques for one fully susceptible and one highly multi-resistant Asian strain established	Demonstration of magnitude of cross resistance within and between chemical classes Y1 and Y2	Project report	Asian partners can supply strains reliably **Met – but ability to maintain colonies was not always met, esp with nerve-insensitivity resistant material**	**Done for strains from the China, Pakistan and India**
1b. Develop insect lines with single mechanisms of resistance	Well maintained lines with single, fully characterised mechanisms Y1, Y2,Y3 and Y4	Report on mechanisms	Colonies with single resistance mechanisms can be reared in the laboratory **This proved difficult – even where such lines had been produced in India**	**Lines with dominant (but not single) mechanisms produced**
1c. Compare, standardise and validate bioassay techniques	Techniques perform well	Methods reported		**Done**
1d. Create full dose response lines for two representative pyrethroids and two organophosphates (chosen by structure) one carbamate and one cyclodiene 1e. Create full dose-response lines for two representative 'resistance busting' chemicals (e.g spinosad) 1f. Identify resistance mechanisms for each of the main cross-resistance groupings.	Understand and report implications of individual resistance mechanisms for toxicology of commonly used insecticides	Report on cross-resistance mechanisms available, and used to focus the IRM tactics	Possible to maintain strains reliably **Met**	**Done** **Done for spinosad and indoxacarb** **Done (see text for details)**
2a Develop integrated resistance management strategies, based on fully characterised cross-resistance patterns	Strategies produced	Strategies reported	Cross resistance can be overcome using pesticides available in the three countries **Partially Met**	**Understanding of patterns of resistance magnitude and underlying mechanism/ cross resistance supported the development of field strategies later shown to be highly effective in reducing resistance, reducing**

				production costs and greatly increasing cotton profitability
2b Contribute to the handbook of *H.armigera* management being prepared by the CFC project, arrange and contribute to workshops	Demonstration of resistance management tactics (in separately-funded fieldwork)	Handbook produced		**Handbook with printer**
3a Synthesis of insecticide-specific haptens for use in the development of insecticide quantity and quality detection kits.	Antisera molecules conjugated with endosulfan, cypermethrin, fenvalerate, quinalphos, phoxim, thiodicarb and carbaryl	Report on insecticide haptan protein synthesis	Synthesis of some molecules may affect structural features of the insecticide **Early constructs improved on for eventual insecticide quality kits**	**Protein/ insecticide conjugates produced and sent to Indian collaborator.**
3b Purification of hapten-protein conjugates for cross reactivity testing	Molecules available for Rothamsted/CICR India	Molecules used successfully in development of insecticide quality kits	Molecules respond to antisera methods **Met**	**Done** **Kits developed at CICR for pyrethroid, endosulfan and carbamates. Organophosphate kits under development. Patents applied for in the name of ICAR**
4a Review the existing literature on pesticidal effects on non-target organisms (beneficial arthropods in particular) and identify the 'softer' and 'harder' chemistries.	Reprints and literature assembled Y1	Review completed and published (Y2) and written up for publication in the the Handbook (Y4)	None envisaged	**Review and tabulation of all published literature on all major chemicals undertaken. Results available in software decision tool '*Helibase*' and in Handbook Chapter.**
4b Synthesise the data into a report	Protocols defined for non target effects	Report provided and implications integrated into strategy Y1	None envisaged	**Results available in software decision tool '*Helibase*' and in Handbook Chapter.**
4c Formulate guidelines on rational decision making using 'competing' chemicals with the same apparent activity spectrum.	Guidelines produced	Strategies incorporated into national programmes based on chemicals least disruptive to beneficials	Price and availability in the market allows farmers to select more appropriate chemicals **Met. There are moves away from older chemistries now into narrower spectrum, more IPM compatible, insecticides and to the deployment of Bt cotton (which is however, still sprayed for bollworms)**	**Guidelines developed in collaboration with national partners, to become national extension recommendations in all countries and the underpinning science of the 50,000 farmer Indian Govt IRM project (2002-7)**
5a Review the existing work on cotton spraying methods	Reprints and literature assembled	Review completed	None envisaged	**Review undertaken with Punjab Agricultural University.**
5b Synthesise the data into a report on minimum recommendations for cotton spraying equipment	Spray application data fully reviewed	Report produced for Handbook Y3	None envisaged	**Review disseminated as a report, a farmer manual and Handbook chapter**

Output 1: Understanding of the principles underlying cross-resistance patterns due to different metabolic resistance mechanisms, which will inform practical *H.armigera* and insecticide resistance management strategies for Asia and Africa.

(This current report is a modified abstract of the much lager and more detailed report of this highly technical work appended to this document).

During the course of the parent CFC project, very significant advances have been made in our understanding of resistance mechanisms in Asian *H. armigera*. Most of this work took place under the current project though collaborative work between the CFC project partners, in particular Rothamsted Research, Nanjing Agricultural University (China), the Central Institute for Cotton Research (India) and the Central Cotton Research Institute (Pakistan). The current project supported UK staff, equipment and consumables at Rothamsted Research, while the CFC project supported the production and testing of relevant insect strains in Asia and the costs of Asian scientists working at Rothamsted (18 person months per country CFC funded and a further two years of work supported by the Chinese Ministry of Science and Technology). As a consequence we now know more about resistance mechanisms in this insect than in any other non-medical species, and this has profound implications for control strategies. [Apart from the concentration of specialist skills, the rationale for undertaking the work at Rothamsted was that UK was able to accept strains from all over Asia, as *H.armigera* is not a pest species in UK, and because pathogenic virus outbreaks were far less likely in a country with no large resident population of the species. This latter assumption proved too optimistic, and stunt virus imported with a Pakistani strain caused considerable disruption to the work in its early stages.]

The rationale for this work was the need to understand how individual classes of insecticide were resisted in the different parts of the species' range (given that the major mechanisms selected may depend on the contingent history of the particular insecticide use pattern to which the local insects were exposed). When the major mechanisms (and their contribution to field resistance) was clear, and the patterns of cross –resistance known (i.e. was a particular resistance mechanism for pyrethroids – say enhanced esterases – cabable of providing resistance to organophosphates) then it would be possible to suggest materials, and sequences of materials, which did not enhance resistance (and indeed hopefully undermined it). The current project was charged with providing this information to the Asian partners for the development of field programmes.

When this project was formulated, it was thought that pyrethroid resistance in the Indian sub-continent was mediated through multiple mechanisms: metabolic (cytochrome p450 and esterases), detoxification, reduced cuticular penetration and target site insensitivity, and that resistance to organophosphates was mediated through insensitive acetylcholinesterases and enhanced esterase activity. It is now clear that, in practical terms, the situation is considerably less complicated and that many of these putative mechanisms are of no, or little importance from a management standpoint.

The extent of cross-resistance, and its importance to field control was not clear. In this study, simple comparisons of resistance factors derived from field populations collected from a variety of localities in India and Pakistan suggests that the resistance exhibited by some compounds is particularly well correlated (e.g. most pyrethroids, or most organophosphates), but that in others the relationship is not clear.

There was a fear that common metabolic mechanisms such as esterases might cause widespread cross-resistance between the pyrethroids, OPs and cyclodienes and that a common target site modification (insensitive AChE) might confer resistance to a wide range of OPs and carbamates. In fact, the current evidence suggests that esterases are not the major resistance mechanism for Asian *H. armigera* and that oxidases play the major role. This is quite different to the situation that is described for Australian populations – the best characterized until now – and closer to the recently obxserved situation in W.Africa.

It is the case however, that in Asian *H. armigera* populations, insensitive forms of AChE give broad cross-resistance to many carbamates and organophosphates. However, this mechanism is ubiquitous and is therefore of no great importance in a managerial sense. It occurs in parallel with other mechanisms and occurs in all regions. There is little opportunity therefore to select against it.

