Resistant Pest Management Newsletter

A Biannual Newsletter of the **Center for Integrated Plant Systems (CIPS)** in Cooperation with the **Insecticide Resistance Action Committee (IRAC)** and the **Western Regional Coordinating Committee (WRCC-60)**

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Letter from the Editor

The Scope of Resistance in North America

In 2001 the world's pesticide market exceeded $34 billion while in the US it is projected to have exceeded $11 billion. It has been estimated by Pimentel et al. (2002) that pesticide resistance surpasses $1.4 billion in environmental, ecological, and human impact costs. What are some of the features driving resistance development in North American societies? Consumerism certainly drives much of the globalism and free-market decisions in North America today. Bi-lateral trade agreements and falling tariffs have opened the way to new markets and products. Both pesticide regulations and the enactment of the Food Quality Protection Act (1996) are seen by some as emergent properties of consumerism and the environmental movement. Consumers demand blemish-free fruit and vegetables. Federal and state regulations require wholesome and labeled products as well as numerous other quality-related characteristics. Thus, consumerism in its myriad forms has swiftly overtaken outdated forms of production, marketing, and sales of agricultural products. Consumers have power in the market place today, and their power is partially translated into increased pressure toward "perfect" product quality that can only be delivered through increasingly intense pest management systems.

The environmental movement has also fostered new awareness and a drive toward new legislation and regulations targeting pesticides in agriculture and health protection. Environmental concern has also been linked to the consumer movement in western societies and together they are global in scope, extending even into third world countries. Environmentalism transects the demographics of western societies and strongly affects the regulatory policies in the U.S., Canada, and Mexico. It is projected that environmentalism will extend well into the twenty-first century.

Environmentalism and consumerism together have several pest management and resistance management impacts. The rise of global marketing, consumerism, and environmentalism together medicate much of western society's modern conscience. First, North American societies source products globally and transport these goods rapidly into the country. Second, more than 60% of North American pests historically have been introduced, with new introductions occurring almost weekly. At this rate, will North American societies eventually import most of the ecologically compatible global pest species despite our phytosanitary barriers?

Emerging with consumerism on a global scale, market access through non-tariff phytosanitation barriers has become a gauntlet that every entrepreneur must overcome. Both the introduction of invasive species and phytosanitation requirements dictate additional pesticide applications and potentially accelerate resistance selection.

Within this context is resistance, where the genetic-based adaptation of pests to man's effort to control them has become more and more important as globalism, consumerism, and market access concerns drive pesticide use. From this point of view, it is not difficult to believe that resistance problems will plague agriculture and human and animal health protection for the foreseeable future.

As editors, we represent applied ecology and insect toxicology. In our view, it is difficult to look past the inference that resistance is a symptom of a dysfunctional ecosystem. That is, agricultural production systems are often defined as disrupted ecosystems (Southwood, 1978). Resistance can logically be viewed as a symptom or indicator of an ecosystem that has been disrupted beyond its natural equilibrium, resulting in an ecologically negative outcome. Therefore, resistance is a consequence of pesticide overuse, or selection susceptibility genes in a utilitarian and reductionist sense.

This perspective could also be adopted in human and animal health protection where the problem with anti-microbial resistance has surfaced in the popular media repeatedly. It is with some irony that media would focus on antibiotic resistance and human health while less immediate resistance issues with insecticides, herbicides, and fungicides in food production rarely surface. Insecticide, acaracide, and filaricide resistance is also a critical issue for human health protection in North America but the media surprisingly overlooks it too. The media even ignores efforts by the US Centers for Disease Control (CDC) and the World Health Organization (WHO) to track various disease vector resistance development in the Americas, Asia, and Africa. With the recent media attention in North America on the introduction of the mosquito-vectored West Nile Virus into suburban and urban population centers, one might expect a somewhat broader articulation of the fragile nature of human health protection, including vector resistance. A further irony, some might note, is that North American media in concert with environmental and consumer movements would skewer certain insecticide use like

organophosphates in food production, yet approve - or even champion - the direct exposure of large numbers of people during mosquito vector control operations. Apparently, it is not appropriate to expose people to minuscule residues in the diet, but inhalation and contact exposure for human health protection is not newsworthy.

When addressing the scope of North American resistance development, new regulations dealing with resistance are of critical interest. For example, with the promulgation of regulations governing the registration of genetically modified plants containing insecticidal proteins, resistance management plans were required as a prominent portion of the registration portfolio. With one exception, all of the current conditional registrations for genetically modified plants containing insecticidal proteins have a resistance management plan based on high dose and refugia strategies (the single exception is Mom 863 for corn rootworm control).

The European Union has also recently taken some recent strides to require resistance management guidelines in its regulatory system. The EU-EPPO-PP1/213(1) guidelines require resistance risk assessment, development, and implementation of a resistance management plan and baseline monitoring of resistance for all new registrations within the EU. The 1996 Food Quality Protection Act (FQPA) also has a provision for resistance monitoring contained in its details. Essentially this prescription for resistance monitoring is worded much like a series of recommendations of the US Board on Agriculture of the National Research Council, one of which states that, "Federal agencies should support and participate in the establishment and maintenance of a permanent repository of clearly documented cases of resistance" (Dover and Croft, 1986). However, to our knowledge no divisional program within USEPA has ever followed up on this part of the FQPA law other than voluntary reporting of resistance development by registrants.

Presumably one measure of the impact of recent regulations on the availability of resistance management tools is the number of different formulations, pesticide and biopesticide modes of action, effective natural enemies, and other management strategies, tactics, and tools. Approximately 6,000 pesticides have been cancelled or significantly mitigated since the passage of the FQPA. On the other hand, the FQPA and related activities of the USEPA have accelerated the registration of reduced-risk pesticides and organophosphate alternatives. Unfortunately however, this legislation has also practically eliminated the experimental use permit process whereby land grant universities, private technical service providers, and commodity researchers have historically adapted new pesticide tools to various production systems. In addition, the FQPA has provided an array of new risk-science developments estimating the aggregate exposure to pesticides that exhibit common modes of action, the cumulative human pesticide exposure over a lifetime, and the impact of endocrine disruption on non-target organisms. Potentially all of these risk-science innovations could have unique or integrated impacts on resistance and resistance management in North America as the USEPA evolves these policies.

As previously mentioned, resistance is a genetic-based decrease in the susceptibility of a population to a control measure. It has been observed across herbicides, fungicides, and bactericides, as well as insecticides and miticides. An array of evolving pest biotypes or races has also overcome conventionally selected host plant resistance crop varieties. Perhaps even cultural control strategies like crop rotation may be overcome by genetic adaptation in a pest. An array of adapted ecosystems, particularly resistant soils, has also evolved to pesticides. The economic, social, and environmental consequences for the various types of resistance include pest control failures, disrupted pest management systems (including limitations in the development of integrated pest management options), and increased pest control costs. Increased pest control costs have variously been classified as 1) pest managers forced to resort to newer, higher-priced pesticide alternatives and 2) additional applications.

Certainly, there are arrays of environmental, social, and disrupted functional ecosystem consequences of increased pesticide use induced by resistance. Functionally, disrupted ecosystems and environmental impacts could be measured in increased off-target effects on bio-diversity and/or endangered species. Additional social impacts may include consequences on humans from increased pesticide residues, worker exposure, or increased disease spread where vector control is diminished as a result of resistance.

In summary, globalism and environmentalism will likely continue to impact the availability of pesticides as well as the social and economic determinants that will dictate overuse of pesticides leading to resistance. Heightened concerns over homeland security, particularly in the United States, may have collateral effects in terms of fighting bio-terrorism with additional pesticide use. Certainly the emergence of biotechnology and genetically modified organisms with various pest selection processes could result in further expansion of resistance problems. On the other hand, monitoring and diagnostics in resistance management should improve dramatically with the application of new high-through-put technology developed initially for HIV/AIDS and cancer detection. In addition, the pesticide industry, though market and regulatory incentives, is beginning to deliver an expanding array

of novel and ecologically softer pesticides. This fresh collection of new modes of pesticide action should allow pest managers a greater diversity of management tools to focus on target pests, thereby reducing the rate of resistance selection. Obviously the dissemination of various regulations will continue to impact the availability of resistance management tools.

Certainly, society is witnessing the rapid and expansive response of the private sector to reduced-risk and organophosphate-alternative incentives through the USEPA. One might only speculate on the development of new resistance management strategies, tactics, and tools if some of the focus and resources currently employed to regulate pesticides in North American societies are actually allocated to monitoring and measuring resistance, the loss of susceptibility in resistant-prone species, or the dysfunctional ecosystems resulting from resistance development. The CAST Resistance Conference highlighted several efforts to document resistance development in weeds, fungicides, and arthropods. These efforts are essential from our prospective, because "what gets measured gets managed."

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Resistance Management Reviews

Managing Phosphine Resistance in Grain Insects with the Phoscard®

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Export grain is one of the mainstays of Australian agriculture with over 80% of grain produced by Australian growers destined for export. Unfortunately the warm storage conditions in Australia are conducive to the establishment and development of grain insects.

Australia's enviable record as an exporter of clean grain has been achieved through the judicious use of pesticides, fumigants, and general storage hygiene. More recently our customers have begun to demand grain that is free from chemical residues as well as from insects and this has created challenges for grain growers, handlers, marketers, and researchers.

Australia is largely satisfying these markets for residue-free grain with the use of fumigants, particularly phosphine. In Western Australia (W.A.) sealed storages are used on over 60% of farms and a similar percentage of Bulk Handling storages are sealed. Since 1990, all grain has been exported from W.A. without the use of contact insecticides at any stage during storage.

This reliance on phosphine at all stages of storage places a lot of pressure on a single fumigant, particularly with respect to resistance development. Worse still, there are few alternatives - the use of the "fall back" fumigant, methyl bromide, is soon to be heavily restricted or terminated completely.

Researchers around the world have shown that the ineffective use of phosphine in poorly sealed storages can lead to resistance and eventually control failures. In the early 1980's highly resistant strains of lesser grain borer were found in Bangladesh where phosphine had been used for many years.

Early research in Western Australia has shown that grain insect resistance rarely develops in bulk storages because the cost and return of fumigating large amounts of grain is so high that bulk handlers make sure the job is done correctly the first time, every time. In the past, resistance has developed on-farm where grain protectants and fumigants are not always used in accordance with the label. There is a danger that these strains could find their way from the farm into the central handling system or worse still, an export market.

Bulk handlers routinely monitor the gas concentrations in storages under fumigation throughout the fumigation period; this is the key missing factor from farm fumigations.

Gas detection/monitoring equipment is often seen by farmers as being too expensive, too difficult to maintain and calibrate, and too sensitive to the rigours of day-to-day farmer use.

