



THE ICAC RECORDER

International
Cotton
Advisory
Committee

Technical
Information Section

VOL. XXIII No. 4
DECEMBER 2005

Update on Cotton
Production Research

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Introduction

Intellectual property rights (IPRs) are important to protect new developments from misuse and obtain sustained benefits for a long period of time. Many countries still do not have legal protection against misuse of intellectual rights, and multinational companies are hesitant to extend technologies to these countries. Currently, IPRs are territorial in nature and they are acquired and enforced on a country-by-country basis. The three main international organizations working on IPRs are the World Intellectual Property Rights Organization, the International Union for the Protection of New Varieties of Plants and the Food and Agriculture Organization of the United Nations (FAO). FAO published the International Treaty on Plant Genetic Resources for Food and Agriculture, which is one of the latest in a series of international guidelines to formulate local IPRs. Member governments of the World Trade Organization are required to have some basic structure under the on-going negotiations. Research has shown that protection of rights to plant varieties increased yields and enhanced the rate of development of new varieties. The need for IPRs has increased with commercialization of biotech varieties of cotton, and there is a strong need for harmonization of intellectual property rights. The intellectual property rights issue is discussed in the first article.

Over the last 13 years, the ICAC has sponsored many projects with funding from the Common Fund for Commodities (CFC). Projects covered all aspects of cotton production and marketing and were located in the ICAC member and non-member countries. The ICAC is a recognized International Commodity Body, and all cotton projects must be approved by the ICAC before they are considered and approved for funding by the Common Fund for Commodities. CFC/ICAC projects have provided additional funding for cotton research, and they have greatly enhanced collaboration among institutions and countries. One project 'Sustainable Control of the Cotton Bollworm (*Helicoverpa armigera*) in Small-scale Cotton Production Systems' was located in China (Mainland), India, Pakistan and the UK. The project CFC/ICAC-14 began in October 2000 and concluded in December 2005. Some residual activities, including publication of reports, are continuing but all the research work has been completed and the project is

technically over. The project involved three institutions from India, two from the UK and one each from China (Mainland) and Pakistan with supervision from the ICAC. The project set an excellent example of international cooperation on cotton research under the leadership of Dr. Derek Russell of the Natural Resources Institute of the UK. More than five years of work of seven institutions directly involved in the project is summarized in the 2nd article.

The project CFC/ICAC-14 mapped the patterns of bollworm resistance to the major chemical groups and concluded that precise pattern in any one place is contingent on the historical use and sequence of chemicals applied. On the mechanisms of resistance, the project concluded that amongst the metabolic mechanisms, enhanced oxidases are of considerable importance in pyrethroid resistance in India and China and to a lesser extent in Pakistan. The project concluded that if resistance to pyrethroids is metabolically based (most cases), the mixture of phosphorothionate organophosphates with a pyrethroid can restore the efficacy, but the efficacy of a new mixture may be short lived. The project concluded that laboratory-measured resistance is reasonably constant, and there is a useable relationship between the mortality of 3rd instar larvae in laboratory assays and the ability of that chemical to control *H. armigera* in the field. The project developed an insecticide quality kit and a resistance detection kit that are in the process of being commercialized through a patent by the Indian Council of Agricultural Research. Many details about project achievements are given in the 2nd article. The project published a handbook covering all aspects of sustainable control of cotton bollworm. Other results of the project were demonstrated to researchers in the project countries and outside the region, particularly in the West African countries where insecticide resistance has been an issue for some time.

Meeting of the Southern and Eastern African Cotton Forum (SEACF)

The Technical Information Section of the ICAC has a mandate to facilitate communications among researchers. The mandate is achieved through various means including regional net

working and world cotton research conferences. ICAC supports four networks in four regions. The Southern and Eastern African Cotton Forum (SEACF) has announced that it will meet from March 7-9, 2006 in Pretoria, South Africa. The Institute for Industrial Crops of the South African Agricultural Research Council will host the meeting. The three-day program comprises country reports from the region, a one-day sticky cotton workshop and a field trip. Membership in the network is voluntary and all countries and researchers in the region are invited to attend the meeting. For additional information on the meeting and registration please contact

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World Cotton Research Conference-4

The World Cotton Research Conference-4 will be held in Lubbock, Texas, USA from September 10-14, 2007. The theme of the conference is 'Nature's High-Tech Fiber.' As of December 12, 2005, 349 researchers from over 30 countries had pre-registered for the conference. Free online pre-registration is available at <http://www.icac.org/>. The full registration package along with more details on the program and hotel booking will be available in mid-2006. Full registration will be announced to those who have pre-registered for the conference. Most information on the conference is available on the ICAC web page. However additional information can be obtained from the following.

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Intellectual Property Rights: Need for Overhaul

Intellectual property rights are legal rights granted by governments to protect certain products of human intellectual effort and ingenuity. National and international intellectual property rights systems have evolved slowly into their present form. It is an ongoing process, particularly in the international perspective. The two basic ideologies that shape the structure of intellectual rights are exclusive rights reserved for authors/inventors for their discoveries, and moral and economic benefits to inventors. Intellectual property rights is a wide-ranging issue that covers all walks of life, but in this article the main emphasis will be on questions related to cotton varieties.

There has always been a need for intellectual property rights, but the need has significantly increased in agriculture over the last 10 years due to biotechnology applications in agriculture. Compared to conventional breeding, biotechnology research and its applications are characterized by heavy investments in research and development, technological change, an increasing focus on the output market and ownership of intellectual property rights. Plant biotechnology research is not a single activity but a sequence of activities that begins with generating and testing ideas, developing commercially viable products and successfully marketing these products. Developing and testing ideas is common to biotech and non-biotech research, but biotech research is relatively expensive and carries a higher risk of unsuccessful results. Research for the sake of research may be fine, but the chances of coming up with a commercially viable product are very critical in

biotechnology. Developmental stages are always expensive and time consuming and that is true in biotechnology today. With the slowdown in technological developments in the last few years, it would seem that biotechnology research will remain expensive for quite some time, in spite of the fact that transformation processes have become easier and transgenic lines can be developed now with a higher degree of success than only 7-8 years ago. Furthermore, a great many breakthroughs have been achieved, and will serve as building blocks for the commercial products of tomorrow. The third aspect, which is successful marketing, has deteriorated in the last decade and become almost irrelevant in many local and international markets, thus emphasizing the need for adherence to intellectual property rights (IPRs).