Part of the original plan of the project was to produce strains of *H. armigera* possessing single, defined mechanisms of resistance using a combination of selection by insecticides and selective breeding. In fact, the project discovered that insensitive AChE and the rdl gene that has been linked to endosulfan resistance were ubiquitous globally and even in museum specimens from early in the 20th century. Moreover, it was shown that nerve insensitivity associated with pyrethroid resistance and illustrated electophysiologically in India (and historically in China) was not connected to any known kdr or super kdr mutation. A comparison of glutathione-s-transferase (GST) titres in resistant and susceptible insects also showed that this was probably not an important mechanism of resistance. Given these discoveries, the original plan in which isolines containing many different mechanisms would be maintained in the laboratory was no longer tenable.

Perhaps the most important finding of this research is that only oxidative and esterase-mediated resistance mechanisms account for most of the variation in resistance that is seen. It is therefore mainly these mechanisms that are manipulable and therefore of practical importance in terms of cross-resistance patters and of pest management.

Although McCaffery (1998) wrote "…*it is disappointing that such [research on mechanisms] have not yet resulted in commercially viable products* [diagnostic tests]…" it now seems clear that the only tests that might be of real use would be those identifying specific oxidase or esterase isozymes imparting pyrethroid and organophosphate resistance. Given that specific tests for oxidase isozymes are still some way off, esterase markers are proving to be the most practically useful of those diagnostics. The collaborators at the Central Institute for Cotton Research in India are developing just such unique esterase isozyme kits based on the different isozymes involved in organophosphate and carbamate resistance.

The major types of resistance likely to be important in *H.armigera* were known from earlier work with this species (McCaffery 1999) and other insects. However, the importance and ubiquity of the various mechanisms in different populations within Asia was not known. It was expected that the patterns would reflect the historical use of various materials in different orders in different areas, which would have been selecting for different mechanisms. In India the situation for pyrethroids is given in Kranthi et al (2001a) and for organophosphates and carbamates in Kranthi et al (2001b). Recent results on enzymatic detoxification of pyrethroids are reported in Yang (2004 and 2005) and Chen *et al.* (2005).

Conclusions on mechanisms of resistance
Metabolic mechanisms
- GSTs (Glutathione-S-transferases) play only a minor role (RF<10)
- Oxidases are very important in pyrethroid resistance
- Esterases are less important in pyrethroid resistance (not true in Australia) but are involved with OP/carbamate/endosulfan resistance
- Different isozymes are involved in resistance to particular groups of chemicals (e.g. E9 for Methomyl)

Target site mechanisms
- The rdl '(dieldrin resistance) 'mutation'is ubiquitous in *H. armigera,* even in museum material from prior to the introduction of endosulfan. Rdl is likely to confer background tolerance to endosulfan but doesn't currently appear to account for variation in endosulfan resistance between field populations, which now seems more likely to be metabolically based.
- Modified AChE – insensitive forms (at various levels in different populations) also appear ubiquitous in field populations, conferring basal resistance to OPs and carbamates but perhaps not the primary cause of variations in response between strains
- Knockdown resistance to pyrethroids (kdr) – electrophysiological evidence for nerve insensitivity is strong in heavily sprayed populations in India (China and Pakistan are working on this now) but it does not seem to be attributable to any of the known mutations.

Penetration reduction
- Present in China and Pakistan and probably India. It may well have a multiplicative effect on the impact of metabolic mechanisms.

Table 2 summarises what is now known of the distribution and importance of the three major types of mechanisms in *H. armigera* in Asia.

Table 2: Distribution and relative importance of resistance mechanisms in Asian *H.armigera*

	METABOLIC			TARGET SITE			PENETRATION REDUCTION
Mechanisms	**Oxidases**	**Esterases**	**GSTs**	**Ache**	**Nerve Insens**	**rdl**	
Chemicals Affected	*Pyreth.*	*OP/Carb Endo/Pyreth*	*Pyr*	*OP/Carb*	*Pyreth*	*Endo*	*Pyrethroid (others?)*
India	***	**	*	*	**	*	?
China	***	**	*	*	*	*	*
Pakistan	**	**	*	*	*	*	*

Careful crossing of lines of resistant insects at the Central Institute for Cotton Research in India (under CFC funds) elucidated the inheritance pattern of these resistances (3 and 4)

Table 3: Genetics of resistance inheritance (India)

Insecticide	**SR**	**RS**	**Nature of the resistance allele**	
Cypermethrin	0.84	0.84	Inc-dom	Autosomal
Endosulfan	0.58	0.64	Inc-dom	Autosomal
Quinalphos	0.59	0.57	Inc-dom	Autosomal
Methomyl	0.96	0.92	Dominant	Autosomal
Spinosad	0.13	0.11	Recessive	Autosomal

Table 4: Genetics of resistance mechanisms in Indian *H.armigera*

Insecticide	**Mechanism**	**Nature**	**Frequency**
Quinalphos	Esterase	Recessive	20%
	Insens-AchE	Inc-domi	80%
Methomyl	Esterase	Dominant	90%
	Insens-AchE	Semi-dom	30%
Pyrethroid	Esterase	Inc-recessive	
	MFO	Inc-dominant	
	Nerve-Ins	Recessive	

The relatively dominant nature of inheritance of most resistances is unfortunate. The phenotypic role of particular mechanisms is affected by their inheritance pattern

Understanding the mechanism of resistance to the different classes of compounds provides a theoretical underpinning for patterns of cross resistance which were confirmed in the field in Asia. Where, for example, a particular pyrethroid is not susceptible to esteratic cleavage e.g. bifenthrin, it will not be cross resistant to most other pyrethroids by that mechanism. The pattern of cross resistances below emerges Table 5.

Cross Resistance of the Main Chemical Groups

Pyrethroids are broadly cross resistant to each other. Unfortunately most 'resistance-busting' pyrethroids such as bifenthrin are not registered on cotton. There is no cross resistance to organophosphates or endosulfan, but some evidence of cross resistance to carbamates, probably through shared metabolic detoxification mechanisms (particularly enhanced esterases.

Spinosad and Ixdoxacarb are the only members of their groups currently significantly used in cotton. They are not cross resistant to each other or to other classes of insecticide (though there is some doubt with respect to Spinosad and OP metabolic resistance).

Table 5: Patterns of cross-resistance in Asian *H.armigera*

	Pyrethroid	**OP**	**Carbamate**	**Cyclodiene**	**Spinosyn**	**Indoxacarb**
Pyrethroid	Yes *but some exceptions*	No	No?	No	No	No
OP		Yes *but not Monocrot-ophos*	Yes *variable*	No	Some doubt	No
Carbamate			Yes *Methomyl/ Carbaryl* No *Thiodicarb*	No	No	No
Cyclodiene				Only one - Endosulfan	No	No
Spinosyn					Unknown	No
Indoxacarb						N/A

Organophosphates also show broad cross resistance within the major phosphothiorinate group, which includes almost all cotton OPs. Monocrotophosis the major exception. It is a phosphate OP which does not need to be activated by mixed function oxidases at the P=O group and is therefore not cross resistant to the other OPs by enhanced oxidases. There is some cross resistance with carbamates as they share both oxidase and esterase resistances. However, the inheritance of resistance to these insecticides is very different. Not only is resistance though enhanced esterases much more important in carbamates than OPs because of its higher dominance, the specific esterase isozymes responsible for the resistances turn out to be different (indeed these differences were used as the basis for rapid resistance mechanism detection kits for carbamates and OPs under the CFC project).

Carbamates show cross resistance between methomyl and carbaryl but not, surprisingly, to thiodicarb which could therefore be used as an independent material in resistance management rotations. There is some (weak and variable) cross-resistance to organophosphates by metabolic mechanisms.

Endosulfan – the only cyclodiene still widely used on cotton, is not cross resistant to the other groups, confirming its continuing value in cotton insecticide rotations (especially as resistance to endosulfan is low to moderate, especially early in the season.)