As an alternative to monitoring, the current recommendation is that farmers pressure test their storages to assess the gas-tightness of the structure prior to fumigation. If the storage is sufficiently well sealed, we know that an effective fumigation will take place provided the storage is kept sealed for 7-10 days. Unfortunately, from a farmer perspective, this pressure

testing bears little relationship to the actual fumigation that appears successful because all the adults are dead. The trap of course is that the eggs and pupae will very likely have survived the fumigation and are waiting in the wings to turn into adults and reinfest the grain within days.

With support from the Department of Agriculture Grains Program and the Grains Research and Development Corporation, we decided to develop an extension tool in the form of a farm fumigation card that would outline key points for conducting a safe and effective fumigation. The card would also have an indicator strip that would give farmers a "no frills" assessment of the standard of their fumigation.

The fact that phosphine gas corrodes copper is well known, the label even warns against using phosphine around copper electronic components. Early quarantine fumigation manuals from the United States recommend placing a shiny penny inside rail wagons before fumigation to give an indication of success at the end.

We began experimenting with various forms of copper until we found one that gave an obvious response after exposure to lethal concentrations of phosphine for at least 7 days. Copper used in electronic circuit boards gave the best results and could be readily applied as a strip to plastic card.

We tested the cards in over 20 sealed and unsealed farm storages. The copper strip consistently indicated successful fumigations. Figures 1 and 2 show typical results for good and poor fumigations carried out over 7 days.

The Phoscard® is styled on a credit card with the front side bearing 5 key fumigation principles (Figure 3). The backside (Figure 4) has a copper strip that is protected from tarnishing by a layer of transparent adhesive tape that must be removed before use. There is a hole to attach string for retrieval and instructions for use.

Farmers can place a Phoscard® in storage prior to fumigation. If the copper strip is exposed to sufficient phosphine concentrations for at least 7 days, the shiny copper strip will turn almost completely black. In fact, it could end up looking like the inside of a car exhaust pipe - evenly coated with a black sooty substance, and in some cases verdigris may develop giving the copper the distinctive blue/green colour often seen on corroded copper pipes. There is a colour chart printed alongside the copper strip to allow easy comparison.

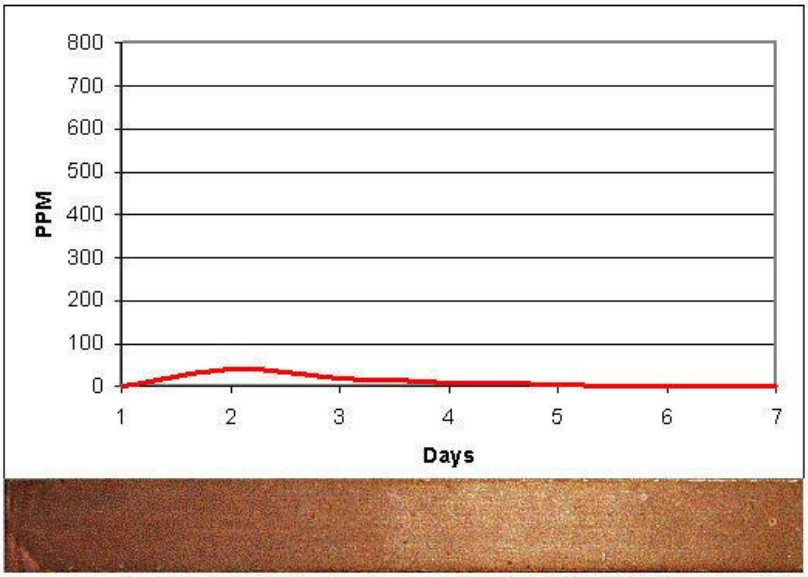

Figure 1. Poor fumigation in unsealed farm storage, 0 second pressure decay half-life. Inset copper strip showing very little corrosion.

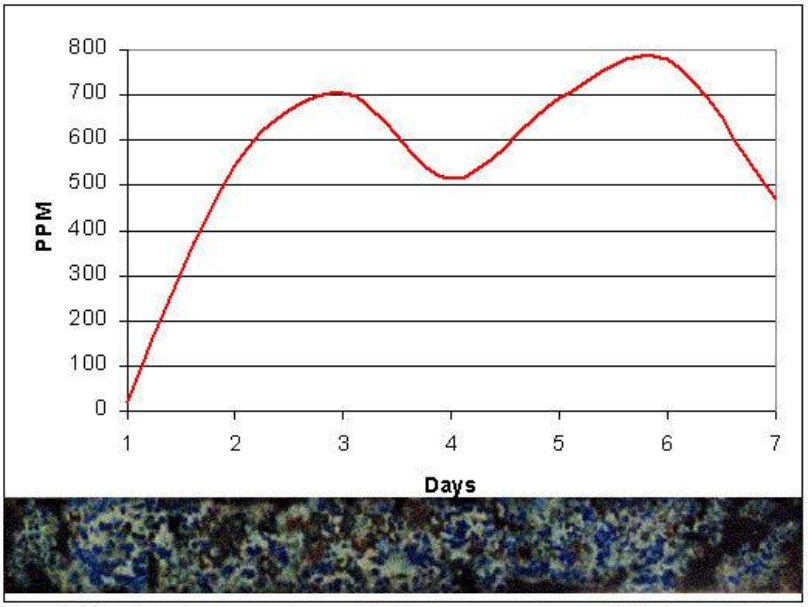

Figure 2. Good fumigation in sealed storage with a 70 second pressure decay half-life. Copper strip is heavily corroded and verdigris developing.

If the strip has not turned black at the end of the fumigation something has gone wrong: usually a leaky storage or insufficient number of aluminium phosphide tablets being placed in the storage. Remember, fumigate the storage space not the grain, use the same dose whether the storage is full or empty, and regularly inspect and replace rubber seals.

The Phoscard® is an extension tool for farmers. It will not replace phosphine monitoring equipment and is not intended to be used by fumigators or bulk handlers. However, we are hopeful that farmers experiencing fumigation failures indicated by the Phoscard®, will seek advice on how to improve their fumigations. The cards will introduce farmers to the value of fumigation monitoring and may even

encourage them to purchase one of the digital phosphine meters to more accurately monitor their fumigations.

The cards can be stored over long periods while the protective tape remains in place, but they can only be used once. Production costs are about US$0.40 each for an order of 5,000. Over 15,000 Phoscards® have been produced by the Department of Agriculture and Co-operative Bulk Handling Western Australia and distributed among farmers.

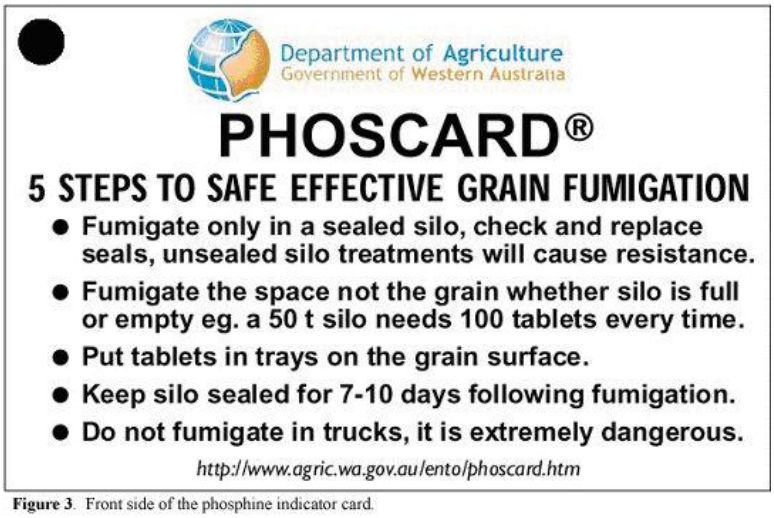

Figure 3. Front side of the phosphine indicator card.

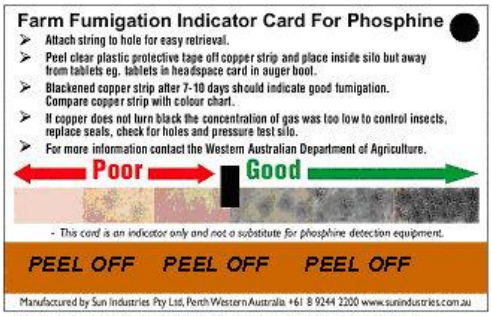

Figure 4. Rear side of the phosphine indicator card with transparent adhesive tape printed with "PEEL OFF" removed from copper strip.

Sample Phoscards® are available from:

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International QoI Working Group of FRAC: Detection of Resistance to QoI Fungicides in *Septoria tritici* in Wheat in Europe

Fungicide Resistance Action Committee (FRAC)
Klaus Gehmann
Syngenta

Sensitivity Monitoring for Septoria Leaf Spot (Septoria tritici) in Wheat in 2002

Extensive monitoring programmes were carried out BASF, Bayer, and Syngenta throughout the wheat growing areas of Europe in 2002 using both regional monitoring approaches and targeting analysis of strains from high risk trial sites.

Field performance across Europe was good under high disease pressure. However, in a few locations in South West Ireland disease control was lower than expected. This was associated with severe disease infections, which were accentuated by agronomic factors coupled with adverse weather conditions. In these sites, resistant isolates were found.

In the monitoring programmes the vast majority of tested isolates was sensitive across Europe. A low frequency of resistant isolates was detected at specific sites in the UK and to a lesser extent in Germany and France.

The G143A mutation was identified for the individual resistant isolates in 2002. In retrospective PCR analysis of some isolates collected in 2001a very low frequency of the G143A was found indicating that the 2002 observations are the result of an ongoing

selection process. Also, in 2001the field performance had been good.

Due to the epidemiology of *Septoria*, the spread of resistance is expected to be much slower than that observed for wheat powdery mildew. Nevertheless, it is critical in order to maintain the effectiveness of QoIs to strictly implement, in practice, the guidelines given conferred a low resistance factor. Studies are in progress in order to investigate the significance of these isolates under practical conditions.

Guidelines for Using QoI Fungicides on Cereal Crops in 2003

1. Apply QoI fungicides according to manufacturers recommendations for the target disease (or complex) at the specific crop growth stage indicated. Effective disease management is a critical parameter in delaying the build-up of resistant pathogen populations.
2. Apply use rates recommended by the manufacturer in order to ensure solid disease control and resistance management. The FRAC QoI working group is concerned with the trend towards the application of decreased dose rates.
3. Apply a maximum of 2 QoI fungicide containing sprays per cereal crop. Limiting the number of sprays is an important factor in delaying the build-up of resistant pathogen populations.
4. Apply the QoI fungicide preventively or as early as possible in the disease cycle. Do not rely only on the curative potential of QoI fungicides.
5. Apply QoI fungicides in mixtures to control cereal pathogens. At the rate chosen each mixing partner on its own has to provide effective disease control. Refer to manufacturers recommendations for rates.
6. Split / reduced rate programmes, using repeated applications, which provide continuous selection pressure, must not be used.