The International Scene

International IPRs are practically non-existent. Currently, IPRs are territorial in nature, and they are acquired and enforced on a country-by-country basis. Biotech varieties are the best example in the cotton industry. The inventors of biotech varieties, even those employing the same Cry1Ac gene or any other gene, have to apply to governments individually for protection and for permission to commercialize the same variety or one with a gene that has been in exploitation in some other country for more than 10 years. Recent international efforts, especially through international treaties and the World Trade Organization (WTO), have sought to achieve at least a modest harmonization in procedures, but these efforts are still far from success.

The World Intellectual Property Rights Organization (WIPO) is a specialized agency of the United Nations that is responsible for the protection of IPRs throughout the world. It organizes conferences of government officials and provides them with technical assistance as well as liaison with other national IPR organizations. WIPO has been instrumental in creating the Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore. The International Plant Genetic Resources Institute of the Consultative Group on International Agriculture Research (CGIAR) is the world's largest institution devoted to conservation, management and preservation of plant genetic resources. The Food and Agriculture Organization of the United Nations organized a conference in 2001 that produced the International Treaty on Plant Genetic Resources for Food and Agriculture. The Trade-Related Aspects of Intellectual Property Rights (TRIPS Agreement) is different from other agreements. TRIPS was adopted in 1994 and is administered by the WTO. The reason TRIPS seems to supersede all earlier agreements is that it seeks to establish minimum standards in member countries. However, TRIPS is not a stand-alone agreement; it is linked to prior and more general trade-related agreements. TRIPS was adopted during the Uruguay Round of trade negotiations. Through TRIPS, the industrialized nations received a commitment from developing nations to formulate basic standards for legal protection of IPRs. Conversely, the developing nations received assurances that industrialized nations would open their markets to goods from developing countries. All member countries of the WTO are bound to comply with TRIPS by virtue of their membership in the WTO. In the area of plant varieties, TRIPS has gathered and organized a considerable amount of useful information concerning national government practices. If a government believes that another government has failed to meet its obligations under the TRIPS agreement, the claimant may attempt to settle the issue through consultations under the Dispute Settlement Understanding. If the issue is not resolved, the claimant may request that the WTO convene a three-member expert panel. The panel must then arrive at a decision within six months and the WTO Dispute Settlement Body will adopt the decision. The defendant has the right to take the decision of the WTO Dispute Settlement Body to the Appellate Body, which must hand down its decision within three months. The Dispute Settlement Body will recommend that the defendant bring its domestic laws into compliance with TRIPS. If the country fails to do so, the claimant may then call for arbitration proceedings to fix the amount of compensation (Helfer, 2004). However, the dispute settlement provisions are more focused on trade and subsidy issues and the case between Brazil and the USA is the best example in the cotton industry.

Patenting of varieties is still not permitted in most countries. In the European Union, the accepted language prohibits patents for plant or animal varieties or essentially biological processes for the production of plants and animals. Consequently, the

current patent system is hard to extend to encompass biotech characteristics. Thus, lately, patents have been allowed when an invention applies to more than the established form of a variety, such as biotech traits. In many cases, any variety developed through insertion of a gene construct would be dependent, and commercialization would require permission from the variety rights holder. Dependency is again a relative term. It may vary from very low to a rather high percentage of the original parent characteristics in a new variety. No limits can be set on relatedness as far as a patent is concerned. Varieties may be different by a single gene or by many genes. Sometimes, even one gene is enough to differentiate two varieties and other times many new genes may not be enough. A single gene showing no morphological identification (like a Bt gene) could have a large impact. Thus, it is apparent that it is difficult to use greater or lesser relatedness as a basis for plant variety protection.

Intellectual property rights may apply to all living organisms and technologies. As far as planting seeds are concerned, developed countries have generally tried to enforce their IPRs in developing countries in order to protect their products. Conversely, the developing countries have insisted on maintaining a loose property rights system in order to provide their farmers with easy and low cost access to technologies and seeds. Consequently, in a majority of developing countries, where most of the world's cotton is produced, IPRs have either not been developed or, where they have been developed, have not been implemented in the true spirit of the concept. Still, there are countries where patent protection is not available and seed companies have to rely on the only form of protection that exists, Plant Variety Protection Certification. The name of this kind of certification may vary from one country to another and may include simple registration, but such rights do exist in most countries. The International Convention for the Protection of New Varieties of Plants of 1978 has been quite instrumental in propagating the idea of plant variety certification.

Plant Varieties and Intellectual Rights

The four mandatory criteria that must be met for a new plant variety to be patented are: novelty, distinctiveness, uniformity and stability. Any given variety or plant-related innovation meeting those criteria could acquire plant variety protection rights under any of the international agreements discussed above. Any of these "established" approaches to protection achieve, in different ways, the principal policy goal of the IPR system, which is the creation of adequate incentives to encourage plant breeders to develop and market new varieties. Whether any of these established approaches is appropriate for a particular country will depend on the needs of that country's agricultural industry and its desire to encourage foreign investment.

Depending upon the nature of the invention or development,

IPRs may allow free limited use or exclusively licensed use. The terms and conditions of licensing vary depending on the product and its end users, particularly if the proprietary product has been developed using public funding. However, no IPRs can be implemented successfully unless there is an effective mechanism by which to enforce those rights.

Commercial cotton hybrids are grown in some countries, and heterozygous and heterogeneous genotypes may acquire exclusive rights. Governments need to develop a mechanism for distinction between two or more hybrids. There may also be a need for different levels of protection tailored to the requirements of domestic industries. Under such a differentiated regime, there could be different levels of protection for commercial and non-commercial breeders, with a higher level for the former to compensate them for their investment of capital and their distribution costs.

There may also be a need for privileging the use of varieties derived from germplasm of local origin. This privilege, which could take the form of either an exemption or a compulsory license, would permit the residents of a state, without the permission of the right holder, to use plant varieties derived from germplasm collected in that state. Such an exemption could work against researchers who collect exotic germplasm in their own country and then attempt to have it protected under the country's local seed laws. If the exemption does not operate in a country, the breeding program could suffer due to the limited supply of germplasm. However, if the exemption is applied only to foreign breeders, such a limitation would violate the national treatment rule and thus be incompatible with the core obligations of TRIPS.