These cross resistances were measured empirically but also emerge logically from the patterns and sharing of mechanisms, though it is important to note that, for example, the esterase-based resistance of quinalphos as an organophosphate and methomyl as a carbamate, are not based on the same esterase isozyme and it does not necessarily follow that chemistries resisted by the same overall mechanism will show cross resistance. For example, bifenthrin and related pyrethroids; or monocrotophos and related phosphatic OPs, have structures sufficiently different to give them a different resistance profile from that of most of their respective groups.

Output 2: Formulation of informed strategies for reducing insecticide use and combating resistance based on control failures in the field, based on interpretation of toxicological data and identification of the resistance mechanisms responsible.

The conclusions of the collaborative work at Rothamsted in relation to the field application of the results were:

1. Despite (or perhaps as a result of) decades of extremely severe selection pressure, there remain few mechanisms of prime importance in Asian *H. armigera*. All the available evidence now suggests that particularly oxidases and esterases are of major practical importance..

2. Although, with regard to all the traditional compounds in use (endosulfan, carbamates, organophosphates and pyrethroids) the only mechanisms of note are oxidases and esterases, there exists considerable complexity and geographic variation within these mechanisms. However, resistance is a dynamic evolutionary process and, at intervals, the situation should be reassessed.

3. In all countries, pyrethroid resistance is the most intractable problem. It is caused by a mixture of esterase and oxidase mediated mechanisms and the relative importance of these appears to vary by region. In general, it is oxidases that seem likely to provide the greater part of that resistance (although oxidase-mediated resistance seldom, if ever, exists in isolation).

4. The use of single applications of cypermethrin, deltamethrin and fenvalerate against *H. armigera* are likely to be ineffectual. Insecticide mixtures however, utilising some OPs (such as triazophos) are likely to restore efficacy, at least in the short term.

5. With reference to the use of mixtures, resistance to both OPs and pyrethroids is widespread, and that continuous selection by a mixture is likely to select rapidly for resistance to both constituents of the mixture. This has been demonstrated within the CFC project at Nanjing Agricultural University, where selection with an OP/Pyrethroid mixture (phoxim/fenvalerate) rapidly selected for two separate mechanisms – oxidases for pyrethroid resistance and esterases for phoxim resistance. Resistance to the pyrethroid component continued to build rapidly, although the overall resistance to the mixture grew more slowly.

6. Other pyrethroids (bifenthrin, lambda cyhalothrin) which are less susceptible to the particular enzymatic breakdown mechanisms currently prevalent in Asia appear to be less severely resisted and may still be of some use when used alone.

7. If the use of some pyrethroids is stopped, the situation should be monitored to discover whether susceptibility can be restored. Work in the field IRM programme in India has shown that, with restraint in their application over relatively modest areas (say 20Km in diameter), virtually full susceptibility to pyrethroids and other insecticide classes can be restored. However, the genes conferring resistance may be present at higher levels in such populations and it is likely that resistance could be rapidly selected again were the materials brought back into widespread use.

8. In all countries, organophosphate and carbamate resistance is mediated by esterases. There is little evidence for the involvement of oxidases. It is likely therefore, that continued use of these compounds should not continue to select for pyrethroid resistance. Rotation of OPs with the pyrethroids that retain some efficacy should be encouraged. OP / pyrethroid mixtures with proven efficacy could also be used where agronomically appropriate.

9. In almost all cases, endosulfan resistance is not severe. The mechanism of resistance remains unclear (in part due the lack of highly resistant strains to work with) but there does not appear to be any cross-resistance with the pyrethroids or the organophosphates. We recommend therefore that endosulfan is used in rotation with OPs, OP / pyrethroid mixtures and with those pyrethroids that retain some efficacy.

10. Amongst the newer insecticides which are effective for bollworm control, spinosad and indoxacarb are effectively not cross resistant to other older chemistries or to each other and form a extra tools to use in rotation strategies.

The results derived from output 1 now tell us which materials it is safe to use sequentially in a bollworm spray programme without exacerbating resistance problems (Table 6). The number of different resistance groups is smaller than might have been hoped, limiting the scope for rotations.

Table 6: Materials which could be rotated in an IRM strategy

	Major mechanisms	**Minor mechanisms**	**Potential Rotation groups**
Pyrethroids	Oxidase	Esterase Nerve insensitivity Penetration	• Most pyrethroids • Bifenthrin and similar structures
Organo-phosphates	Insensitive Ache	Esterase	• Phosphatic – (monocrotophos) • Phosphothioronate (quinalphos, phoxim and most others)
Carbamates	Esterase	Insensitive Ache	• Methomyl/carbaryl • Thiodicarb
Endosulfan	Esterase (sequestration)	Rdl	• Endosulfan
Spinosyn	None todate		• Spinosad
Organotin	None to date		• Indoxacarb

Of course, which actual materials are recommended will depend on efficacy against the particular pest complex present at the time, (including consideration of the level of resistance to that particular chemical) cost and availability. This information is therefore incorporated into somewhat different recommendations in the different parts of the three countries.

Based largely on theoretical work, several operational tactics have been proposed for retarding the spread or combating the impact of resistance to insecticides. The following classification of tactics into three categories is only one of the proposed schemes but is useful in the present context:

Management by moderation. Reducing selection for resistance by less frequent applications, short-lived residues, and the creation of untreated 'refuges'. These have yielded some benefit for agricultural situations since, for example, a refuge can be deliberately created or may arise *de facto* through the occurrence of a pest on host plants other than the crop being treated.

Management by saturation is aimed at overcoming resistance by the use of doses sufficiently high to kill resistant insects from the outset, or by combining insecticides with synergists to overpower specific resistance mechanisms. The second of these obviously requires a sound knowledge of the mechanisms present. Prospects of achieving the former depend critically on the potency and dominance of mechanisms, but not necessarily on a knowledge of their biochemical basis. The reasoning behind the tactic is that even if resistant homozygotes are sufficiently tolerant to withstand an insecticide treatment, heterozygotes (which predominate in the early stages of selection) may succumb to applications set as far above the tolerance range of susceptible insects as economic, health and/or environmental constraints permit. This tactic is considered of dubious relevance to agricultural systems, is likely to be financially and environmentally costly, can only be regarded primarily as preventative, and will not be effective if resistance is already established.

<u>Management by multiple attack</u> involves using two or more unrelated insecticides in ways that reduce the selection or impact of resistance to any one chemical. The compounds could be applied simultaneously as mixtures, alternated over time, or used in more complex spatial patterns known as mosaics. The key to success is being able to ensure that ‘partner’ compounds run no risk of cross-resistance, and being able to implement and co-ordinate such tactics amid the commercial and socio-economic pressures that normally drive the choice and use of insecticides. Ideally, when using mixtures, the components would be non-resisted at the outset, have similar toxicities and field persistences, in order to avoid one being exposed alone to selection by the insect. In practice these conditions have never been fully met. Nonetheless, the use of mixtures (usually organophosphates and pyrethroids) os widespread in cotton management where resistance is prevalent, especially in China

Implications of management by multiple attack: In West African strains of *H. armigera*, pyrethroid resistance is thought to be conferred largely by the increased activity of MFOs. This is supported by the ability of piperonyl butoxide (PBO) to suppress resistance in laboratory bioassays), and by biochemical studies showing that deltamethrin resistance is correlated with MFO titres. The esterases that now seem largely responsible for pyrethroid resistance in H. *armigera* from Australia and certain Asian countries appear to play a lesser role in pyrethroid resistance in West African strains.

Despite the apparent lack of esterase-based pyrethroid resistance in West Africa, researchers in Côte d’Ivoire have shown mixtures of deltamethrin and triazophos to be particularly potent against *H. armigera*. They argue that such an effect may be explained by (1) an interaction between triazophos and MFOs that interferes with the MFO-mediated detoxification of pyrethroids, and/or (2) more rapid activation of triazophos to triazophos-oxon (the active analogue) by the enhanced MFOs that confer pyrethroid resistance.