Insecticide Resistance Action Committee
Current Overview

Alan Porter and Gary D. Thompson
IRAC

INTRODUCTION The Insecticide Resistance Action Committee (IRAC) is one of the sponsors of the MSU Resistant Pest Management Newsletter and it was considered an appropriate time to publish an overview article outlining the background and objectives of IRAC and update readers with some of the ongoing current activities.

IRAC was formed in 1984 to provide a coordinated crop protection industry response to prevent or delay the development of resistance in insect and mite pests. The mission of IRAC is to facilitate communication and education on insecticide resistance and to promote the development of resistance management strategies in crop protection and vector control so as to maintain efficacy and support sustainable agriculture and improved public health.

The organization is currently implementing comprehensive strategies to confront resistance through a range of activities. In terms of organizational structure, IRAC along with the other Resistance Action Committees is a task force or working group of CropLife International and as such is recognized by The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) of the United Nations as an advisory body. The group's activities are coordinated via the IRAC Executive Committee, IRAC International and Country or Regional Committees with the information disseminated through meetings, workshops, educational materials and the IRAC Website (www.plantprotection.org/irac). Groups are comprised primarily of key technical personnel from the agrochemical companies affiliated with CropLife through membership in the relevant National Associations (ECPA, CropLife America etc).

OVERVIEW of ACTIVITIES The IRAC groups are actively involved and, on certain occasions, provide funding for a variety of resistance management projects around the world. These are generally driven or coordinated by the local country group and in some cases a specific project group is set up to lead and ultimately report results and findings into the public domain. Examples of these have been the long term monitoring of mosquitoes resistance in Mexico and the monitoring of pyrethroid resistance of *Helicoverpa armigera* in West African cotton. A new project group was set up recently within IRAC International investigating codling moth resistance in a number of countries around the world. Other activities focus on issues relating to education, communication, and regulatory approvals as well as providing expert technical support. These more general activities are wide ranging but can be grouped under the following headings:

The Resistance Database - IRAC carried out and published an international resistance survey a number of years ago but this is gradually becoming out of date. The survey is now being replaced by a new database being developed at Michigan State University and sponsored in part by IRAC. This is a major effort and will be the subject of a separate article in the Resistant Pest Management Newsletter.

Resistance Monitoring Methods - Reliable data on resistance, rather than rumors or assumptions, is the cornerstone of successful resistance management. Key to this is the availability of sound baseline data on the susceptibility of the target pest to the toxicant. A large number of bioassay and biochemical tests are employed to characterize resistance but are not necessarily comparable because different parameters and criteria are used. IRAC has evaluated and validated a wide range of testing methods that are published and are also freely available on the IRAC website. New methods are being evaluated and added to the list all the time.

Regulatory Approvals and Support - IRAC (along with HRAC and FRAC) have taken a leading role as an expert group providing industry responses to proposals from regulatory bodies. For example there is now a regulatory requirement in the EU under Directive 91/414/EEC for companies to provide an assessment of the potential risk of resistance being developed by target organisms and for management strategies to be introduced to address such risks. The Resistance Action Committees (RACs) have been instrumental in developing workable guidelines for companies resulting in the publication of an official Guidance Document. Similarly the US Environmental Protection Agency and the Pest Management Regulatory Agency of Canada have been developing a voluntary pesticide resistance management labeling scheme based on mode or target site of action on the pest. The RACs have been heavily involved in classifying pesticides into specific groups and families to enable the scheme to work. Development has been carried out under the auspices of the North American Free Trade Association and has resulted in the issue of a Pesticide Registration (PR) Notice in the US.

Education and Communication - IRAC has always believed that education and communication plays a key role in the global management of resistance and has taken many steps over the years to provide resources to academia, researchers, industry, and growers. A new project being undertaken this year is to update the existing IRAC Education Kit that comprised of a video, 35 mm slides, and supporting documentation to conduct an introductory workshop. Details about the kit will be announced in later editions of the Resistant Pest Management Newsletter. Most of the IRAC Country groups, as well as utilizing centrally developed resources, have their own educational programs in place, tailored to meet their local needs. IRAC US for example publishes articles on a regular basis in grower magazines while IRAC Brazil holds training workshops in different locations. Other IRAC Groups such as Australia, South Africa, Spain, and India have similar initiatives ongoing.

IRAC Website - The existing IRAC website has now been on-line for four years and has become the main home for IRAC information. Data available on-line includes the Monitoring Methods, MOA classification scheme, Project and Country Group updates, Meeting Minutes, Member Contact details, Useful Links, details of Published Articles, and copies of new posters recently produced.

CONCLUSION IRAC is proud of its contribution to the Crop Protection Industry by improving awareness and management of resistance issues. For this to continue it requires not only the cooperation and support of manufacturers, regulators, extension workers, consultants, sellers, and users, but also effective communication and compromise between technical and commercial departments of all companies marketing crop protection products. IRAC, with the support of member companies, intends to continue its role in facilitating this process.

Historical Perspectives on Insecticide Resistance Research - New Study Investigates Developments in Scientific Approaches from First Known Cases to the Present Situation

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The case of insecticide resistance provides an illuminating window into the workings of scientific research and the complex interactions between disciplines and institutions. Beginning with the first

scientific publications of the 1910s and continuing to the present, the resistance question has drawn researchers from fields as diverse as population genetics and organic chemistry and in institutional contexts ranging from agricultural experiment stations to industrial research laboratories. To be sure, insecticide resistance research has been primarily the domain of economic entomology, yet both the ubiquity and complexity of the phenomenon have brought together researchers with diverse backgrounds and research agendas.

Detailing this history is the goal of a new research project funded by the Swiss National Science Foundation conducted by Christian W. Simon, professor of history at the University of Basel, and John S. Ceccatti, a post-doctoral research associate on the project. One of the central questions being addressed is how scientists have utilized field observations, laboratory experiments, and theoretical frameworks to develop explanations of insecticide resistance that were consistent with current biological thought. Another area of interest is to compare the various research approaches taken by scientists from various disciplines and institutional settings.

From the current scientific vantage, the ability of insects to develop resistance to insecticides is hardly a contested fact. But even into the 1940s and 1950s, the phenomenon of insecticide resistance continued to challenge many deeply held convictions among scientists. For many economic entomologists, for example, insecticide resistance ran against the long-standing idea of the fixity of biological species in nature. Resistance also contradicted a guiding principle of the insecticide industry that once a chemical compound was proven effective is would remain so indefinitely. Resistance is also a sticky concept in the general public - and, by extension, those corporate managers, policy-makers, and other 'thought leaders' without scientific training. One need only look at on-going discussions (and confusions) about the related issue of antibiotic resistance to see that general beliefs in technological 'fixes' to complex problems have strong staying power.

To write the history of insecticide resistance research, the authors draw on a variety of sources ranging from scientific journals, industry trade magazines, unpublished technical reports from company archives, as well as personal communications with scientists currently or formerly involved with the resistance question. On this latter point, the authors welcome additional input and any interested persons can contact the authors by email at john@conceptualresearch.com. A synopsis of the research findings will be presented in the next issue of the RPM Newsletter.

Resistance Management from Around the Globe

Baseline Resistance Information

Native Resistance to Cry1Ac Toxin in Cotton Bollworm, *Helicoverpa armigera* (Hubner) in South Indian Cotton Ecosystem

Genes from *Bacillus thuringiensis* coding for crystal (Cry) toxins of the Cry1A group have been transferred to and expressed in a number of crops in order to confer resistance against lepidopteron insect pests (1,2,3). Bt transgenic cotton was cleared by the Department of Biotechnology, Government of India for commercial cultivation for the year 2002, after long debate and discussion. The primary target pest of this technology in India and several other countries is the cotton bollworm, *Helicoverpa armigera* (Hubner), which causes economic losses up to about Rs. 250 billion in India (3,4). Lately, the problems of pest management in cotton and other crops have been compounded by the development of resistance to insecticides in *H. armigera* (5). Outbreaks of *H. armigera* in south Indian cotton and pigeonpea ecosystems usually lead to severe socio-economical disturbances, including several reports of suicide by farmers. Introduction of insect resistant transgenic crops, especially Bt transgenics, are expected to be of immense value in management and effective control of lepidopteran pests with a significant reduction in the overall use of insecticides. However, long-term exposure to Bt transgenic crops is likely to render lepidopteran pests resistant to the Cry toxins due to continuous selection pressure (6). Moreover, with the introduction of transgenic plants, expressing a Cry toxin under the influence of constitutive promoters is likely to hasten this process. The development of resistance to Bt toxins can be quite distinct, depending upon the species, selection regime, or geographical origin of the founder colony (7). Hence, initial surveys to assess the susceptibility of test insects to the Cry toxins will establish a baseline that can be used in monitoring resistance development in future. We report the resistance of *H. armigera* to Cry1Ac toxin in 11 distinct geographic populations representing the entire south Indian cotton ecosystem.

The Cry1Ac protein was produced according to the method in Albert et al. (8) from an *E. coli* strain containing the hyper-expressivity recombinant plasmid vector pKK223, kindly provided by Daniel R. Zeigler, Ohio State University, USA. The toxin was purified from over expressing cells by sonication and extensive washing with sodium bromide. Proteins were quantified according to Lowry et al. (9) and the toxin was quantified by SDS-PAGE densitometry before preparing dilutions (ranging from 10 to 20000 fold) in distilled water (10). Forty percent of the protein extracted the recombinant *E. coli* cultures were found to comprise Cry1Ac toxin. LC50 values were determined for the toxin.

Laboratory strains of *H. armigera* were established from those collected in cotton fields during the cropping season of 2001-02 from major cotton growing regions of the south Indian cotton ecosystem: Nagpur and Nanded (Maharastra); Guntur, Madhira, and Nalgonda (Andhra Pradesh); Dharwad, Raichur, and Mysore (Karnataka); Coimbatore, Madurai, and Kovilpatti (Tamil Nadu). These 11 sampling locations represent the cotton growing ecosystems of south India (Fig. 1). An insecticide susceptible *H. armigera* obtained from ICRISAT, Patancheru, Hyderabad was used as a baseline susceptible strain for comparison. Larvae were reared on a chickpea-based semi-synthetic diet (11), individually in 32-well multicavity trays until pupation. Moths were kept in glass jars at 270C ±10C and 70% RH and fed with a 10% honey solution. A layer of muslin cloth was placed on the inner surface of the jars for oviposition.