Some additional compromises need to be considered. The critical question is whether the act of extracting a gene from a seed is, in itself, a sufficient alteration of the seed's genetic material such that the extracted genetic product is no longer "in the form" received from the original source. The same question might also be asked about the addition of a single gene or a limited number of genes into an existing genotype. According to one view, the denial of IPRs would extend to raw germplasm, not to individual genes or DNA fragments that are isolated, purified and thus altered from their natural state.

International Convention for the Protection of New Varieties of Plants

The International Convention for the Protection of New Varieties of Plants held in December 1961 recognized the importance of protecting new varieties of plants, not only for the development of agriculture but also for safeguarding the interests of breeders. Participating governments agreed to settle property rights issues in accordance with uniform and clearly defined principles. Governments also agreed to establish the International Union for the Protection of New Varieties of Plants (UPOV), which is based in Geneva, Switzerland. The convention applied to only botanical genera and species and

the parties undertook to cover all such species within eight years of its entry into force. Provisions of the convention required that a new variety must be clearly distinguishable by one or more important characteristics from any other variety whose existence is a matter of common knowledge at the time when protection is applied for. The convention encompasses morphological or physiological characteristics but does not talk about genetic variations, an issue that has become of greater concern in biotech varieties. DNA fingerprinting, capable of identifying point-by-point variation among varieties, is not mentioned. DNA fingerprinting is the most authenticated method for establishing distinctions among varieties, but it is still far from being recognized as a tool for verification of differences among varieties. Breeders themselves may be reluctant to accept DNA fingerprinting, as a basis for variation because it could also uncover the origin of a variety which breeders otherwise may not want to disclose. Breeders continue pushing for morphological differences as basis for distinction among varieties, which are much harder to detect, particularly in cotton, since it is so sensitive to growing conditions.

The provisions of the convention were revised in 1972, 1978 and 1991. The provisions clearly defined that prior authorization shall be required from the breeder for production, offering for sale and marketing of a reproductive or vegetative propagating material, as such, of the new variety.

The International Treaty on Plant Genetic Resources for Food and Agriculture was adopted in November 2001 and came into force as of June 29, 2004. One hundred and nine countries are signatories to the treaty. All countries that ratified are expected to develop the legislation and regulations needed to implement the Treaty. The objective of the treaty is to establish an efficient, effective and transparent multilateral system to facilitate access to plant genetic resources for food and agriculture, and to share the benefits in a fair and equitable way. The Multilateral System of the International Treaty on Plant Genetic Resources for Food and Agriculture applies to over 64 crops including legumes and forage plants but unfortunately not to cotton. The criteria for inclusion of crops in the Multilateral System were:

- 1) contribution of the crop to global food security and,
- 2) interdependence of countries on each crop's genetic resources.

What is a Variety?

Researchers usually define variety as a group of similar behaving and true breeding plants. In commercial terms, a strain, line or genotype becomes a variety as soon as it is officially approved for commercial production in any area. It is understood that morphologically similar and true behaving plants could come only from within a species. The International Union for the Protection of New Varieties of Plants has defined a variety as "a plant grouping within a single botanical taxon of the lowest known rank, which grouping, irrespective of whether

the conditions for the grant of a breeder's right are fully met, can be defined by the expression of the characteristics resulting from a given genotype or combination of genotypes, distinguished from any other plant grouping by the expression of at least one of the said characteristics and considered as a unit with regard to its suitability for being propagated unchanged." The International Union for the Protection of New Varieties of Plants (UPOV) currently has 60 member countries. Some important cotton producing countries like Greece, India, Pakistan, Syria, Turkey and Turkmenistan are not yet members of UPOV.

Need for Access to Genetic Resources

Breeders make use of variability in a population by crossing various varieties. But, they also create different combinations of characters, which could be regarded as an act of creation of variability with respect to more than one character or a group of characters. Plant breeding is a fundamental aspect of the best use and development of genetic resources. Breeders create new combinations, combinations not existing in nature, to use them in their own programs, but also for use by others. Breeders cannot invent new characters but they can create rare combinations and some times even impossible combinations as when interspecies barriers are crossed. Creation of new combinations (and particularly rare combinations) should not consume extra efforts and must avoid duplication of time and resources. UPOV considers access to genetic resources to be a key requirement for sustainable and substantial progress in plant breeding. Breeders must have free access to plant genetic resources. Without such access, progress in breeding would inevitably slow. The concept of the breeder's exemption, in the UPOV Convention, is for the purpose of breeding new varieties and not for any commercial transactions. At the time of registration of varieties, breeders are usually required to indicate the origin and parentage of breeding lines. Breeders must faithfully provide information concerning the breeding history and genetic origin of the variety with acknowledgement of the origin of either of the parents. The new variety should not be a true copy of either parent, but should meet the criteria of novelty, distinctness, uniformity and stability. Moreover, when countries pass their own seed laws that affect free access to germplasm by other breeders, access to genetic resources rests with the national governments and is subject to national legislation. The International Treaty on Plant Genetic Resources for Food and Agriculture also recognizes the concept of the breeder's exemption but only for further research and breeding.

Beyond research and development purposes, UPOV provisions allow farmers to keep part of their harvest of a protected variety for planting the following year's crop. This is what had been practiced in most countries and did not raise concerns until the development of biotech varieties. Even in countries where biotech varieties are grown on a large scale, planting of 'self-seed' has been a common practice. The most

supportive argument in such a practice has been the price of the seed. No doubt the countries who are members of UPOV can adopt solutions specifically suited to their agricultural circumstances; farmers are obliged to sign agreements with biotech seed supply companies to the effect that they will not retain seed for planting the next crop. Free access to breeding lines for research and development purposes must ensure, on the other hand, that there is a continued incentive for the development of new varieties which could be lost if varieties or seeds are available free to anyone for any purpose.

In the absence of an international institute for cotton research, there is no formal way to exchange cotton germplasm. Good germplasm is the basis for any successful breeding program and, unfortunately, there is no information available on what kind of germplasm is available. The International Plant Genetic Resources Institute does not deal with cotton because cotton is not included in the CGIAR's mandate. The lack of access to germplasm is hampering breeders' efforts and narrowing the genetic base of commercially grown varieties in most countries. There is a need to establish an international cotton germplasm bank. Such a facility would collect germplasm from all countries, hold and preserve it and supply it on request to researchers. The protocol to be followed to supply and/or receive specific accessions could be decided separately once such a facility becomes available. The national plant variety protection laws of many countries can be viewed at <http://www.upov.int/en/publications/npvlaws/index.htm>.