Even in those countries where esterases are considered to be the major mechanism of resistance, such pyrethroid / organophosphate mixtures may prove of great utility. In Australia, pyrethroid resistance in *H armigera* is considered to be primarily a consequence of the overproduction of esterases that sequester (and possibly hydrolyse) pyrethroid esters. Biochemical studies with Australian strains have shown that esterases associated with pyrethroid resistance bind to and are inhibited by a range of organophosphorous compounds, including ethion, chlorpyrifos, profenofos, and acephate. As a consequence, co-application of these organophosphates with pyrethroids may lead to striking suppression of pyrethroid resistance, and may offer an additional tool for managing pyrethroid resistance under field conditions (Gunning et al 1999). This fact has not escaped the notice of Asian farmers. Ethion (an OP which is not itself effective against bollworms) and Cypermethrin (a pyrethroid normally strongly resisted) have been successfully deployed as a bollworm controlling mixture in the Indian Punjab for some years. We now understand and can generalise on that finding.

The efficacy of such mixtures of insecticides (particularly organophosphate/ pyrethroid mixtures) and the impact of their deployment on the development of resistances to both components of the mixture –separately and together, was the subject of a component of the CFC project (led from Nanjing Agricultural University). The results are presented in the final technical report of the CFC project (attached) and in Russell (2005). Very briefly, although the OP component of such mixtures can indeed undermine metabolic resistance to the pyrethroid component, deployment of the mixture does not prevent the rapid rise of resistance to the pyrethroid component (and at a slower level, the organophosphate moity). It would seem that mixtures select for multiple mechanisms of resistance and that some of these processes can be rapid.

Example Resistance Deployment Strategys

Based on the accumulating finding of the project the first of the following strateies developed for the highly successful Indian Insecticide Resistance Management programme (Govt. of India, Cotton Technology Mission, Mini Mission II 2002-2007) which is being implemented in 28 districts of the 11 cotton states in India, in 565 villages with 46,431 collaborating farmers by 2005-6. Deployment of that strategy resulted in a 46 % reduction in Insecticide use by the programme farmers and an 11% average yield increase, providing a financial benefit of c.$175/ha. The Central Cotton Research Institute (our project partner) provides the technical backstopping to that programme. The materials (and rates) recommended in the 'windows' combine known efficacy (from output 4) against the pest complex present at the time, with an appreciation of the resistance profile and cross-resistance mechanisms, to reduce sequential application of materials resisted by the same mechanism.

Simple Indian IRM Programme Recommendations 2002-5

(developed with K.Kranthi, Central Institute for Cotton Research, India)

(These have been used very widely over a number of years with great financial success by over 200,000 farmers. They use only readily available and moderately inexpensive materials)

Sucking Pests	Bollworm Window 1	Bollworm Window 2	Bollworm Window 3	Bollowrm Window 4
0-60 days	60-90 days	90-105 days	105-120 days	120-140 days
Zero sprays	Endosulfan (Neem/HaNPV)	Spinosad/ Indoxacarb	Organophosphate/ Carbamate	Pyrethroid

Note: Windows 2 and 3 are commonly run together, using only OP/carbamates, by the more resource poor growers.

<u>Current Indian IRM Programme Recommendations 2005 onwards</u>

(From K.Kranthi, Central Institute for Cotton Research, India)

(Note that there is an increase in the number of windows and materials used here. The newer materials are beginning to be available and utilized).

A. Early sucking pests: NO SPRAY up to 60 DAS

Aim: to mitigate early season damage while conserving natural enemies

Strategies: Jassid resistant genotypes

Seed treatment: Imidocloprid (7g)/ Carbosulfan (20g) or Carbofuran (25g/Kg)

B. Window 1: 60-90 DAS: Initial bollworm infestation

Aim: to suppress the first generation of *H.armigera* while minimizing the effect on beneficials

Strategies: Scouting – ETL 0.5 larvae/plant
Biologicals – Trichogramma/ Neem/ HaNPV
IGR - Novaluron, Lufenuron if available
Endosulfan – ‘soft’ on beneficials, resistance level low early season

C: Window 2: 75-90 DAS Mid-Reproductive stage: Bollworm infestation:

Aim: to protect boll formation by controlling the mixed instars of overlapping *H.armigera* generations. while minimizing the effect on beneficials

Strategies: Scouting – ETL 1larvae/plant
Spinosad – No resistance so far
Indoxacarb – No resistance so far

D: Window 3: Mid bollworm: 90-110 DAS Bio-selective and least resisted insecticides.

Aim: to suppress the first generation of *H.armigera* while minimizing the effect on beneficials

Strategies: Scouting – ETL 0.5 larvae/plant
Biologicals – Trichogramma/ Neem/ HaNPV
IGR - Novaluron, Lufenuron if available
Endosulfan – ‘soft’ on beneficials, resistance level low early season

E: Window 4: peak bollworm: 110-140 DAS: Conventional insecticides.

Aim: to suppress the first generation of *H.armigera* while minimizing the effect on beneficials

Strategies: Scouting – ETL 0.5 larvae/plant
Biologicals – *Trichogramma*/ Neem/ HaNPV
IGR - Novaluron, Lufenuron if available
Endosulfan – ‘soft’ on beneficials, resistance level low early season

F: Window 5: Pink bollworm: >140 DAS: Pyrethroids.

Aim: to suppress the first generation of *H.armigera* while minimizing the effect on beneficials

Strategies: Scouting – ETL 0.5 larvae/plant
Biologicals – Trichogramma/ Neem/ HaNPV
IGR - Novaluron, Lufenuron if available
Endosulfan – ‘soft’ on beneficials, resistance level low early season

<u>Full Indian IRM Programme Recommendations for 2006 onwards</u>

(From K.Kranthi, Central Institute for Cotton Research, India)

(These are useable only by those having considerably above average financial resources – but represent a goal to work towards as yields and incomes improve)

A. Early sucking pests: NO SPRAY up to 60 DAS

1. Cultivation of sucking pest tolerant genotypes to help in delaying the first spray, thereby conserving the initial build-up of natural enemies.

2. Chemical seed treatment to help delay the first spray (Imidacloprid 70 WS or Thiomethoxam 70WS @ 5-7g/Kg seed are useful for hybrids in protecting the crop against leafhoppers up to 30-60 days (Kairon and Kranthi 1998; Surulivelu et al., 2000).

3. Inter-cropping with cowpea, soybean and black gram was found to encourage natural enemies (Rao et al. 1994).
4. Stem application of acetamiprid or thiomethoxam or imidacloprid (confidor) at 40 DAS.

5. Avoidance of broad-spectrum organophosphates such as Monocrotophos, Methyl demeton, Phosphomidon, Acephate etc. especially as early season sprays as these strongly disrupt the natural enemy populations (Kranthi, unpublished data).

6. **Emergency**: ETL based spray of diafenthiuron (POLO) against jassids or whitefly or aphids.

B. Window 1: 60-75 DAS: Initial bollworm infestation: Mostly eggs and young larvae: biological and biopesticides window

7. Release of Trichogramma egg parasitoids at 70 DAS.

8. The use of soft chemistry biopesticides such as *Bacillus thuringiensis* or HaNPV (Nuclear polyhedrosis virus of *Helicoverpa armigera*) or Neem (*Azadirachta indica*) based insecticides can be used as initial sprays to help conservation of natural enemies.

9. Do not spray against the cotton leaf folder, *Sylepta derogata* and cotton semilooper, *Anomis flava*. The larvae cause negligible damage to cotton but serve as hosts for parasitoids such as *Trichogramma* spp., *Apanteles* spp and *Sysiropa formosa*, that also parasitise *H. armigera*.