Laboratory cultures were established for each population from 500-650 moths and reared to get homogenous F1 populations before conducting bioassays. Bioassays were carried out in 32-well multicavity culture trays. Six-day-old juvenile larvae (ca: 30-40 mg) were tested, one per well, on cotton leaves dipped in different concentrations of the toxin. In all, 30 larvae in three replicates were tested for each treatment. Mortality was recorded daily for six days. All assays were repeated three times and pooled data were subjected to statistical analysis. Assays were performed in the laboratory at conditions at 270C ± 10C and 70% RH. Median lethal concentrations (LC50) presented in Table 1 were derived from log dose probit calculations (12) using the MLP 0.38 statistical package (13).

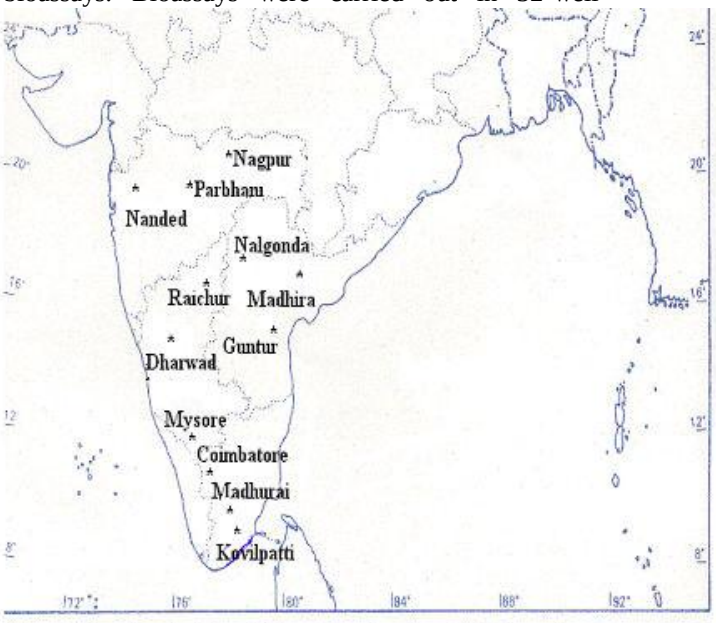

Figure 1. Sampling location of *H. armigera* from in south Indian cotton.

Cry1Ac protein was found to be toxic to all geographic populations tested (Table 1). The insecticide susceptible ICRISAT laboratory strain was the most susceptible. Compared with the others, geographic populations from Nagpur, Nanded, Guntur, Nalgonda, Madhira, and Raichur were found tolerant to the toxin. Mortality of different populations is presented in Table 1. LC50 values for Cry1Ac ranged from 0.147 to 1.095 µg/ml. The fiducial limits (at P=0.95) of the probit assay data indicated that there was a good deal of variability in response of different populations to Cry1Ac. The Kovilpatti (Tamil Nadu; extreme southern most part of south Indian cotton ecosystem) population was found to be as susceptible as the laboratory strain for Cry1Ac. The Coimbatore and Dharwad populations were similar to each other at a resistance factor (RF) of 1.5. Geographic populations of Guntur and Nanded recorded the highest RF of 8.03 and 8.42, respectively. The LC50 values of the test populations could be considered as the baseline susceptibility LC50 values for these individual populations and could be used for monitoring resistance in the future.

For resistance management programs to be effective, monitoring, surveillance, and early detection of resistant phenotypes in the field populations are important pre-requisites in order to initiate timely remedial measures and to evaluate the effectiveness of resistance management strategies. Traditionally, log dose probit assays and recently diagnostic dose assays, have been routinely used to monitor development of resistance to insecticides (3, 14, 15). Diagnostic or discriminatory dose assays are normally employed to identify individuals in a population resistant to the toxin (16), whereas log dose probit assays are useful to assess the level of resistance of a population as a ration over a reference strain or a population, usually a susceptible check. Therefore, for monitoring resistance built up in a population, diagnostic dose assays and log dose probit assays are the most appropriate (10,16,17). The results of the present analysis, showing significant differences in susceptibility to Cry1Ac toxin among

geographical locations of the south Indian cotton ecosystem, is consistent with the studies of Gujat et al. (8) in Maharastra and Karnataka. Geographical variation in susceptibility to Bt toxins was earlier reported for the related species *H. virescens* and *H. zea* (10).

One of the important exercises in the success of Bt transgenics is to assess and monitor baseline resistant levels in representative geographical populations of the target insect and to ensure that it does not cross the present values. It is obvious that this value would vary for each location/area. Data shows that even before the use of Cry1Ac transgenics, levels of resistance were 8.4 fold in the Nanded population followed by followed levels of 8.03, 7.70, 7.13,and 6.80 respectively for Guntur, Nalgonda, Madhira, and Raichur. It was as low as +1.131 fold in the Kovilpatti population located in the extreme south. This is hard to explain. Even where Bt sprays are used to some extent as a component of Integrated Pest Management (IPM) programs carried out in Andhra Pradesh and Tamil Nadu by the state department agencies, RF values are not indicative of a definite trend. Apparently, there is some relationship in slope and RF value indicative of heterogeneity and levels of resistance, respectively. Heterogeneity within a geographical location is expected due to migratory nature of the *H. armigera* and lack of selection history for Cry1Ac toxin in these geographic populations. Inter-population variation is difficult to explain in a species like *Heliothis* (19). Notably, variability for response to the Cry1Ac toxin does exist in the target population, whether or not previously exposed to the toxin. Except for the Raichur district where Bt constitutes for 9.03% of the total insecticides used (unpublished data), Bt sprays hardly constitute 0.1% of the total insecticides used on cotton in these districts.

The introduction of Bt transgenic crops is an important addition to the existing components of Integrated Pest Management. The technology is perceived to be effective and eco-friendly. However, much of its success will depend on the sustained susceptibility of the target pests to the Bt toxins used in transgenic crops. Bt transgenic crops, which express Cry1Ac, were found to cause 100% and 75-90% mortality in susceptible *H. virescens* and *H. zea* respectively, in the United States of America (20). The same level of expression caused less than 90% mortality of *H. armigera* and *H. punctigera* in Australia (21), indicating that *Helicoverpa* species appear to have certain levels of tolerance to the Bt toxin Cry1Ac, whether or not previously exposed to Bt toxin, when compared with the *Heliothis* species. It is important to note that in this study as well, a few individuals of *H. armigera* in almost all the populations tested were found to survive even the highest concentrations of Cry1Ac tested. This would suggest

Table1. Log dose probit response of south Indian cotton ecosystem field populations of *H. armigera* to Cry1Ac toxin.

Location	LC_{50}	95% FL		Slope	RF*
	mg/ml	Lower	Upper		
ICRISAT†	0.130^{f}	0.08	0.18	1.15	1
Nagpur	0.927^{bc}	0.688	1.087	4.43	7.131
Nanded	1.095^{a}	1.032	1.479	4.13	8.423
Guntur	1.044^{ab}	0.98	1.154	8.67	8.031
Nalgonda	1.001^{ab}	0.909	1.204	4.99	7.7
Madhira	0.927^{bc}	0.688	1.087	4.43	7.131
Raichur	0.884^{c}	0.647	0.975	4.57	6.8
Dharwad†	0.191^{e}	0.094	0.277	1.07	1.469
Mysore†	0.260^{d}	0.121	0.408	0.87	2
Coimbatore†	0.191^{e}	0.094	0.277	1.07	1.469
Madurai†	0.177^{ef}	0.061	0.302	0.77	1.362
Kovilpatti†	0.147^{ef}	0.057	0.306	0.54	1.131

†Test for heterogeneity; χ^2 significant at 5% level of significant. LC_{50} values designated by different letters are significantly different from each other through non-overlap of 95% fiducial limits[25].

Abbreviations: LC_{50} is the median Lethal Concentrations expressed in µg/ml diet; FL are the Fiducial Limits; RF are Resistant Factors calculated using ICRISAT strain as susceptible reference.

that, under field conditions, tolerant individuals are likely to persist despite high expression of the Cry1A toxins and may subsequently contribute to the resistant gene pool. Daly (22) reported that transgenic cotton plants in Australia killed susceptible larvae early in the season but the effect significantly declined later (95-100 days after sowing), when an increasing proportion of first instar larvae placed on transgenic leaves survived to late instars. The studies on Bt cotton in the USA and Australia have shown that Cry1Ac protein production decreased over the growing season and that the bio-efficacy of the protein was reduced by interaction with increasing levels of secondary plant metabolites (23,24). Differential expression in plant tissues may contribute toward a reduced efficacy of the Bt transgenic crops. If proper resistance management strategies are not implemented, the efficacy of pest management through Bt transgenic crops will be seriously diminished due to widespread development of resistance. Such strategies have not yet been developed for the small farmer and predominantly non-irrigated cotton growing systems found in India and elsewhere.

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Insecticide Resistance in Cotton Bollworm, *Helicoverpa armigera* (Hubner) in South Indian Cotton Ecosystems

Cotton occupies only 5% of the total cultivable area in India but consumes more than 55% of the total insecticides used in the country, accounting for about 250 billion rupees (Kranthi et al., 2001). Plant protection continues to rely heavily on chemical pesticides, a not very viable, long-term strategy if one looks at recent failures against cotton bollworms and several other crop pests. Large-scale failures to control *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) in the major cotton-growing region of South India in 1987 have been traced to insecticide resistance (McCaffery et al., 1989). To combat the unprecedented *Helicoverpa armigera* pest pressure, many farmers in the region applied synthetic pyrethroid, endosulfan, or organophosphate insecticides, sometimes as mixtures, at 2-3 days intervals during critical periods. This resulted in over 30 sprays (against the 8-10 recommended) during the season (Rakila et al., 1995), but growers were unable to achieve effective control with any of the available insecticides.

The phenomenon of resistance to insecticides in *Helicoverpa armigera* that surfaced under different agro-ecosystems of South India is the major negative side effect of the chemical control strategy. Identification of baseline resistance to each of the insecticide used in the region to control bollworm would be indispensable for formulating effective IRM strategies.