Plant Variety Protection

Most countries have some form of law relating to plant variety protection (PVP). Countries that do not have any form of PVP are moving forward to devise at least some form of PVP for use as an alternative to intellectual property rights. The Trade Related Aspects of the Intellectual Property Rights agreement of the World Trade Organization has made protection of intellectual property rights an integral part of the multilateral trading system. Member governments of the WTO are required to meet their intellectual rights obligations for which some countries are using PVP as a form of intellectual property rights protection. PVP has its own advantages and according to Naseem *et al.*, (2005) plant variety intellectual property protection led to improved productivity and resulted in the development of a greater number of cotton varieties in the USA. They analyzed cotton data for 50 years (1950-2000) across 14 cotton producing states and proved that PVP increased the number of varieties introduced into the market each year, thereby providing farmers with more varietal choices. Kesan and Gallo (2005) compared the intellectual property rights relating to corn and soybeans in Argentina and the USA. They reviewed the evolution of the PVP certification process in Argentina beginning in 1935 and included the latest modifications made in 1994. Kesan and Gallo (2005) showed that, since the inception of the Seed Law in 1994, there was a sharp increase in the number of new corn varieties while the registration of soybean varieties remained low. The

nature of breeding, out-crossing and self-pollination in corn and soybeans, respectively, played a big role in boosting the number of new varieties. It is evident that frequent releases of new varieties would reduce the commercial life of an existing variety in the field.

After the introduction of biotech varieties, the use of PVP certificates in lieu of intellectual property rights has given way to utility patents. Only a few utility patents have been issued, but they seem to be important as over 80% of the cotton area was planted to biotech varieties in the USA in 2005. At this stage in biotechnology, utility patents are not a replacement for intellectual property rights or for a variety protection system. Intellectual rights will remain a key issue in the introduction and protection of new varieties.

Limitations to Plant Variety Protection

Plant variety protection seems to have a positive impact on yields, which in turn accelerates the process of development of new varieties. However, the process of obtaining PVP for a new variety is cumbersome in most countries, as is the case in the USA under the Plant Variety Protection Act. It has been found that in most countries post-issuance licensing and enforcement activities are almost non-existent. Seed travels freely across borders and is used in breeding programs. The lack of facilities for formal germplasm exchange only adds to the gravity of the problem. According to Janis and Kesan (2002), PVP has been used as a marketing tool rather than as property rights protection.

Private seed companies cannot survive unless they have the right to protect the varieties developed by their breeders. This is one of the reasons that private breeding of cotton varieties is still not popular in most cotton producing countries. Producing, processing, packing and marketing planting seed of commercial varieties, in competition with other companies, are not a cost-effective endeavor for many companies. It is estimated that almost 600 varieties have received PVP in the USA, which is the secret for a successful private cotton breeding program. PVP has been used in place of intellectual property rights but it has worked to protect varieties within companies.

Many researchers working with other crops observed the opposite of what Naseem and his colleagues found (2005). It is quite possible that variety protection rights may have a different impact on cotton if farmers have to buy planting seed every year, as opposed to other crops where they can use self-

seed for years. According to Naseem *et al.*, (2005), on average it costs US\$1,500 to obtain a PVP certificate for a variety in the USA. Though the price would vary greatly among countries, it would be, nevertheless, expensive and time consuming to get PVP certification.

PVP certification prohibits the illegal spread of varieties within countries and, more particularly, across borders. Consequently, resource-poor growers in developing countries are prevented from using technological developments achieved in other countries. In some developing countries, domestic resources are not available for sophisticated but useful productive research, and farmers suffer for the lack of easy and less expensive access to newer technologies.

Free-for-all use of PVP certified varieties discourages multinational private companies from taking heavy investment risks, and they would rather not go into markets where PVP certification rules are not followed. Companies may pull out from such markets, as was the case with the insect-resistant biotech soybean in Argentina (Burke, 2004). The illegal spread of biotech cotton seeds have also led to underutilization of biotech developments with respect to cotton in Argentina.

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Insecticides in Sustainable Control of the Cotton Bollworm (*Helicoverpa armigera*) in Small-scale Cotton Production Systems

(Report of the Project CFC/ICAC-14)

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Introduction

Crop protection costs were increasing during the late 1990s throughout Asia, the bulk of this increase was in insecticide costs which were 44% of growing costs and 16.5% of total production costs in 1997/98 in the three countries [China (Mainland), India and Pakistan] covered in this project (Table 1 and 2). In all these countries insecticide use was increasing strongly without concomitant yield increases. This was well documented to be due in large part to evolved resistance to the major chemicals available to control insect pests, and particularly the cotton bollworm (*Helicoverpa armigera*) which is the major pest of cotton throughout the Old World and Australia, occurring in 29 cotton producing countries (ICAC-CCRI 1999). Despite the enormous amount of research directed towards non-chemical control of this species, the impact across the region was still small, due to efficacy, reliability, availability and cost concerns in relation to the developed practices. Understanding how to minimise

the impact of insecticide resistance on production costs, in ways that could be applied by farmers, represented a very considerable and immediate contribution to the support of the commodity and the many millions of farming families, which depend on it across Asia and Africa. Given the scale of production, even a very small improvement in productivity or reduction in input costs could provide very large-scale benefits. China (Mainland), India and Pakistan, with nearly half the world's cotton area and production, were chosen as the project partners for the Common Fund for Commodities (CFC) support, with the UK providing project management. The UK's Department for International Development provided support for technical inputs.

Objectives and Expected Outputs

The overall objective of the project, which ran from Oct 2000 to Dec 2005 at a total cost of US\$4 million, was to develop and disseminate cropping systems and pest management practices for cost-effective and sustainable control of the cotton bollworm. The project aimed to build on existing knowledge and experiences for the further development of efficient methods, resulting in substantially reduced uses of hazardous pesticides and increased profitability for cotton producers. Although direct project activities are focussed on the resource-poor Asian producers/production systems, throughout the project, due emphasis was to be given to ensure that the ultimate outcome of the project would also be relevant and applicable to the small African producers who were also facing higher production costs and crop losses due to the cotton bollworm.