10. **Emergency**: ETL based spray 50 % plants showing flared up squares: Endosulfan may be used if none of the biological control or biopesticides alternatives are available.

C: Window 2: 75-90 DAS: Bollworm infestation: Mostly younger larvae: Bioselective and least resisted insecticides.

11. ETL based spray: 50 % plants showing flared up squares: Use of Novaluron (Rimon) or Lufenuron (Match) or Endosulfan during the initial cropping phase representing the first window for bollworms. Results from monitoring clearly indicated low levels of resistance in *H. armigera* to almost all groups of insecticides initially in the cropping phase. Novaluron, Lufenuron and Endosulfan are considered to be relatively benign on beneficials. Because of the low resistance frequencies, the use of this insecticide at the early cropping stage is proposed as the appropriate choice against *Helicoverpa armigera* or leaf hoppers. For north Indian conditions, wherein, the spotted bollworm occurs initially in the season, spinosad would be the preferred insecticide in this window.

D: Window 3: Mid bollworm: 90-110 DAS Bio-selective and least resisted insecticides.

12. ETL based spray: 90-100 % plants showing flared up squares: Spinosad and Indoxacarb are highly effective on pyrethroid resistant *H. armigera*. Emamectin benzoate, which is likely to be commercially approved soon in India, is yet another ideal option in this window. Apart from their toxicity to *H. armigera*, Spinosad and Emamectin benzoate are also effective on *E. vittella* and jassids and hence are preferred first over indoxacarb. All the three insecticides have a high selective toxicity towards the target pests while being less toxic to many beneficial insects in the cotton ecosystem. These insecticides are ideally suited in eco-sustainable insecticide resistance management programmes. Thus far there is no evidence of any resistance against Spinosad or Indoxacarb. However, if the molecules are overused, there is every likely chance that resistance will render the molecules less useful.

E: Window 4: peak bollworm: 110-140 DAS: Conventional insecticides.

13. ETL based spray: 90-100 % plants showing flared up squares: Organophosphates or carbamates can be used as effective larvicides. Resistance levels against certain organophosphate group of insecticides (Quinalphos, Chlorpyriphos & Profenophos) and carbamates (Thiodicarb and methomyl) have been found to be low in most populations tested. These insecticides are very effective for bollworm control but have low ecological selectivity and can be harmful to beneficial insects. The populations of beneficial insects in cotton ecosystem are generally low in this window and hence the application of organophosphates and carbamates is rational.

F: Window 5: Pink bollworm: >140 DAS: Pyrethroids.

14. ETL based spray: Eight pink bollworm moths per trap per night for 3 consecutive nights. The application of pyrethroids as late season sprays would be effective for pink bollworm management. Pyrethroid resistance in *H. armigera* is generally high, but pyrethroids are very effective against pink and spotted bollworms and are ideally suited for the late season window.

<u>Basic IPM context promoted in all IRM work, for ensuring most effective pest control.</u>

1. Destruction of crop residues to prevent carry over of pest populations and summer ploughing to destroy resting stage insect populations. Especially useful for pink bollworm management. Immediately after the season allow animal grazing in fields and ensure timely removal and destruction of cotton stubbles, followed by deep ploughing to expose the carry-over population of bollworms. Do not stack cotton stalks near fields.

2. Avoid growing American cotton in orchards as it favours whitefly outbreaks. Grow only *Gossypium arboreum* cotton or Cotton Leaf Curl Virus (CLCV) resistant varieties in CLCV hot-spot areas. Only recommended varieties/hybrids from reliable sources must be procured. Avoid growing tur, moong and bhendi in and around cotton field as these harbour insect pests. Off-season hosts must be discouraged. Weeds such as *Sida* sp., *Abutilon* sp and *Xanthium* sp. must be uprooted to prevent initial build-up of spotted bollworm, whitefly and CLCV.

3. Hybrids must be grown in medium-deep soils having good drainage. Apply 5-10 tonnes of well-decomposed compost or FYM /ha before sowing. Delint the seed with 100 ml sulphuric acid /kg seed for two minutes, wash with water and soak for two minutes in Calcium carbonate (5g/ltr water). Treat seeds with Ceresan wet or Agallol @ 1 g/ltr water, Captan or carbendazim @ 2g/kg, imidacloprid or thiomethoxam. Early sowing on ridges and furrows, especially in areas with drip facility, could be adopted. Sowing must be completed by the third week of May in North India and mid July for central and south India (except Tamil Nadu). Sowing can be done at a row spacing of 67.5 cm with 30 cm plant-plant spacing or preferably wider for varieties and 75cm for hybrids.

4. Application of weedicide Stomp 30EC or Basalin @45EC 2.5 lt/ha and harrow immediately to prevent degradation. Harrowing must be done twice after pre-monsoon showers and field should be leveled.

5. Apply fertilizers considering the crop history, previous crop and its fertilizer use pattern. Nitrogen rates recommended for *G. hirsutum* varieties range from 40-60 Kg/ha in rain fed and 60-90 Kg/ha in irrigated cotton. For hybrids, 90 Kg/ha in rain fed and 100-120 Kg/ha in irrigated. P and K doses depend on soil test values or in their absence N: P: K is used at a ratio of 2:1:1.

6. Gap filling must be completed within 10 days after sowing. Thinning should be done within 20 days after sowing. First hoeing can be done 30-40 days after sowing followed by second after 15-20 days.

7. Spotted bollworm can cause damage to growing points initially, hence scouting is necessary during the first two months and removal of affected parts helps in minimizing future damage.

8. Handpicking of larvae 2-3 days after insecticide sprays effectively eliminates any surviving population, which can cause future resistance problems.

9. Always use insecticides as need based applications as per threshold levels. Always target younger stages of *Helicoverpa* as younger stages of resistant larvae are known to be killed at normal recommended doses

Effect of the programme on resistance in *H.armigera*

The field programme for insecticide resistance management has been in operation on an expanding scale since 1996 (originally under other CPP projects). Continuous application of the evolving programme has been going on for longest in the Wardha district of Maharashtra, where a cluster of villages has been part of the programme since 1997. Resistance values for the major groups are given below in Table 7.

Table 7: *H.armigera* resistance frequencies 1999-2003 in the IRM district of Wardha, Maharashtra (figures are resistance frequencies (RF)*)

	1997 Status	**1997 RF**	**1998 RF**	**1999 RF**	**2000 RF**	**2001 RF**	**2002 RF**	**2003 RF**	***2003 Status***
Pyrethroid *(Cypermethrin)*	Medium	96	7	10	6	7	9	3	v.Low
Cyclodiene (*Endosulfan)*	Medium	29	35	7	5	3	2	1	None
OP: *(Qunialphos)*	Low	2	3	1	1	1	1	1	None
Carbamate *(Methomyl)*	None	1	1	1	1	1	1	1	None

* *RF – multiple of dose killing 50% of susceptible population which is required to kill 50% of the current population.*

By 2003 resistance was practically undetectable in the district and has remained low since. Limited use of pyrethroids can therefore be made in late season with the confident expectation that efficacy will be as high as it ever was. This, despite the fact that the area covered by the programme has a radius of only 40km or so. This suggests that long term effects can be achieved even in quite restricted areas, despite the long-distance flight potential of *Helicoverpa armigera*.

Output 3: Participation in the development of insecticide quality and quantity testing kits for commercialisation and use in India, Pakistan, China and elsewhere (in association with certain components of the CFC project).

Poor quality insecticide is a major problem in India and to a lesser extent in Pakistan and China but the capacity to enforce national quality standards is poor, leaving farmers at the mercy of unscrupulous or incompetent manufacturers and dealers. When insecticide failure can be also be blamed on resistance, a diagnostic to separate quality issues is extremely useful. Although a national insecticide quality control system is theoretically in place in India, in practice testing centres do not have the equipment or funds to make this routinely available to individual purchasers of insecticides. The CFC project set out to develop a rapid quality check by vendors, extension agents and farmers, using an immunoassay 'dip-stick' kit approach(similar to pregnancy detection kits) at CICR Nagpur for the major chemical groups in current use against cotton bollworm. The work under this project supported this aim. The kits allow the user to determine if the correct active ingredient is in a pesticide tin and whether it is at least of the specified concentration.