The larvae of *Helicoverpa armigera* (second to sixth instar) collected from 12 different geographical locations of South India (Fig. 1) were reared in the

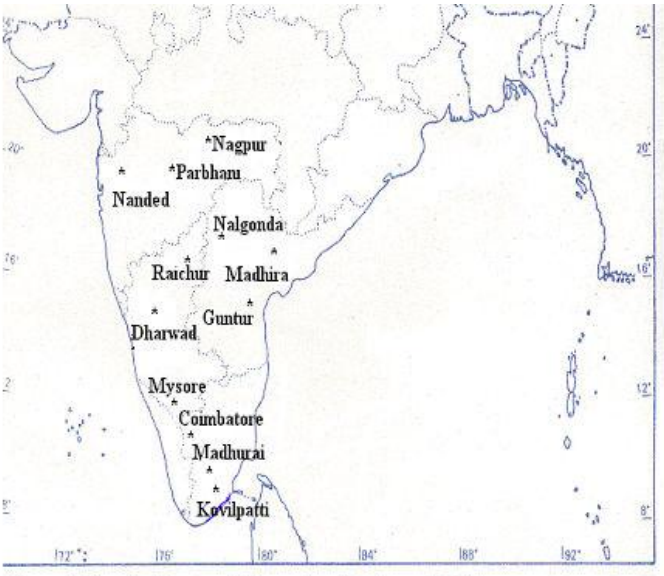

Figure 1: Sampling location of *H. armigera* from in south Indian cotton.

laboratory on a semi-synthetic diet to get F1 homogeneous larvae for bioassays.

Larvae of 30-40 mg were used for bioassays. Six insecticides viz., monocrotophos, chlorpyriphos, Endosulfan, carbaryl, cypermethrin, and quinalphos that are extensively used to control bollworm in cotton ecosystems of South India were used to determine baseline resistance. The technical grade chemicals of these insecticides were obtained from M/S De Nocil Crop Protection Ltd., M/S Syngenta Company, and M/S Aventis Crop Science. Different concentrations of these selective insecticides were prepared in analytical grade acetone and a Hamilton microapplicator was used to deliver a 1.0 µl drop to the thoracic dorsum of each third instar larva. The control larvae were treated with acetone alone. The concentrations of each insecticide were varied to obtain 20-80 % mortality. Immediately after expose to insecticide/acetone, each of 30 larvae (for each insecticide) was kept individually in a 30 ml plastic cup with fresh artificial diet and mortality was assessed 72hr after treatment. The dose-mortality regression was computed by using MLP 3.08 software (Ross, 1987).

Table 1. Response of geographical population of cotton bollworm for monocrotophos bioassay.

	Monocrotophos						
Location	LD_{50} µg/µl	Fiducial limits		Slope	Chi-square Value	RR*	RR**
		Lower	Upper				
Nagpur	13.690	10.8846	16.8953	0.24	16.49	44.48	68.5
Nanded	7.275	6.0886	8.4884	0.39	13.08	23.64	36.4
Guntur	7.027	5.9360	8.1259	0.41	4.93	22.83	35.1
Nalgonda	7.291	5.6571	8.7829	0.40	7.30	23.69	36.5
Madhira	2.140	1.8184	2.4300	0.65	3.27	6.95	10.7
Raichur	2.233	1.7034	2.8402	0.46	6.93	7.26	11.2
Dharwad	4.226	3.5758	5.0147	0.89	1.14	13.73	21.1
Mysore	6.115	4.8259	7.4531	0.60	8.63	19.87	30.6
Coimbatore	0.777	0.6578	0.8906	0.50	6.04	2.52	3.9
Madurai	0.452	0.3617	0.5472	0.66	6.69	1.47	2.3
Kovilpatti	0.308	0.2310	0.3846	0.55	0.96	1.00	1.5

* Susceptible Kovilpatti strain
** Susceptible ICRISAT strain

Table 2. Response of geographical population of cotton bollworm for endosulfan bioassay.

	Endosulfan						
Location	LD_{50} µg/µl	Fiducial limits		Slope	Chi-square value	RR*	RR*
		Lower	Upper				
Nagpur	4.571	3.9129	5.1376	0.5741	3.15	6.17	20.80
Nanded	3.299	2.6086	4.0185	0.3432	11.81	4.46	15.00
Guntur	13.156	11.1393	15.4543	0.5888	12.38	17.77	59.80
Nalgonda	13.241	10.6351	15.3325	0.6861	6.19	17.88	60.20
Madhira	2.676	2.2767	3.1255	0.7248	1.33	3.61	12.20
Raichur	4.165	3.1640	5.1557	0.6637	7.27	5.62	18.90
Dharwad	4.428	3.8579	4.9458	0.8594	7.56	5.98	20.10
Mysore	2.294	1.5739	3.2371	0.3071	7.98	3.10	10.40
Coimbatore	1.025	0.7983	1.2586	0.3247	3.36	1.39	4.70
Madurai	0.740	0.5864	0.9080	0.5033	4.98	1.00	3.40
Kovilpatti	0.906	0.6330	1.1140	0.6322	7.51	1.22	4.10

* Susceptible Madurai strain
** Susceptible ICRISAT strain

Table 3. Response of geographical population of cotton bollworm for quinalphos bioassay.

	Quinalphos						
Location	LD_{50} µg/µl	Fiducial limits		Slope	Chi-square Value	RR*	RR**
		Lower	Upper				
Nagpur	2.840	2.4536	3.2856	0.52	0.18	5.68	16.70
Nanded	3.320	2.7993	3.8283	0.46	5.69	6.65	19.50
Guntur	12.564	10.8071	14.5113	0.41	4.10	25.15	73.90
Nalgonda	20.937	18.7695	23.3873	0.68	4.10	41.91	123.20
Madhira	2.193	1.6794	2.4744	1.90	6.20	4.39	12.90
Raichur	4.369	3.5399	5.1044	0.76	1.70	8.75	25.70
Dharwad	4.385	3.7964	4.9821	0.87	2.70	8.78	25.80
Mysore	4.155	3.0172	5.5478	0.39	3.62	8.32	24.40
Coimbatore	1.071	0.9097	1.2166	0.59	7.73	2.14	6.30
Madurai	0.500	0.3605	0.6469	0.36	10.98	1.00	2.90
Kovilpatti	0.941	0.7510	1.1999	0.50	5.22	1.88	5.50

* Susceptible Madurai strain
** Susceptible ICRISAT strain

Monocrotophos: The Nagpur population recorded a maximum LD50 value to monocrotophos (13.690 µg/µL) followed by the population from Nalgonda (7.291 µg/µL), Nanded (7.275), Guntur (7.027), Mysore (6.12), and Dharwad (4.22). The lowest LD50 value was observed in the population from Kovilpatti (0.308) followed by Madurai (0.452 µg/µL) and Coimbatore (0.777). The resistance ratio (RR) against the ICRISAT susceptible strain was found to be highest for the population of Nagpur (68.5 fold) followed by Nalgonda (36.5), Nanded (36.4), Guntur (35.1), Mysore (30.6), and Dharwad (21.1). The least resistance ratio was observed in the population of Kovilpatti (1.5) followed by Madurai (2.3) and Madhira (3.9) (Table 1).

Endosulfan: The Nalgonda population recorded a maximum LD50 value to endosulfan (13.240 µg/µL) followed by the population from Guntur (13.155 µg/µL). The lowest LD50 value was observed in the population from Madurai (0740 µg/µL) followed by

Table 4. Response of geographical population of cotton bollworm for chlorpyriphos bioassay.

Chlorpyriphos							
Location	LD50 µg/µl	Fiducial limits		Slope SE (±)	Chi-square	RR*	RR**
		Lower	Upper				
Nagpur	2.586	2.2551	2.9454	0.53	1.19	7.19	18.50
Nanded	1.761	1.3650	2.1321	0.53	7.57	4.89	12.60
Guntur	11.038	9.2306	13.8978	0.52	8.57	30.69	78.80
Nalgonda	9.479	6.9735	11.5751	0.43	0.92	26.35	67.70
Madhira	2.110	1.7305	2.4459	0.82	1.95	5.87	15.10
Raichur	2.345	1.8394	2.8643	0.63	2.07	6.52	16.80
Dharwad	4.676	3.9971	5.2890	0.95	0.91	13.00	33.40
Mysore	2.220	1.5669	3.0326	0.33	2.37	6.17	15.90
Coimbatore	1.128	0.9481	1.2940	0.55	9.92	3.14	8.10
Madurai	0.360	0.2842	0.4350	0.55	3.24	1.00	2.60
Kovilpatti	0.463	0.4044	0.5121	1.18	0.11	1.29	3.30

* Susceptible Madurai strain

** Susceptible ICRISAT strain

Table 5. Response of geographical population of cotton bollworm for cypermethrin bioassay.

Cypermethrin							
Location	LD50 µg/µl	Fiducial limits		Slope	Chi-square Value	RR*	RR**
		Lower	Upper				
Nagpur	1.628	1.3901	1.8652	0.47	3.39	11.42	27.60
Nanded	2.333	1.8584	2.9282	0.50	10.53	16.36	39.50
Guntur	10.917	9.5684	12.4475	0.59	4.33	76.59	185.00
Nalgonda	9.984	8.2207	11.2847	0.82	1.33	70.05	169.20
Madhira	3.038	2.0706	7.4659	0.63	0.76	21.31	51.50
Raichur	22.400	19.9492	24.1182	2.25	3.20	157.15	379.70
Dharwad	4.759	4.0020	5.5898	0.93	6.94	33.38	80.70
Mysore	4.054	3.1452	5.2449	0.35	0.08	28.44	68.70
Coimbatore	2.472	2.1859	2.7581	0.61	6.66	17.34	41.90
Madurai	0.143	0.1073	0.1779	0.51	3.04	1.00	2.40
Kovilpatti	0.192	0.1418	0.2516	0.36	3.11	1.35	3.30

* Susceptible Madurai strain

** Susceptible ICRISAT strain

Table 6. Response of geographical population of cotton bollworm for carbaryl bioassay.

Carbaryl							
Location	LD_{50} µg/µl	Fiducial limits		Slope	Chi-square Value	RR*	RR**
		Lower	Upper				
Nagpur	2.922	2.5382	3.3196	0.43	9.51	3.74	14.60
Nanded	2.149	1.7070	2.6071	0.44	10.35	2.75	10.70
Guntur	8.728	7.1038	10.7135	0.39	8.14	11.17	43.60
Nalgonda	9.132	7.2087	11.0628	0.42	9.37	11.69	45.70
Madhira	1.122	0.9466	1.3139	0.57	1.77	1.44	5.60
Raichur	13.356	10.5676	16.0296	0.61	4.98	17.09	66.80
Dharwad	4.838	4.1725	5.6177	0.67	8.13	6.19	24.20
Mysore	5.151	3.8977	6.6522	0.39	2.72	6.59	25.80
Coimbatore	1.347	1.0444	1.5610	0.62	7.45	1.72	6.70
Madurai	0.781	0.5571	0.9865	0.48	11.46	1.00	3.90
Kovilpatti	0.873	0.6771	1.0661	0.47	8.10	1.12	4.40

* Susceptible Madurai strain

** Susceptible ICRISAT strain

Kovilpatti (0906) and Coimbatore (1.025). The resistance ratio (RR) against the ICRISAT susceptible strain was found to be highest for the population of Nalgonda (60.2 fold) followed by Guntur (45.7). The least resistance ratio was observed in the population of Madurai (3.4) followed by Kovilpatti (4.1) and Coimbatore (4.7) (Table 2).