Table 1
Global Cost of Cotton Insecticides at the Farmer Level

Region	US\$ Millions
Asia	811
USA	340
South America	312
Africa	194
Australia	57
Europe	5
Global Total	1,719

Data from James, (2002)

Table 2
Cotton Production Situation in the Region before the Project Began (1997/98)

	China	India	Pakistan	Total
Cotton area 1997/98 (million ha)	4.5	8.8	3.0	16.3
Area as % of world area	13.3%	26.0%	8.9%	48.2%
Production in 1997/98 (million tons)	4.6	2.6	1.6	8.8
% of World production	23.1	13.1	8.9	45.1%
Average yield/ha (Kg lint)	1,016	294	582	524
Insecticides as a % of growing costs	46%	44%	42%	44%
Insecticides as % of total production costs	17%	15%	18%	16.3%
% of total insecticide use which is dedicated to cotton	38%	50%	80%	47.3%

Figures from ICAC (1998)

The project laboratories in the four countries worked as a team on the components of the project (Table 3), with a great deal of collaborative input from most partners into most components. This was especially true of the component on resistance mechanisms where scientists from the collaboration Asian laboratories worked together in the laboratories of Rothamsted Research where colonies of *H. armigera* from across Asia could be maintained without quarantine issues.

Table 3
Project Technical Partners

Institution	Project Component/Area of Technical Leadership
Central Institute for Cotton Research (CICR), Nagpur, India	<i>Components 7 and 8</i> - Development of cheap and rapid kits for the detection of resistance and the quality of insecticides
Entomology Department, Tamil Nadu Agricultural University (TNAU), Coimbatore, India	<i>Component 2</i> – Compilation of resistance data from across the region
Entomology Department, Punjab Agricultural University (PAU), Ludhiana, India	<i>Component 6</i> – Minimum acceptable insecticide application practices
Entomology Dept., Nanjing Agricultural University (NAU), Nanjing, China (Mainland)	<i>Component 4</i> – Insecticide mixtures
Central Cotton Research Institute, Multan, Pakistan	<i>Component 5</i> – Measured resistance level and field control (NRI took over the lead role)
Rothamsted Research (Previously called (Institute of Arable Crops Research), Rothamsted, UK	<i>Component 3</i> - Mechanisms of resistance and cross-resistance to insecticides
Natural Resources International and Natural Resources Institute of the University of Greenwich, UK	<i>Component 1</i> - Experience globally with the main bollworm chemicals <i>Component 5</i> – Relation between laboratory measured resistance and field control with insecticides <i>Component 10</i> – Handbook of control of <i>H. armigera</i> <i>Component 11</i> – Workshops in Asia and Africa <i>Technical management of the project</i>
All collaborators in China, India and Pakistan	<i>Component 9</i> - Practical implementation of the developed control strategies taken up and paid for by the national governments

1. 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 other insects 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 same mechanisms in insects.

Information from the 557 significant, peer reviewed, published reports on pesticide performance, were summarized 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 control (Table 4).

This table can be used to make decisions on which insecticide to use (in conjunction with the information below 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 database 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 database and instructions for its use can be obtained on CD from the ICAC's Technical Information Section and from the author.

2. Kits to Identify Pesticide Quality

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 also be blamed on resistance, a diagnostic to separate quality issues is extremely useful. As a rapid quality check by vendors, extension

Table 4
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
Fenprothrin (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

agents and farmers, immunoassay ‘dip-stick’ kits (similar to home pregnancy detection kits) were developed at CICR for the major chemical groups in current use against cotton bollworm. These 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. A diagnostic part of the insecticide of interest is conjugated with a protein to form a hapten that is injected into rabbits. The resulting antisera are 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. 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 color change and a visible band. In principle, a single dipstick can be used for quality detection of a number of insecticides. Prototype kits for cypermethrin and endosulfan have been tested in the 26 laboratories of the Indian Insecticide Resistance Management (IRM) network. 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.

3. Acceptable Insecticide Application Practices

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 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 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.

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* 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, and 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 behavior 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 5 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 6 were necessary for adequate control. Work with water-sensitive papers showed that this occurs only in the

Table 5
Spray Patterns Obtained in Canopies on Mature Plants Using Hollow Cone Nozzles with a Knapsack Sprayer on Cotton in Punjab, India

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 6
Minimum Effective Insecticide Application Practices Required to Obtain an Adequate Kill Rate of *H. armigera* in Light Air Movement

Parameter	Practices for obtaining minimum effective control
Equipment	Tractor mounted, manually operated knapsack and motorized 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/ha 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

India, Shen *et al.* (1995) and Tan (1999) for China (Mainland) 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). Results naturally vary with the area of the country and the history of insecticide use in each area (Kranthi *et al.*, 2001c). As a preliminary to this project, ICAC held a Regional

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 undersurface 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).

4. Resistance to Insecticides Across Asia

A global history of insecticide resistance is given in Kranthi *et al.* (2005). China (Mainland), India and Pakistan have been monitoring resistance of *H. armigera* to the common insecticides since the late 1980s (Armes *et al.*, 1996) for

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). The regional summary results of the recent work were presented at the World Cotton Research Conference-3 (Regupathy *et al.*, 2004). In brief summary, the regional resistance to the commonly used chemicals is shown in table 7.

In summary, the synthetic pyrethroids are highly resisted, and 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 has 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

Table 7
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

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.

5. How Laboratory Measured Resistance Relates to Field Control Failures

The strength of resistance to an insecticide is most easily measured by taking a sample of the pest from the field and measuring mortality of one particular standard life stage to a range of doses of the insecticide, thus generating a 'dose-response line' which tells us the quantity of insecticide required to kill any particular percentage of the population (LD_{50} , LD_{99} , etc). Apart from being very labor intensive, the problem is that this procedure removes (deliberately) almost all the variables that affect insecticide efficacy in the field – mixed life stages, concealment on or off the plant, temperature variations, rainfall, enhanced susceptibility to natural enemies etc. Much skepticism has been expressed as to the value of such laboratory measurements for assessing the field efficacy of insecticides and thus for deciding appropriate field rates for applications or for withdrawing highly resisted material. Measuring insecticide mortality directly in the field is, however, vastly more labor intensive and the results

more variable. The project set out to see whether laboratory-based resistance measurements to a range of rates to the common insecticides could be used to predict *H. armigera* mortality in the field. This involved a very large and labor-intensive operation over two seasons at five sites (one in Pakistan, three in India and one in China) having differing resistance profiles to these chemicals. Large replicated field trails were laid out with 5 rates of representative pyrethroid, organophosphate, carba-mate and cyclodiene cantering on the field rate. Eggs from the field were reared to 2nd instar. Laboratory dose/response measurements made on part of the population at 3rd instar. Others were placed in the field on 100 plants per plot, and counted 24 hours later when they had settled on the plant. They were then sprayed as 3rd instars with the dose of insecticide and survivors counted 24 hours later to calculate spray mortality. Survivors were collected and assayed for resistance.