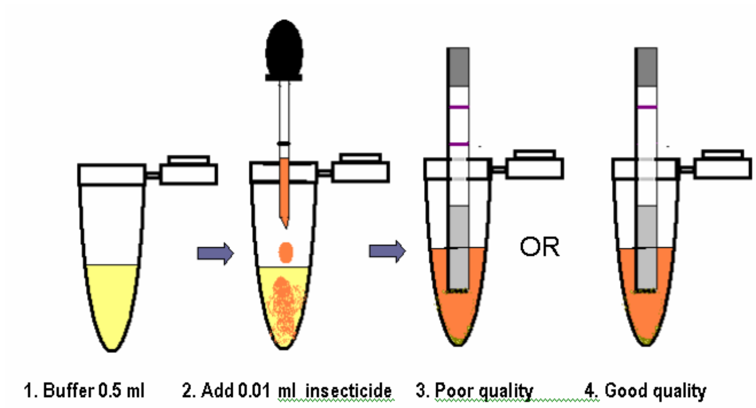
There had been some previous work on insecticide/protein conjugates but they had not been made for the materials of interest here. The Chemistry Dept of the University of Greenwich developed modified methods to link diagnostic parts of pyrethroids and endosulfan to the standard mammalian immune system-detectable proteins (Keyhole Limpit Protein and Bovine Serum Albumen) to produce hapten usable for the generation of antisera in rabbits. These were provided to CICR at the end of year one of the project as intended.

At CICR the protein conjugates were injected into rabbits. Some excessive cross reactivity with insecticide degradation products caused problems which were solved by refining the target moity on the insecticide and re-producing the haptens. Further refinements were necessary for the more 'difficult' organophosphates. The use of monoclonoal antibodies was considered but in the end proved unnecessary. At CICR the antisera was placed in the 'pad' at the base of the stick and the antigen (the insecticide itself) in a strip half way up the 'stick'. The stick is dipped in the insecticide for testing (Figure 1). If the active ingredient is not sufficient it cannot bind the antiserum in the pad. The antiserum therefore runs up the stick and conjugates with the antigenic stripe of insecticide causing a colour change and a visible band. In principle, a single dip-stick can be used for quality detection of a number of insecticides.

Prototype kits for cypermethrin and endosulfan have now been tested in the 26 laboratories of the Indian IRM network and other kits are under development. The kits are cheap (cents per test), reliable, rapid (few minutes), robust and very user-friendly. It is expected that these will empower farmers to demand quality improvements from producers and dealers.

Patenting of the IP in these kits was always envisaged under delegated authority from CFC to the Indian Council of Agricultural Research. Negotiations to put this in place have taken longer than the work itself, but the Indian patents were finally applied for in Jan 2006, with international patents (esp. China and Pakistan) to follow.

Figure 1. 'Dip-stick' immunoassay kits for insecticide quality ('dip-sticks', buffer, vials and pipettes provided in the kits)



The method has also been used to develop diagnostic tests for resistance to insecticides in *Helicoverpa armigera*, brought about by metabolic mechanisms mediated by enzymes (esterases or oxidases – which are proteins). For example a methomyl resistance detection kit has been produced based on the specific esterase isozyme found to mediate resistance to that chemical. The IP in these kits has been separately patented by ICAR.

Similar kits for detection of adequate quantities of insecticidal proteins in Bt cotton tissues were developed and patented simultaneously under other funding.

Output 4: Synthesis of existing knowledge of the efficacy of insecticides used against *H.armigera*, and how they affect natural enemies

Choice of Insecticides for *H.armigera* control

Many materials have been used for *H.armigera* control over the years. Not all are equally effective, many have impacts on others insects in the pest and beneficial complex and many are to a greater or lesser extent harmful to human health.

In the late 1990s four chemical classes dominated cotton crop protection in Asia. The synthetic pyrethroids (cypermethrin etc), the organophosphates (quinalphos, phoxim etc), a single cyclodiene (endosulfan) and the carbamates (esp. methomyl). These individual chemicals have been used as representatives of their classes in many of the studies reported here. Toxicity and range of efficacy varies between chemicals but those of the same class are generally (but not always) metabolized and resisted by the sme mechanisms in insects.

The Natural Resources Institute summarized the information from the 557 significant, peer reviewed, published reports on pesticide performance. This information was used in a number of ways.

The published results were categorized to give a score for efficacy (out of 6) (A), a score for harmful impact on beneficial insects (out of 4.5) (B) and a score for mammalian toxicity (5 is the least toxic) (C). These have been combined as an Overall Score (= A – B + C). The higher the Overall Score, the more suitable the insecticide is for cotton bollworm a control (Table 8):

Table 8: Insecticide performance and Overall Score of suitability for H.armigera control (not taking account of resistance to any material)

Insecticide	Control efficacy A	Impact on natural enemies B	WHO mammal toxicity class C	Overall score A- B+C
NPV products (Bio)	4.4	1	5	8.40
Bt products (Bio)	4	2.2	5	6.80
azadirachtin (Bot)	4	2	4	6.00
thiodicarb (Carb)	6	2.5	2	5.50
fluvalinate (Pyr)	6	2.8	2	5.20
fenpropathrin (Pyr)	6	3.8	2	4.20
chlorpyrifos (OP)	6	3.9	2	4.10
bifenthrin (Pyr)	6	4	2	4.00
cyfluthrin (Pyr)	6	4	2	4.00
endosulfan (OC)	5.2	3.5	2	3.70
fenvalerate (Pyr)	5.2	4	2	3.20
deltamethrin (Pyr)	5.2	4.2	2	3.00
flucythrinate (Pyr)	6	4	1	3.00
cypermethrin (Pyr)	5.2	4.3	2	2.90
profenofos (OP)	5.4	4.5	2	2.90
malathion (OP)	4	4.3	3	2.70
methomyl (Carb)	6	4.3	1	2.70
quinalphos (OP)	4.8	4.4	2	2.40
monocrotophos (OP)	5.2	4.1	1	2.10
carbaryl (Carb)	4	3.9	2	2.10
BHC (OC)	4	4.1	2	1.90
triazophos (OP)	4.4	4	1	1.40
acephate (OP)	2	3.7	3	1.30
lambda-cyhalothrin (Pyr)	4	4.7	2	1.30
carbofuran (Carb)	2	3.8	1	-0.80
parathion-methyl (OP)	2	4.3	1	-1.30

This table can be used to support decisions on which insecticide to use (in conjunction with the information from the current projects on resistance management strategies and application methods and taking into account other pest species present). Several insecticides obtain high Overall Scores indicating that they would be good all round choice products for *Helicoverpa* control.

A queriable data base of all this literature, *'Helibase'*, has been created in Microsoft Access which can be used for more sophisticated searches. For example, all papers concerning aphid mortality or all papers touching on the impact of chlorpyrifos on lacewings can be instantly accessed. This data base and instructions for its use have been distributed within China, Pakistan and India, both at the national final workshops and to individuals. *Helibase* and its manual is attached to this report and can be obtained on CD from the ICAC Technical section and from the author at NRI.

The findings of this work have been written up as a chapter in the CFC project *Helicoverpa Management Handbook* and submitted for publication in the journal literature.

Output 5: Definition of minimum spray application equipment and practices for effective application of pesticides to cotton.