Quinalphos: Maximum resistance to quinalphos was observed in the Nalgonda population (20.937µg/µL) followed by the Guntur population (12.564 µg/µL). Least resistance was noticed in the Madurai population (0.5 µg/µL). High resistance to quinalphos was recorded by the population from Nalgonda (123.2 fold) followed by the Guntur population (73.9 fold) as against the susceptible ICRISAT population (Table 3).

Chlorpyriphos: Maximum resistance to chlorpyriphos was recorded in Guntur (11.038 µg/µL) followed by Nalgonda (9.480 µg/µL). Minimum resistance was observed in the Madurai population (0.36 µg/µL) followed by the Kovilpatti population (0.463) and Coimbatore (1.128). The resistance ratio against the ICRISAT susceptible strain was found to be highest for the population of Guntur (78.8 folds) followed by Nalgonda (67.7) and Dharwad (33.4). The least ratio was recorded in the population from Madurai (2.6) and Kovilpatti (3.3) (Table 4).

Cypermethrin: The Raichur population recorded a maximum LD50 value to cypermethrin (22.40 µg/µL) followed by the population from Guntur (10.92) and Nalgonda (9.984). The lowest LD50 value was observed in the population from Madurai (0.143µg/µL) followed by Kovilpatti (0.192) and Coimbatore (2.472). The resistance ratio (RR) against the ICRISAT susceptible strain was found to be highest for the population of Raichur (379.7 fold) followed by Guntur (185.0) and Nalgonda (169.2). The least resistance ratio was observed in the population of Madurai (2.4) followed by Kovilpatti (3.3) (Table 5).

Carbaryl: The Raichur population recorded a maximum LD50 value to carbaryl (13.36 µg/µL)

followed by population from Nalgonda (9.13 µg/µL), Guntur (8.73), Mysore (5.15), and Dharwad (4.84). The lowest LD50 value was observed in the population from Madurai (0.78µg/µL) followed by Kovilpatti (0.87), Coimbatore (1.35), and Madhira (1.12). The resistance ratio (RR) against the ICRISAT susceptible strain was found to be highest for the population of Raichur (66.8 fold) followed by Nalgonda (45.7), Guntur (43.6), Mysore (25.8), Dharwad (24.2), Nagpur (14.6), and Nanded (10.7). The least resistance ratio was observed in the population of Madurai (3.9) followed by Kovilpatti and Madhira (Table 6).

The general LD50 values recorded were far higher compared to the recommended dosages indicating the existence of resistance as was reported earlier (Armes et al., 1992). However, the resistance drastically differs from location to location within the South Indian cotton ecosystems. The resistance levels in the Guntur, Nalgonda, and Raichur regions (heavy insecticide usage areas) are due to heavy dependence on insecticides. This clearly explained that resistance levels were proportionate with the usage of pesticides. The study conducted by Forrester (1990) also clearly revealed that resistance levels rose when pyrethroids were used but fell significantly when they were withheld. Thus, the pesticides were creating very high selection pressure for resistant genotypes. This suggests that indiscriminate use and heavy dependence on pesticide will further complicate the already worsened situation and hints at aiming for insecticide resistance management strategies.

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Development of Resistance in Insects to Transgenic Plants with *Bacillus thuringiensis* Genes: Current Status and Management Strategies

INTRODUCTION There is a continuing need to increase food production as the world population is expected to exceed 6 billion by 2050. In both the developed and undeveloped countries, the cost for achieving production has become too high because of the need to incur costs for controlling insect pests that cause an estimated loss of $10 billion annually. The difficulties experienced in controlling insect pests over the past 30 years have been largely due to the over-use of pesticides. Indiscriminate use of insecticides has led both to the development of resistance in insects and the destruction of natural enemies. Bio-pesticides such as Bt (*Bacillus thuringiensis*) products are widely regarded as being the least harmful to natural enemies. Because of its selectivity and environmental safety, usage of Bt is increasing, particularly in IPM programs. Foliar application of Bt breaks down quickly under field conditions due to UV sensitivity and rainfall. With the advent of recombinant DNA technologies, insecticidal proteins present in Bt have been expressed in crop plants to ensure durable insect resistance. There is a considerable increase in global area under transgenic crops from 1.7 million hectares in 1996 to 52.6 million hectares in 2001, in which the share of Bt crops was 15% of the total area (James 2001). Although much progress has been made in the discovery of new genes for introduction into plants, only Bt genes have been exploited so far.

Considerable efforts have been made to incorporate delta-endotoxins from Bt into cereals, root crops, leafy vegetables, forage crops, and horticultural crops (Schuler et al. 1998). Of the $8.1 billion (US

dollars) spent annually on insecticides worldwide, it was estimated that nearly $2.7 billion could be substituted with Bt biotechnology applications (Krattiger 1997). Economic advantage gained during 1999 by Bt cotton alone has been estimated to be $213 million in the USA. Cultivation of transgenic crops has led to a reduction in pesticide use and significant increase in yield (Cannon 2000). Unfortunately, there are also concerns that the benefits of genetically transformed plants will be short-lived (McGaughey & Whalon 1992). Despite the potential advantages of using Bt crops, the possibility of their widespread use has raised some potential problems. Decades of indiscriminate insecticide use have demonstrated that exposing insect populations to high levels of toxins results in evolution of resistance to insecticides (Roush & McKenzie 1987). Recently, several species of insect pests have been selected for resistance to Bt in the laboratory, indicating that biological pesticides can suffer the same fate as the chemical pesticides (Liang et al. 2000, McGaughey et al. 1998a).

DEVELOPMENT of RESISTANCE in INSECTS to Bt GENES

Several studies have shown that insect pests can adapt to Bt toxins under laboratory conditions (Shelton et al. 2002). Certain pests such as *Plodia interpunctella* (McGaughey 1985), *Heliothis virescens* (Stone et al. 1989), *Plutella xylostella* (Tabashnik et al. 1990), *Spodoptera exigua* (Moar et al. 1995), and *Ostrinia nubilalis* (Huang et al. 1997) have been shown to develop some degree of resistance to *B. thuringiensis* under laboratory conditions. Evolution of insect resistance to insecticidal proteins produced by Bt would decrease our ability to control agricultural pests with genetically engineered crops designed to express genes coding for these proteins (Gould et al. 1992). Information on development of resistance in insects to Bt toxins has been summarized below.

Indian meal moth, Plodia interpunctella:

The first studied case of resistance to Bt-strains was *P. interpunctella*, which had developed 100-fold resistance following 15 generations of laboratory selection with Dipel (McGaughey 1985). On further selection, after 36 generations, the resistance levels reached 250-fold (McGaughey & Beeman 1988). *Bacillus thuringiensis* sub sp. *kurstaki* caused a narrow spectrum resistance to Cry1Ab and Cry1Ac toxins, while sub sp. *aizawai* and *entomocidus* strains caused broad-spectrum resistance to Cry1Aa, Cry1Ab, Cry1Ac, Cry1B, Cry1C, and Cry2A (McGaughey & Johnson 1994).

Diamondback moth, Plutella xylostella

Although there are no instances of insects developing resistance to Bt transgenic plants in the field, diamondback moth, *P. xylostella*, is the first insect known to have evolved high levels of resistance to Bt as a result of repeated use of formulated Bt insecticide (Tabashnik et al. 1990). A diamondback moth colony derived from field population in the Philippines that was regularly exposed to Dipel showed more than 200-fold resistance to Cry1Ab (Ferre et al. 1991). As much as 1640-fold resistance to Bt has been recorded in localized populations of diamondback moth from Hawaii, Florida, and Asia (Tabashnik et al. 1992). In field populations of *P. xylostella*, resistance to Bt sub sp. *kurstaki*, containing Cry1A(a,b,c), Cry2A, and Cry2B toxins and to a lower extent Bt sub sp. *aizawai*, containing Cry1A (a,b), Cry1C, and Cry1D toxins has been observed in various countries (Tabashnik 1994). Laboratory selection of *P. xylostella* using Cry1Ca protein and in later generation transgenic broccoli expressing Cry1Ca, increased Cry1Ca resistance to 12400-fold (Zhao et al. 2000b). Resistance to Cry1A toxins from Bt sub sp. *kurstaki* caused cross-resistance to Cry1F, but not to Cry1B or Cry1C (Tabashnik et al. 1996). Contrary to the assumption that independent mutations are required to counter each toxin in *P. xylostella*, an autosomal recessive gene conferred extremely high resistance to Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F (Tabashnik et al. 1997). In a *P. xylostella* colony possessing 1500-fold resistance to a commercial formulation, the resistance rapidly fell to 300-fold in the absence of selection, but remained stable at this level in subsequent generations (Tang et al. 1996).

Cotton bollworm/ legume podborer, Heliothis /Helicoverpa

Helicoverpa armigera is capable of developing resistance to Cry1Ac in 7 to 8 generations (Kranthi et al. 2000). Highly mobile polyphagous pests such as *Helicoverpa* may develop resistance to Bt on one transgenic crop and then disperse, nullifying the effectiveness of a wide range of Bt transgenic crops expressing the same or similar Cry proteins. Pests with resistance to CryIA proteins in transgenic plants may also display significant resistance to Bt biopesticides. A laboratory strain of *H. virescens* developed resistance in response to selection with the Bt toxin CryIAc. In contrast to other cases of Bt-toxin resistance, this strain exhibited cross-resistance to Bt toxins that differ significantly in structure and activity (Gould et al. 1992). Over 10000-fold resistance to Cry1Ac was obtained in *H. virescens* colony on selection with Cry1Ac protoxin (Gould et al. 1995). The insecticidal activity of Bt in leaves and squares of transgenic cotton plant was high during the second generation of the insect, but declined in the third and fourth generations of *H. armigera* in North China. The surviving third and fourth generation larvae, after feeding on flowers of Bt cotton, fed on the bolls until pupation, which caused selection in field populations of *H. armigera*. The increase in resistance was 7.1-fold

after 17 generations of selection in the laboratory (Zhao et al.1998). Liang et al. (2000) found that the resistance ratio of *H. armigera* to Bt transgenic cotton, after selection for 16 generations was 43.3, and inheritance of resistance was controlled by a single autosomal incomplete recessive allele.