Figure 1: Log Dose/ Mortality Response Lines for *H. armigera* with Quinalphos Near Nanjing, China

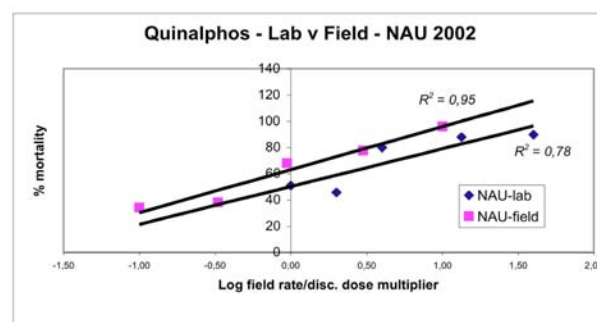


Figure 1 shows the results for just one chemical and one site in one season. The laboratory dose response line has its zero at the discriminating dose (the level at which practically all the susceptible insects and none of the resistant insects would be killed). The field response line has its zero set at the normal recommended field rate for the chemical (notice that the kill at this rate is only 70% in this resistant population). The overall analysis of all chemicals and sites showed:

- Field lines from areas with different resistance had different slopes (data not shown)
- The dose response in both the laboratory and the field appear to be log- linear
- The lab line can be ‘mapped’ onto the field line
- It is therefore possible to use lab results to predict field failure at recommended rates, to recommend more appropriate rates or the withdrawal of the chemical.

Note: A single selection event routinely enhances average resistance levels (as measured in survivors) by 10-40%.

6. Mechanisms of Resistance to Insecticides

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).

The Approaches used were:

- Correlations in response to different insecticides
- Use of synergists to block enzymes potentially involved in insecticide detoxification
- Direct comparisons of the amounts of detoxifying enzymes present
- Comparisons of metabolism rates
- Looking for DNA mutations in target-site proteins known to occur in other species
- Neurophysiology to detect direct evidence for nerve insensitivity
- Comparisons of penetration and excretion rates using radio-labelled deltamethrin
- Reciprocal selection experiments (does selection with compound A also increase resistance to compound B?)

Table 8 summarizes what is now known of the distribution

and importance of the three major types of mechanisms in *H. armigera* in Asia.

Conclusions on mechanisms of resistance

Metabolic mechanisms

- GSTs 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.
- 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 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.

With what is now known of cross resistances and their bases (for example OPs and carbamates are undermined by enhanced esterase production but it is not the same esterase isozyme which is important in the two cases) it is now possible to suggest which materials can be used with confidence next to each other in chemical use rotations in the three countries. Given that the inheritance patterns and effective dominance

of these mechanisms have been worked out (esp for India by CICR), we are now in a position to propose these sequences with some confidence.

Genetics of Resistance

Very careful crossing of multiple insect lines selected for particular resistances at CICR, showed the following (Table 9 and 10).

Table 8
Distribution and Relative Importance of Resistance Mechanisms in Asian *H. armigera*

Mechanisms Chemicals Affected	Metabolic			Target Site			Penetration Reduction
	Oxidases Pyreth	Esterases OP/Carb Endo/Pyreth	GSTs Pyreth	AChE OP/Carb	Nerve Insens Pyreth	rdl Endo	Pyrethroid (others?)
India	***	**	*	*	**	*	?
China	***	**	*	*	*	*	*
Pakistan	**	**	*	*	*	*	*

Table 9
Genetics of Resistance Inheritance (India)

Insecticide	SR	RS	Nature of the Resistance Allele	
Cypermethrin	0.84	0.84	Incomplete dominance	Autosomal
Endosulfan	0.58	0.64	Incomplete dominance	Autosomal
Quinalphos	0.59	0.57	Incomplete dominance	Autosomal
Methomyl	0.96	0.92	Dominant	Autosomal
Spinosad	0.13	0.11	Recessive	Autosomal

Table 10
Genetics of Resistance Mechanisms in Indian *H. armigera*

Insecticide	Mechanism	Nature	Frequency
Quinalphos	Esterase	Recessive	20%
	Insens-AChE	Insens-AChE	80%
Methomyl	Esterase	Dominant	90%
	Insens-AChE	Semi-dom	30%
Pyrethroid	Esterase	Inc-recessive	
	MFO	Inc-dominance	
	Nerve-Insensitivity	Recessive	

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

Cross Resistance Patterns

These were measured empirically but also emerge logically from the patterns and sharing of mechanisms (Table 11), 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. Bifenthrin and related pyrethroids, and monocrotophos and related phosphatic OPs have structures sufficiently different to give them a different resistance profile from that of most of their groups.

Table 11
Patterns of cross-resistance in Asian *H. armigera*

	Pyrethroid	OP	Carbamate	Cyclodiene
Pyrethroid	Yes <i>but some exceptions</i>	No	No?	No
OP		Yes <i>but not Monocrotophos</i>	Yes <i>variable</i> Yes Methomyl/ Carbaryl No Thiodicarb	No No
Cyclodiene				Only one - Endosulfan

Table 12 indicated which materials are safe to use sequentially in a bollworm spray program without exacerbating resistance problems. The number of different resistance groups is smaller than might have been hoped, limiting the scope for rotations.

The information above was used in the design of field insecticide use programs. The success of the Indian program in particular has been spectacular in enabling reduced insecticide use while enhancing effectiveness and consequently yields and profitability in the 10 Indian states where it is being used on a large scale.

7. Kits to Identify Resistant Insects

Appropriate pesticide use strategies, incorporating an understanding of local resistance patterns, pre-supposes the ability to rapidly and accurately identify particular resistances (and mechanisms of resistance) in insects from the field.