Despite insecticides being very widely used for bollworm control, and despite the large sums of money being devoted to identifying new active molecules for these pests, there has been very very little work on insecticide application equipment and practices in recent years. Application is generally very poor, with less than 20% of the spray liquid impinging on the target sites. This work, led from Punjab Agricultural University under the CFC project, with support from Nanjing Agricultural University in China and the Central Cotton Research Institute in Pakistan, set out to specify minimum application equipment and practice specifications which could deliver acceptable kill rates (defined as >85%) with a non-resisted chemical (spinosad). It is worth noting that in all the trials in this project, *H.armigera* larval kill rates in the field were never over 90%. This is due to concealment from the insecticide of a proportion of the larvae on the plant.
Ascertaining which equipment and practices were suitable within the parent CFC project comprised:

(a) Selecting the best of the available equipments commonly used by the cotton grower.
(b) Identification of target sites both in determinate and indeterminate cotton varieties/hybrids based on preferred ovipositional and feeding niches.
(c) Obtaining droplet characteristics and mortality data for the range of equipment.
(d) Fine tuning of the existing equipment and calibration by standardizing.
(e) Selection of nozzle
(i) Nozzle placement
(ii) Discharge rates
(iii) Minimizing application rates
(iv) Defining swath widths
(v) Height of nozzles/boom from plant tops.
(vi) Application speed.

(f) Integration of minimum effective application technology in IPM modules to maximize the appropriateness of deployment of insecticides.
(g) Safety precautions in pesticide application
(h) Maintenance and repair of equipment

Within the current project, a preliminary report on the current knowledge of effective spraying for bollworm control was produced by NRI (J. Cooper supported by D.Russell – report attached) in year one, and support provided to PAU in the design, implementation and analysis of experiments to determine the values of key parameters necessary for setting up equipment to provide effective control. This included training for PAU staff at NRI and the International Pesticide Application Research Centre of Imperial College at Silwood Park. The full findings are presented in the CFC project final technical report. The key findings were:

Identification of target sites: Target sites for pesticide droplet impingement are mostly the ovipositional and feeding niches for different insects. Gravid females normally oviposit on the sites which are also suitable for feeding of young individuals or neonates. The target sites for *Helicoverpa*, *Earias* and *Pectinophora* bollworms are the upper, upper and middle and all plant canopies in the case of sympodial, semi-determinate monopodial and indeterminate monopodial type cottons respectively. In the case of *Spodoptera,* eggs are laid in clusters mostly on the lower side of the upper canopy and middle canopy leaves. First and second instar larvae feed gregariously, then larger solitary phase larvae disperse to other plants. These larvae prefer to feed on young leaves and young fruiting bodies and inflict economic damage. Sucking insects vary greatly in their behaviour but economic damage is mostly determined by their feeding on leaves supporting the fruiting bodies For all practical purposes, target sites for insecticidal deposit for *Helicoverpa*, *Earias*, *Pectinophora* and sucking pests are generally on the same part of the plant.

Droplet characteristics and mortality data: Table 9 shows the droplet patterns obtained with knapsack spraying. Spray table and experimental work with larvae placed on plants in the field, showed that the parameters described in Table 10 were necessary for adequate control. Work with water-sensitive papers showed that his occurs only in the upper and to a lesser extent the middle canopy of the dense, monopodial cottons commonly grown in India and Pakistan, and that impingement is generally poor on the under-surface of leaves. Fortunately, this upper area of the plant coincides with the most important feeding and oviposition sites of the key pests but improvements in plant architecture and/or spray impingement would clearly be welcome.

In both laboratory and field experiments conducted with Indian and Pakistani nozzles, adequate mortality (set at > 85% larval mortality of third instar larvae *H. armigera* with a non-resisted chemical (Spinosad)) was obtained with equipment delivering the following specifications (Sohi *et al.* 2004) Table 10.

Table 9: Spray patterns obtained in canopies on mature plants using hollow cone nozzles with a knapsack sprayer on cotton in the Indian Punjab

Parameter	Top/Bottom of leaf	Upper plant canopy	Middle plant canopy	Lower plant canopy	Ground
Droplet density (no./cm^2)	Top	52-134	66-88	40-47	20-30
	Bottom	4-42	0-4	0	
NMD (μm)	Top	46-60	63-87	50-78	35-52
	Bottom	14-46	0-11	0	
VMD (μm)	Top	107-232	101-216	80-172	50-116
	Bottom	28-89	0-23	0	

Table 10: Minimum effective insecticide application practices required to obtain an adequate kill rate of *H. armigera* in conditions of light air movement

Parameter	Practices for obtaining minimum effective control
Equipment	Tractor mounted, manually operated knapsack and motorised knapsack sprayers
Nozzle type	Hollow cone nozzle made of ceramics
Nozzle placement	Downward
Nozzle discharge	400-600 ml/minute
Nozzle pressure	40-70 psi
Nozzle droplet density	30-55/cm^2
Average % leaf area covered	28-31%
Average NMD (μm)	40-100 μm
Average VMD(μm)	140-190 μm
Application rate	125 L/ha in vegetative phase and 250 L/hr in fruiting phase.
Swath width	10-12 m for tractor operated sprayer, 4 m for motorized knapsack sprayer and 1.4 m for manually operated knapsack sprayer
Height of nozzle/boom from plant	50 cm above crop canopy
Tractor speed	4 km/hr at vegetative stage and 2.5km/hr at fruiting stage

Nozzle specifications in particular were found not be closely adhered too by manufacturers, with what were theoretically the same nozzle from different manufacturers and even from the same manufacturer showing a wide range of exit apertures and angles and consequently delivering an excessively wide range of applications rates and droplet sizes.

The results of the work were presented to equipment manufacturers and the scientific community at a dedicated seminar at Punjab Agricultural University in late 2004 and have formed the basis of farmer and extension staff literature on bollworm control produced by PAU in local languages (Singh *et al.* 2004).

Contribution of outputs to development impact

This project provided key inputs from the UK science base to the larger CFC project of the same name and through it, provided contributions to development impact. It is therefore not possible to separate the development impacts of the CPP project, from that of the larger project of which it was part. Even the CFC projects impacts have been largely achieved through uptake of results and ideas by the national agricultural extension and cotton support systems within India, China and Pakistan. In India in particular, these programmes were already strongly influenced by the results of the preceding DFID CPP programmes on insecticide resistance issues (1993-2000), the current results strengthening and refining earlier findings.

The development impact fall into two areas – the impact of the uptake of project results in the design and implementation of cotton extension programmes, and the development of insecticide quality test kits.

Uptake of project results in national cotton extension systems:

Each of the institutions acting as national principal investigators in the CFC project and the collaborators working at Rothamsted within the current project, are the key providers of advice to the national extension systems in their respective countries i.e. the Central Institute for Cotton Research in India, the Central Cotton Research Institute in Pakistan and Nanjing Agricultural University Dept of Entomology in China (National Key lab for Insecticide Resistance advising the National Agro Technical Extension and Service Centre). This structure enabled rapid national promulgation of the project results across the three counties.

India:

The results from the series of IRM projects in India have been very successfully incorporated into field insecticide-use management programmes from 1993 onwards. The current GOI Min of Agric, National Insecticide Resistance programme (approved 2002 to 2007 at a current cost of $US400,000 per year) was a successor to the DFID-led programmes from 1996-1999 and the larger ICAR projects to 2002. The specifically insecticide use elements of that programme are discussed in the Outputs section above.. CICR continues to technically lead and back-stop the programme. It is a full IPM package based on insecticidal control of over-threshold pests. A 'window'strategy is in place for bollworm chemicals, taking account of efficacy, secondary impacts and resistance issues.