European corn borer, Ostrinia nubilalis

There has been a significant decrease in susceptibility across generations for selected strains of *O. nubilalis* after chronic exposure to formulated Cry1Ab (Huang et al. 1997, Josette et al. 2001). Similarly, a 162-fold increase in resistance to transgenic Cry1Ac has been observed in European corn borer after 8 generations of laboratory selection (Bolin et al. 1999). Event 176 Bt corn hybrids express high levels of Cry1Ab toxin in green plant tissue and pollen, but extremely low levels in the silk and kernels (Koziel et al. 1993), on which second generation *O. nubilalis* larvae have been shown to survive (Siegfried et al. 2001). Zoerb et al (2003) stated that successfully developed *O. nubilalis* larvae have either survived exposure to sublethal doses of Cry1Ab Bt toxin or exploited plant tissues that do not express the toxin, and they further implicated that Event 176 hybrids do not satisfy requirements for high dose that are recommended for resistance management purposes.

Pink bollworm, Pectinophora gossypiella

Field collected pink bollworm quickly evolved resistance to Cry1Ac under laboratory selection (Patin et al. 1999, Simmons et al. 1998, Tabashnik et al. 2000). *Pectinophora gossypiella* selected with Cry1Ac protoxin developed 300-fold resistance to Cry1Ac protoxin, and high levels of cross-resistance to Cry1Aa and Cry1Ab protoxin, and low levels of resistance for Cry1Bb protoxin (Tabashnik et al. 2000a). Three selections with Cry1Ac in artificial diet increased resistance of pink bollworm to >100-fold relative to a susceptible strain (Liu et al. 2001).

Tobacco caterpillar, Spodoptera spp.

In general, *Spodoptera* spp. larvae are not very susceptible to the Cry toxins (Strizhov et al. 1996). However, Cry1C toxin had been reported to be toxic against *S. exigua* (Visser et al .1988) and *Spodoptera littoralis* (Van Rie et al. 1990a). Selection to Cry1Ca caused 850-fold resistance to Cry1Ca and cross-resistance to Cry1Ab, Cry9C, and Cry2A, as well as to a recombinant Cry1E-Cry1C fusion protein in *S. exigua* (Moar et al. 1995), while in *S. littoralis*, 500-fold resistance to Cry1Ca and partial cross-resistance to Cry1D, Cry1E, and Cry1Ab has been recorded (Muller-Cohn et al. 1996).

BASIS for DEVELOPMENT of RESISTANCE Mutations in insects that cause disruption of any of the steps involved in the mode of action could confer resistance to Bt (Heckel 1994). Decreased solubilization of the Bt crystal, decreased cleavage of the full-length Bt protein into an active fragment, increased proteolytic digestion of the active fragment, decreased binding of the active fragment to the midgut epithelium, and decreased functional pore formation are the major changes in the Bt toxicity pathway responsible for evolution of resistance (Gill et al. 1992). Previous genetic and biochemical analyses of insect strains with resistance to Bt toxins has indicated that: (i) resistance is restricted to single group of related Bt toxins, (ii) decreased toxin sensitivity is associated with changes in Bt-toxin binding to sites in brush-border membrane vesicles of the larval midgut, and (iii) resistance is inherited as a partially or fully recessive trait. If these three characteristics are common to all resistant insects, specific crop-variety deployment strategies could significantly diminish problems associated with resistance in field populations of the target pests (Gould et al. 1992). Recent studies have shown that the genetic basis of resistance to Bt toxins in insects is similar to resistance to chemical insecticides, which is conferred by multiple physiological mechanisms under independent genetic control. In *Heliothis*, the existence of separate, independently assorting resistance genes has already been confirmed by linkage analysis with marker loci (Heckel 1994). Heckel et al. (1997) identified a major Bt- resistant locus in a strain of *H. virescens* exhibiting up to 10000-fold resistance to Cry1Ac toxin. Despite many potential mechanisms of resistance, the best-characterized and most widely observed mechanism of resistance to *B. thuringiensis* is reduced binding of toxin to midgut membranes (Van Rie et al. 1990b). Changes in the binding affinities of toxin receptors on the brush border membranes of the insect midgut have been identified in Bt resistant *P. interpunctella* (Van Rie et al. 1990b), *P. xylostella* (Ferre et al. 1991), *H. virescens* (MacIntosh et al. 1991), and *Trichoplusia ni* (Ballester et al. 1994). Cry1Ab and Cry1Ac have the same receptor in the midgut of *O. nubilalis*, with the receptor having a higher affinity for Cry1Ab than for Cry1Ac (Denolf et al. 1993).

Studies on a field population of *P. xylostella* have also suggested that, apart from reduced binding, other biochemical mechanisms are involved in resistance to Bt (Martinez-Ramirez et al. 1995). Some evidence for reduced conversion of protoxin to toxin and increased degradation of toxin also has been reported (Forcada et al. 1996, Oppert et al. 1994, 1997). In *H. armigera*, the excessive degradation of protoxin in midgut juice triggered by receptor binding of activated toxin was presumed to be responsible for low sensitivity of the insect to Bt (Shao et al. 1998). Toxin binding in resistant *T. ni* selected with Cry1Ab did not correlate with resistance, since there was no cross-resistance to

Cry1Ac (Estada & Ferre 1994), which shares the same binding site of Cry1Ab as demonstrated in *O. nubilalis* midgut membrane (Denolf et al. 1993).

When the midgut proteinases from resistant strain of European corn borer were characterized, there was a 35% decreased hydrolyzing efficiency in activation of Bt protoxin compared with the susceptible strain (Huang et al. 1999). However, in studies by Liu et al. (2000), Cry1C toxin was found to be significantly more toxic than was Cry1C protoxin to resistant strain of diamond back moth, but not to susceptible strain. If reduced conversion of Cry1C protoxin to toxin is the sole mechanism of resistance, both susceptible and resistant larvae should be equally susceptible to Cry1C toxin. Further, they observed similar binding of 125I-Cry1C to brush border membrane vesicles from the Cry1C resistant and susceptible strains and concluded that reduced binding of Cry1C to midgut target sites was not a mechanism of resistance in diamondback moth. Mohan & Gujar (2003) also found no differences in proteolytic patterns of Cry1A protoxins in both susceptible and resistant populations of diamondback moth. They also stated that the differences in susceptibility of two populations to *B. thuringiensis* Cry1Ab were not due to midgut proteolytic activity. McGaughey et al (1998b) indicated that apart from toxin solubility and/or proteinase activation in the insect midgut, postbinding events such as receptor aggregation, pore formation, ionic fluxes, and insect recovery may also be involved in resistance development. Following Cry1Ac ingestion by *H. virescens*, similar histopathological changes were observed in midgut epithelium in both susceptible and resistant colony (Forcada et al. 1999, Martinez-Ramirez et al. 1999), suggesting that resistance is due to a more efficient repair (or replacement) of damaged midgut cells (Ferre & VanRie 2002).

Research conducted over the past 10 years has indicated that it is likely that the increased use of Bt toxins from transgenics will result in a rapid evolution of resistance in insects (Gelernter 1997). However, selection of plants for horizontal resistance is more durable rather than vertical resistance, and the current research on transgenic plants, particularly incorporation of the Bt delta endotoxins into crops for control of insects appears to be proceeding on a vertical resistance model, based on complete resistance conferred by one or a few genes. These varieties, like those produced through conventional resistance breeding, may become susceptible to the target pests. This may undervalue the benefits of Bt in IPM approaches (Waage 1996), as it runs the risk of breakdown of resistance in the long-term.

It may be uneconomic to develop Bt-transformed crops unless we develop strategies to extend their usefulness. Wigley et al. (1994) proposed a plan in which the major elements to be considered for deploying Bt genes among crops are: (i) assess the risk of Bt resistant insects evolving and dispersing out of the crop to infest others; (ii) characterize the diversity of Bt protein binding sites in the guts of key polyphagous pests; and (iii) use the above information to deploy Bt genes among different transgenic crops.

RESISTANCE MANAGEMENT Resistant management strategies require detailed knowledge of the toxins' mode of action and genetic response of resistant insects. Unfortunately, insects show great variability in their genetic responses to Bt toxins. Schnepf et al. (1998) emphasized that laboratory selection experiments may give rise to very different outcomes from field situations. However, several resistance management strategies have been proposed to delay adaptation to Bt-transgenic crops by pest populations (McGaughey & Whalon 1992, Raymond et al. 1991, Tabashnik 1994). The most promising with currently available technology is the use of refuges of non-transgenic crops, augmented wherever possible, with high toxin expression in the plants and avoiding mosaics of different toxins and pesticides (Roush 1997a).

THE REFUGE STRATEGY The primary strategy for delaying insect resistance to transgenic crops under large monocultures is to provide refuges of non-Bt crop plants that serve to maintain Bt-susceptible insects in the population. This potentially delays the development of insect resistance to Bt crops by providing susceptible insects for mating with resistant insects (Liu et al. 1999).

The refuge strategy is expected to work if resistance to Bt is inherited as a recessive trait. The basic goals of the mixture strategy are two fold: (i) reduce the difference in fitness between susceptible and resistant insects, and (ii) reduce the degree to which a resistant insect can pass on its phenotypic trait to its offspring. Refuges can consist of fields planted with non-Bt plants or of non-Bt plants within the Bt plants. The large numbers of susceptible insects that survive on the refuge plants are then available to mate with the small number of resistant insects that survive on the Bt plants. The offspring of susceptible (SS) x resistant (RR) matings will be RS, and therefore, will not survive when they feed on high dose Bt plants.

The US Environmental Protection Agency (EPA), which regulates transgenic pesticidal crops, believes that scientifically sound long-term insect resistance management (IRM) strategies are essential to the protection of Bt microbial pesticides, transgenics, and reduction in the risks from the use of pesticides. The EPA has imposed mandatory IRM requirements for Bt cotton. Two structured refuge requirements have been imposed: 4% unsprayed or 20% sprayed crops (Matten 2000), and the refuge fields must be within 0.8 km of their Bt fields (EPA/ USDA 1999). Obviously,

enforcing a similar system for small holding farmers will not be possible in most parts of Asia. In a typical village in Asia, it is unlikely that all farmers will plant Bt crops on all their land, and farmers grow several diverse crops, which serve as hosts for *H. armigera*. In such a scenario, it may not be necessary to enforce the cultivation of refuge crops (Sharma and Ortiz 2001). Bt genes will be one of many factors that the farmers will consider when choosing which varieties to grow. The governments can promote the maintenance of refuges by restricting the number and diversity of Bt cultivars that can be released. For example, in the Indian state of Punjab, rice farmers grow traditional Basmati varieties and modern semi-dwarf varieties. Stem borer damage is higher in basmati varieties, and thus the government could authorize the release of Bt-transformed basmati varieties, but not Bt-transformed semi-dwarf varieties (Cohen 2000).