Where insects are resistant by multiple mechanisms, it makes sense to detect resistance to the major mechanism. Where an immunodiagnostic kit based on a biological marker such as a unique enzyme (esterase, cytochrome P450 or insensitive AChE) can be made available it provides the most user-friendly test. Just such a unique esterase isozyme turned out to be implicated in methomyl resistance and dot-blot kit and a 'dip-stick' style test were developed. A similar kit is under development for quinalphos (organophosphate). In addition molecular markers have been developed into test kits, for example SCAR (sequence characterized amplified regions) for pyrethroid resistance (Table 13). Care needs to be taken that a recessive resistance mechanism is not overestimated by these methods. (Bt toxin quantification kits were developed simultaneously but under other funding).

The intention is for the Indian Council for Agricultural Research to patent the novel elements of these kits under an agreement with CFC in order to ensure that they are commercialized in a way that will provide maximum benefit to small-scale farmers worldwide.

8. Insecticide Mixtures

Insecticide mixtures have been widely used for pest control for several decades. The initial purpose of using insecticide mixtures was to

Table 12
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) • Phosphorothionate (quinalphos, phoxim and most others)
Carbamates	Esterase	Insensitive AChE	• Methomyl/carbaryl • Thiodicarb
Endosulfan	Esterase (sequestration)	Rdl	• Endosulfan

expand the insecticidal spectrum for the simultaneous control of different species in the pest complex, but many are now being used in an attempt to overcome field resistance to one or more of the chemicals in the mixture. China (Mainland) has a very widespread use of mixtures for bollworm control, while the regulatory authorities in India and Pakistan have been much more reluctant to register mixtures. Mixtures can improve the efficacy of pest control to some extent if potentiation occurs between individual ingredients. Potential disadvantages associated with mixtures include disruption of biological control, promotion of resistance in secondary pests, and intense selection for cross-resistance. The principle of mixtures, as a resistance-delaying measure, assumes that the mechanisms for resistance to each chemical in the mixture are different and thus individuals that survive one of the chemicals in the mixture are killed by another. However, it is also possible that one component of the mix may undermine the physiological basis of resistance to another component, thus restoring the efficacy of that component.

Theoretical models of evolutionary response to mixtures of two insecticides assume: (1) resistance to each insecticide in the mixture is monogenic, (2) no cross-resistance occurs between

insecticides in the mixture, (3) resistant individuals are rare, (4) the insecticides have equal persistence, (5) both mixture partners are applied at full rate, and (6) some of the population is untreated. Under these assumptions, resistance evolves very slowly because doubly resistant homozygotes, the only individuals to survive insecticide exposure, are extremely rare. Thus most survivors are untreated, susceptible individuals. In reality, these conditions are highly unlikely to be met, certainly in the deployment of pyrethroid/organophosphate mixtures in Asia.

Studies at Nanjing Agricultural University found:

- Most pairs of a pyrethroid and a thiophosphorate (phoxim and profenofos) are significantly synergistic.
- Toxicity of mixtures between pyrethroids and methomyl show only moderate synergism.
- Toxicity of mixtures between pyrethroids and endosulfan show antagonism.
- The same mixture have different joint action patterns in strains with different resistance profiles.

There are two main mechanisms of synergistic effect between pyrethroids and OPs: (1) OPs can inhibit esterases which

metabolise/sequester pyrethroids, (2) A portion of the mixed function oxidases (MFOs) titre in the insect is used for activation of the thio-OP, leaving less MFO for metabolizing the pyrethroid in the mixture. The relative importance of the two mechanisms varies depending on the particular structures involved. Thus, for example, ethion (not normally a bollworm chemical) effectively restores the efficacy of pyrethroids against *H. armigera* in north India, where the major mechanisms of pyrethroid resistance are metabolic. These effects are also seen in African *H. armigera* (Martin *et al.*, 2003).

Table 13
Immunodiagnostic Kits for Insecticide Quality and Resistance Detection

Insecticide	ELISA	DIP STICK		
	Quality	Quality	Residue	Resistance
Pyrethroid	Complete	Complete	Complete	Developing
Endosulfan	Complete	Complete	Complete	Prototype
Cry1Ac/1Ab	Commerc.	Commerc.	Complete	Prototype
Cry1Ac+Cry2Ab	Complete	Prototype	Complete	Prototype
Carbamate	Complete	Prototype	Prototype	Done
OP, Spinosad, Indoxacarb	Developing	Developing		Developing

Figure 2: Development of Resistance by *H. armigera* to the Two Components (Fenvalerate and Phoxim) of a Mixture Over 16 Generations of Selection

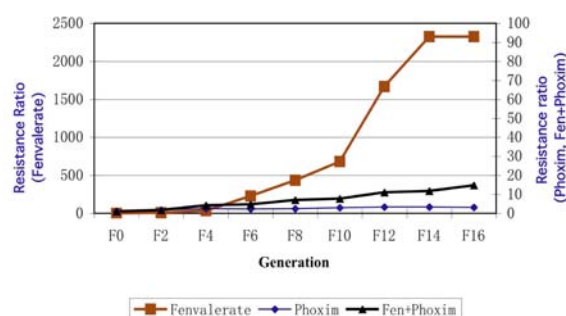
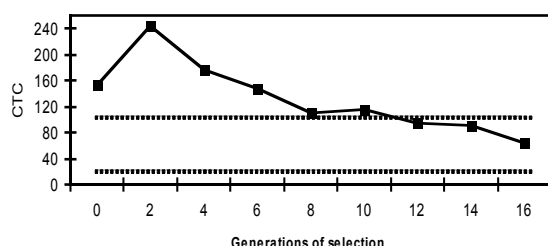


Figure 3: Changes of Co-Toxicity Coefficient (CTC) Between Fenvalerate and Phoxim During the Selection of a Mixture of Fenvalerate+Phoxim (1:10, a.i.) in *H. armigera*.



Synergism: $CTC > 120$; Summation: $80 < CTC < 120$;
Antagonism: $CTC < 80$.

However, selection of the organophosphate/pyrethroid resistant Chinese YS strain over 16 generations with a pyrethroid/organophosphates mixture resulted in rapid and major development of resistance (by an oxidative mechanism) to the pyrethroid component of the mixture (to over 2,000 fold) even though resistance to the mixture as a whole was resisted more moderately (up to 350 fold) (Figure 2). In the field, such selection would result in insects almost totally resistant to pyrethroids applied on their own.