By the 2003-4 season the $US 0.4mill programme was active in the 11 major cotton states. A structure of national, state and district co-ordinators manages the work of village IRM facilitators who live and work directly in the cotton producing villages and train and support growers. In 2003-4, data was collected from 5,372 'core' farmers out of the >18,000 direct participators in 331 villages (there were, in all >50,000 formal and informal participants in the programme). All states showed spray use reductions – average across the states of 50% (dropped from 10.3 to 5.1 applications), yield increases – averaging 24% and consequently net profit increases averaging $US 107/ha or 74% when compared with non-participators in nearby villages (Kranthi *et al*. 2004, Russell *et* *al*. 2004). In the 2003-4 season alone, participating farmers had increased net profits of >$10 million considerably exceeding the total cost of the current project in India and all GOI costs of the national programme since its inception

In 2004-5 the number of states participating increased to include all 11 cotton states. The 28 heaviest insecticide-using districts now participate and over 46,000 growers are active participants. 26 of these districts now have insecticide resistance monitoring laboratories. By the 2005 cotton season (with detailed information from >5,000 growers) the average insecticide use by number of applications was down by 46%, average yield was up 11% and average profit increased by $175 per ha (in many cases from an absolute loss in previous years). In districts using the programme insecticide resistance has fallen and the efficacy of these resisted chemicals has been restored (eg in the Wardha district of Maharashtra the pyrethroid resistance factor fell from 96 to 3 from 1997 to 2003 and endosulfan resistance from 30 to 1 i.e. full susceptibility was restored.)

ICAR recognizes this as the most successful agricultural programme currently running in India and its future funding seems assured. The Minister of Agriculture (Oct 2005) acknowledged this with the launch of special publication on the programme and the lessons to be learnt from it. The President of India, Dr Kalam, made a visit in Jan 2006 to the Wardha village area specifically to examine the programme himself. The influence of the programme and the many associated meetings and discussions have contributed strongly to the swing towards more rational pesticide use in cotton in India and is an important contributor to the rapid decline in national toxic insecticide use. New recommendations are beginning to be implemented integrating Bt cotton into this programme.

Pakistan:

Extension advice in the northern cotton belt of Pakistan is provided by the Chief Minister's Task Force on Agriculture and promulgated through the national extensions systems, radio, TV flyers etc. The Task Force meets and makes recommendations weekly in the cotton season. Project staff at CCRI have always been part of the task force and have fed project advice into the extension recommendations. A number of chemicals have been withdrawn from those recommended for use in cotton because of resistance and susceptibility has been significantly restored to some of these. The recommended rotation pattern for others now follows project recommendations. Pakistani cotton farmers are generally richer (and more literate) than Indian farmers, and the range of chemical groups available in recent years has been larger. Following recommendations has resulted in a significant reduction in the significance of insecticide resistance for Pakistan cotton production. Farmers in the Pakistan Punjab are much more responsive to publicly promulgated extension advice than in India or China. Impacts of the project have therefore been significant, though not directly measurable as in India.

China:

The collaborating Nanjing Agric Univ laboratory provides the insecticide resistance management advice to the National Extension System through NATESC. Both NAU and NATESC were active partners in the CFC project (and in the parallel EC funded project into Bt cotton management, which the same team – including Rothamsted and NRI took part in). Project findings on rotations and use of mixtures have been passed to the NATESC Spray Technology Unit and incorporated into NATESC cotton extension advice to the provinces and implemented there. Rotations and rates follow the project recommendations. However, the use of insecticide mixtures is extremely widespread in China and the influence of the project will be felt more slowly there China's extension system is rapidly moving to a model of more private provision. Nonetheless the national extension advice is widely adhered to and promoted by the Provinces and districts. The use of mixtures has a long history in China and the advent of Bt cotton (now over 90% of plantings in eastern China) have made the measurement of direct project impact very difficult.

Insecticide quality detection kits:

The principles of production of these kits have been demonstrated, prototypes produced and tested, patents sought and commercialization agreements drawn up. However, until their widespread distribution and use, direct development impacts on pesticide quality (thorough pressures on manufacturers) and pest control – through farmer empowerment, has not yet been seen.

a) Market studies needed

The scale of the insecticide quality problem in Asia (particularly north India and to a lesser extent China) is not contested. Independent studies by Punjab Agricultural University and the government testing laboratories found over a quarter of all insecticide samples taken in north India to be inadequate in some way (wrong active ingredient, insufficient active ingredient, poor formulation etc). Prior to commencing the work a study of the nature of the insecticide quality problem in India was undertaken by a consultant Sujith Sandur in 1999 for the DFID CPP programme. He found the government quality control system entirely unable in principle (and utterly incapable in practice) of handling more than a tiny fraction of the testing which needs to be done to provide a genuine deterrent to the production of sub-standard materials. Government testing was a very slow (months or years) and expensive process and despite the significant number of samples failing tests, no manufacturers had been significantly punished for failure. Survey work with farmers, pesticide companies, traders, extension offices, the GOI Plant Protection Advisors Office, the Central Insecticide Board and the State Departments of Agriculture, all produced strong support for the idea of quick, cheap, farmer friendly tests. Members of the pesticide industry's global "Insecticide Resistance Action Committee' – IRAC which co-funded the CFC project, are interested in using these kits. There are ethical questions in negative advertising (saying that the opposition's products are sub-standard) but for quality control they welcome these materials and are interested in their widespread use. Following the Bt test strip example, the current intention is to produce the kits in modest numbers (tens of thousands) and market them through other organizations (farmer bodies, retailer networks etc) until the scale of the demand is clear.

b) How made available

The Indian Council for Agricultural Research made an agreement with the Common Fund for Commodities, that, provided for patenting (if appropriate) and commercialisation (with the aims of maximizing dissemination rather than profit) by ICAR, with initial activity in India and then in China and Pakistan. Discussions on exactly how this should be done and what approvals needed to be in place continued for over 18 months, until ICAR decided simply to submit the patent application without prior agreement in Jan 2006. The reason for seeking patent protection was to ensure that no commercialised organization could patent these ideas and exploit them for maximum profit with the consequent limitation of availability to farmers. Within the year following this patent application in India, patent protection will be sought in Pakistan and China and possibly more widely. Discussions have already been held with potential manufacturers and distributors in those countries. The Central Institute for Cotton Research (CICR), Nagpur, currently produces the antibodies and basic ingredients of the kits. A small company "Innovative BioSciences' was set up to manufacture and disseminate the physically very similar test kits for Bt quality. This company is a possible route to direct marketing to farmers but its poor access to distributor networks makes it more likely that agreement will need to be reached with one of the lager Ag. Input firms.

c) What further development testing etc

The prototype kits have been tested within the 26 laboratory network of the Indian cotton IRM programme. The immunoassay dip-stick kit for endosulfan (a cyclodiene) seems to be performing well with all formulations tested. The pyrethroid quality kit has had some difficulties with certain formulations. It seems likely that materials other than the insecticide active ingredient in those formulations are interfering with the ability of the reactive molecule on the kit surface to bond correctly with the insecticide in the test sample. Although this could be overcome by extracting the insecticide active ingredient before testing, this puts the 'cheap and easy' nature of the kits at risk. There have also been some cross-reactivity issues between organophosphates, in some cases even breakdown products of insecticides have given false positives for quality. These issues need to be conclusively resolved before commercialization.

d) How and by whom will the further stages be paid for

All the technical issues are under continuing study at CICR under Indian Government core funding to the institution. The obtaining and defending of the Indian patent is a contractual obligation of ICAR under the CFC project agreement. CFC has offered to support the patent work in China and Pakistan and this seems likely to go ahead. As for the GM cotton quality test kits, the production and distribution of the insecticide test strips will be self funding from 'profits' on sales. It is not expected that each test of a sample of insecticide would cost more than Rs20 (less than US%0.5). The small excess income over expenditure would (as with the Bt test strips) be returned to CICR for the on-going development of further test kits on the same principles. This model is approved by ICAR and agreed for these kits.

Biometrics: ***The work undertaken in this project was laboratory rather than field based. Conclusions are not statistically based, other than in the use of the normal measures of significance. As agreed at the start of the project, there was no specific project biometrician.***

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