The synergistic action of the mixture (as measured by the Co-Toxicity Co-efficient (Sun *et al.*, 1960), which was initially strong, was rapidly undermined by this selection (Figure 3).

Conclusions on mixtures

- Few mixtures produce more mortality than the most effective component of the mixture on its own.
- Where resistance to pyrethroids is metabolically mediated some mixtures (especially those containing certain

phosphorothionate organophosphates) can undermine the resistance and restore the efficacy of the pyrethroids but this effect is short lived.

- Relatively slow development of resistance to a mixture does not mean slow development of resistance to each component in the mixture.
- The binary mixtures of pyrethroid+organophosphate select intensely for metabolic mechanisms, especially oxidases, in *H. armigera*.
- The employment of mixtures in controlling *H. armigera* from Asia could result in the simultaneous enhancement of multiple resistance mechanisms and significant cross resistance to other compounds.

Mixtures may still be cost-effective for controlling an insect pest complex in cotton, however rational use of mixture, as an insecticide resistance management strategy should be treated cautiously. Development of an anti-resistance mixture should be based on a full understanding of the genetic basis and mechanisms of resistance to each component in the mixture. In West Africa, use of pyrethroid/OP mixtures from the beginning seemed to result in suppressing the development of esteratic resistance to some extent, though oxidative resistance gradually developed in *H. armigera* (Martin *et al.*, 2003). But in most cotton areas of China, Indian and Pakistan, both pyrethroids and OPs have been widely used and already have resistance problems. Employment of Pyrethroid/OP mixtures for resistance management in *H. armigera* is unlikely to be helpful in the long term in this situation.

9. Dissemination of Technical Results

Workshops

Results of the project were presented in 3-day workshops for scientists, industry, extension workers and regulatory officers in Pakistan (Islamabad), China (Beijing), India (Nagpur and a shorter spray technology workshop in Ludhiana) and Burkina Faso (Ouagadougou) during 2004 and 2005. The last workshop was in conjunction with a number of African projects dealing with resistance issues in this and other species. ACD containing the presentations made at the African Workshop and therefore covering both the Asian and African experience with *H. armigera* has been produced by CIRAD and is available from the author.

Publications

The results of the project have been written up in over 20 scientific publications and over 30 papers presented at scientific meetings in addition to farmer bulletins and information brochures in all three countries. The reference list below includes some of the major outputs.

In addition a 180 page 'Manual of Techniques in Resistance Research' has been produced by CICR for the project. This comprehensive manual covers in detail all the important lab and field techniques of sampling, preparing samples,

measuring and analyzing resistance to insecticides and Bt toxins in such a manner as to enable the reader to undertake the measurements.

All the detailed results of all the components of the project have been combined to produce a 200 page Handbook covering all the aspects of the use of insecticides for controlling the cotton bollworm (much of it with applicability to other pests). The CD, the Resistance Techniques Manual (for scientists working in the field) and the Handbook (of wider and more general applicability) are available free for single copies through the ICAC Technical Information Section or by contacting the author, while stocks last.

10. Application of Results in Field Programs

In many small-farmer cotton producing countries, pest control is moving rapidly towards more rational use of insecticides in an IPM context (Russell, 2004). This project contributed strongly to that movement in Asia.

In China, the project laboratory at Nanjing Agricultural University is a national key laboratory for insecticide resistance. Advice on insecticide resistance matters is passed to the National AgroTechnical Extension Service Centre (NATESC) which is responsible for the provision of national extension advice through the provinces to the districts and so to the farmers. The Spray Technology Division and the Pest Control Division of NATESC have incorporated the results of the project into their national chemical management advice since 2002. The growth in the national utilization of Bt cotton for bollworm control (over two thirds of the national crop in 2005) has not had the full expected effect on insecticide use reduction and insecticide applications in Eastern China still stand at an average of 13 per season on Bt cotton (Russell, 2003). IPM training is advancing slowly, especially through the EC Farmer Field School program (2000-2004) and all efforts are being made to incorporate rational insecticide use advice into those programs.

In Pakistan, the Central Cotton Research Institute has a similar role to play in the provision of advice to the extension system and farmers. In particular the input to the Cotton Crop Management Group (CCMG) in their regular meetings throughout the cotton season, meant that project results underpinned advice to the provincial extension systems. Recommendations to withdraw certain resisted pesticides from the extension advice have resulted in rapid declines in the resistance to them in *H. armigera*.

In India, the results of earlier, partially DFID-funded work on insecticide resistance in cotton, had resulted in field programs on an increasing scale from 1996. Rational insecticide use on over-threshold pests and active ingredient rotations were integrated into a full IPM strategy which was then implemented on a village level. By 1999 the program had demonstrated strong insecticide use reductions and yield and profit increases (Russell *et al.*, 2000, Kranthi *et al.*, 2000). It had also shown

that this rationalization of spraying had reduced the impact on bollworm parasitoids by 65-82% depending on the species) on predators by 63-78% and reduced the health implications of spraying for farmers by 76%. This success prompted the Indian Council for Agricultural Research to continue the program in 2000 and 2001 in the four cotton states of Haryana, Maharashtra, Andhra Pradesh and Tamil Nadu. Some 3,000 farmers were enrolled in this program in 2001-2 and the continuing success of the program enabled CICR to successfully propose a national IRM program in cotton from 2002-2007 under the Govt of India's Cotton Technology Support Program, still technically backstopped from CICR and with continuing inputs from the CFC-ICAC project.

By the 2003/04 season the US\$0.4 million program was active in 11 major cotton states. A structure of national, state and district coordinators manages the work of village IRM facilitators who live and work directly in the cotton producing villages and train and support growers. In 2003/04, 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 program). 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 and Russell *et al.*, 2004). The Government of India is committed to this program, which continues to expand rapidly.

Acknowledgements

The Common Fund for Commodities provided fifty percent of the funding under project CFC/ICAC-14. Co-funding came from the UK's Department for International Development (DFID) and from the Insecticide Resistance Action Committee (IRAC) through the provision of active ingredients of many of the most important materials. In addition the national governments of the three Asian countries, China (National Agro Technical Extension and Service Centre), India (Indian Council for Agricultural Research) and Pakistan (Pakistan Central Cotton Committee) provided co-financing of the project through staff, laboratory, equipment and field trial costs. Significant costs were also borne by the collaborating research institutes themselves.

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