Although Bt cotton that produces Cry1Ac toxin has been effective against pink bollworm (Patin et al. 1999, Tabashnik et al. 2000b), the slower development of resistant larvae on Bt cotton as compared to susceptible larvae on non-Bt cotton could reduce the probability of mating between susceptible and resistant insects, and this asynchrony could reduce the expected benefits of the refuge strategy (Liu et al. 1999, Liu et al. 2001, Storer et al. 2001). Though there was slow larval growth, the corn borer larvae were successful in completing development on transgenic corn plants, causing similar amounts of damage as on non-Bt plants (Storer et al. 2001). Each insect/Bt crop system may have unique management requirements because of the biology of the insect, but the studies have validated the need for a refuge (Shelton et al. 2000). Therefore, care must be taken to ensure that refuges, particularly those sprayed with insecticides, produce adequate numbers of susceptible insects. Models and experimental data showed that separate but adjacent refuges might be superior to other strategies for insects that can move between plants in their larval stage (Shelton et al. 2002).

A concern is often raised that insect damage in non-Bt fields will increase after introducing Bt crops. The implication is that farmers will be even less likely to grow non-Bt crops because of the increased damage, and therefore there will be even fewer refuge fields. However, Cohen (2000) suggested that with diamondback moth on Bt collards and the European corn borer on Bt maize, many of the moths that emerge from fields of non-Bt crops would disperse and lay their eggs in Bt fields. In contrast, very few moths will emerge from Bt fields and move from Bt fields to non-Bt fields. As a consequence, insect damage in non-Bt fields may decrease if most fields are planted with Bt crops.

There is also a debate regarding the spatial design of the refuge system (separate/seed-mixture) to be adapted. Roush (1997a) pointed out that seed mixes can actually promote resistance development for insects that move from plant to plant. There is no evidence to show that moths can detect whether or not a plant contains Bt toxin. In some studies, it has been found that after the feeding begins, caterpillars move away from Bt plants faster than from non-Bt plants, but very few larvae crawl far enough to move from one field to another. Ramachandran et al. (1998) found that *P. xylostella* larvae move away from transgenic canola plants within 24 hours. Similarly, *H. virescens* and *H. zea* larvae are known to move between plants, so seed mixtures might not work. For endophytic insects such as *P. gossypiella* and other stem and root-feeding species with limited larval and adult movement, within-field refuge would be best (Gould 1998). Mallet & Porter (1992) pointed out that in seed mixture refuge system, if the pest's feeding stages could move between plants, instead of ingesting a high dose of toxin or no toxin at all, they would often consume intermediate doses nullifying the advantages of high-dose refuge. The same argument can be extended to transgenic plants with tissue specific expression of toxins. Because of the importance of maintaining appropriate refuges, insect biology and behavior should also to be considered for implementing a refuge system that is practical and economic.

Increasing the size of the refuge delays the development of resistance. Some workers have called for refuges as large as 50%, if farmers are allowed to spray them, which may present a dilemma and reduce farm profitability (Gould & Tabashnik 1998). On the other hand, farmers may be reluctant to sacrifice a large number of refuge plants to insects just to maintain susceptible alleles. In China, *H. armigera* naturally possesses a vast refuge as it can feed on corn, soybean, peanut, and many other crops. Studies that have monitored the sensitivity of *H. armigera* field populations to Bt insecticidal protein Cry1Ac from 1998 to 2000 indicated that *H. armigera* is still susceptible to Cry1Ac protein (Wu et al. 2002b). Although development of *H. armigera* on Bt cotton was much slower than on common cotton, there was a high probability of mating between populations from Bt cotton and other sources due to scattered emergence pattern of *H. armigera* adults and overlap of second and third generations. Thus, in a cotton, soybean, and peanut mix system, non-cotton crops provided a natural refuge (Wu et al. 2002a). As indicated earlier in the diverse cropping systems of the tropics (Sharma et al. 2001), where the insects have several alternative and wild hosts, there may not be any need to grow the refuge crops.

FUSION GENE STRATEGY Theoretical models suggest that pyramiding two dissimilar toxin genes in the same plant has the potential to delay the onset of resistance

much more effectively than single-toxin plants released spatially or temporally, and may require smaller refuges (Roush 1997b). Because of diversity among Bt toxins found in nature, one of the most tempting resistance management strategies is to use two or more of these toxins in mixtures, rotations, or sequences. Laboratory as well as field studies have been conducted to evaluate the efficacy of dual protein transgenic crop plants against several lepidopteran pests (Greenplate et al. 2000a, Stewart & Knighten 2000, Stewart et al. 2001). The basis for this strategy is sometimes referred to as "redundant killing" because insects adapted to one toxin may be susceptible to the second toxin. If the plants contain two Bt toxins at a high dose, insects that are able to survive on a plant with one high-dose toxin are rare, and insects that are able to survive on plants with two high-dose toxins will be very rare. If such insects are homozygous for resistance alleles for two different genes, and if the frequency of the allele for resistance to each gene is 10-3, then insects of the genotype R1R1R2R2 will occur at a frequency of only 10^-12, i.e., 1 out of 1 trillion. Because such insects will be very rare, fewer susceptible insects will be needed to ensure that resistant insects do not mate with each other. Therefore, fewer refuge fields will be necessary, although it is still very important to have some refuge fields.

Similar levels of Cry1Ac have been reported in near isogenic lines of cotton expressing either Cry1Ac alone or Cry1Ac and Cry2Ab (Greenplate et al. 2000b). Activity of single and double toxin genotypes remained greater than the conventional cottons against tobacco budworm. However, Bollgard II, with double toxin, may have greater efficacy against lepidoptera that mainly feed on reproductive structures. Increased activity of Bollgard II (Cry1Ac and Cry2Ab) may be due to increased potency of Cry2Ab, increased overall expression level of Cry2Ab, or possibly a synergistic combination of Cry1Ac and Cry2Ab.(Adamczyk et al. 2001). Dual toxin (Cry1Ac and Cry2 Ac) Bt cottons will provide substantially better control of *H. zea*, *S. frugiperda*, and *S. exigua* compared with the existing single toxin (Cry1Ac) Bt cultivars, and may not require supplemental insecticidal applications (Stewart et al. 2001). Hybrid rice plants expressing a fusion gene, Cry1Ab and Cry1Ac, under the influence of rice *actin*1 promoter are highly resistant to the larvae of both leaffolder and yellow stem borer (Tu et al. 2000). The expression level of the fusion gene (20 ng-1mg soluble protein) in the genome was sufficient to control the lepidopteran insects (the LD 50 for yellow stem borer neonate is 7.58 mg-1ml diet, whereas that for striped stemborer is 7.41 mg-1ml diet) (Attotham et al. 1994).

Serine protease inhibitors synergized Bt against four species of moths and *Leptinotarsa decemlineata* (MacIntosh et al. 1990). Lee et al. (1996) found that a combination of Cry1Ac and Cry1Aa exerted a synergistic effect on gypsy moth larvae, whereas a combination of Cry1Aa and Cry1Ab was antagonistic. Hence, while considering a pyramiding approach, an examination of whether co-expression of multiple toxin genes will have a synergistic effect needs to be undertaken. Similarly, if Bt toxin genes are to be integrated with protease inhibitor genes, protease inhibitors that do not affect the protease-mediated cleavage to release activated Bt toxin but that are still capable of inhibiting digestive process of the insect need to be engineered.

The strategy of "pyramiding," i.e., combining two toxins in a single transgenic plant will, at best, substantially reduce the size of the needed refuge and at worst, produce resistance to both toxins in the same amount of time as for a single toxin (Roush 1997b). Cross-resistance among toxins and the ability of insects to develop resistance to multiple toxins will limit the success of this approach (Roush 1998). Studies have shown that there are large differences in the cross-resistance spectrum of the insect species that have been selected for resistance using single toxins or toxin mixtures. Polygenic inheritance and the existence of multiple mechanisms of resistance may be involved in broad-spectrum resistance, and may limit the use of multiple toxin strategies for managing resistance (McGaughey 1994). Although, the independence of Cry1C resistance from Cry1A resistance in diamondback moth suggests that Cry1C and Cry1A toxins might be useful in rotations or mixtures for delaying resistance (Liu & Tabashnik 1997), the dominance of resistance can vary for a given pest from different locations. However, pyramiding of two or more insecticidal genes in the same plant is a promising long-term strategy for delaying resistance, and one which is more forgiving on refuge size. The so-called, high dose strategy, combined with the use of refuges, is widely agreed to be the best technical approach for managing resistance, and evidence is accumulating that 'separate' refuges are more effective at conserving pest susceptibility than 'mixed' refuges (Cannon 2000).

THE HIGH-DOSE APPROACH Doses of toxins that do not make life hard for susceptible individuals, either by killing them or by reducing their reproductive output, do not select for resistance. On the other hand, doses that are sufficient to kill all individuals in a population, including the most resistant genotypes, do not select for resistance either, because no one is favored by discrimination. However, slow decay of toxin residues means that there will almost certainly be a time period where discrimination works strongly in favor of resistant individuals in a population (Tabashnik & Croft 1982). Low-dose insecticide applications have been shown to create high risks of resistance

development (Georghiou & Taylor 1977) and the theoretical potential for spraying crops with extremely high doses of one or more insecticides has been discussed often (Roush 1989, Tabashnik & Croft 1982). The "high-dose refuge" strategy is the most widely used and has been implemented in North America (Alstad & Andow 1995). When an insecticide spray kills 95% of the susceptible (SS) individuals, the survival of RS individuals is likely to be significantly higher, unless the alleles governing resistance happen to be phenotypically recessive (i.e, the RS and SS insects are physiologically identical). Instead of hoping that resistance is phenotypically recessive, the high dose approach attempts to make resistance alleles "effectively recessive" even if they are not phenotypically recessive (Gould 1998). Similarly, dose that is insufficient to kill the insects bearing one copy of a major resistance allele renders resistance functionally partially dominant. Hence, the only commercially available approach to reduce the likelihood of resistance development is the use of a high dose of a single gene, producing 25 times the toxin concentration needed to kill susceptible insects in combination with a refuge.

High concentrations of Cry1Ac in bolls of transgenic cotton are essential for achieving functionally recessive inheritance of resistance (Liu et al. 2001). Further, extensive planting of transgenic corn hybrids having sub-optimal production of the toxin and resulting in only moderate effects on *H. zea* would raise concerns about the rapid evolution of resistance (Storer et al. 2001). If transgenic plants could be made to express enough toxins to overcome all homozygous resistance alleles, the crop in question would become a non-host. The lack of a "high dose" in current Bt cotton cultivars for *H. armigera* and the small scale production systems of cotton indicates that the "high dose/refuge" resistance management strategy is not feasible for Bt cotton in northern China (Zhao et al. 2000a). Under these circumstances, supplemental control of *H. armigera* with insecticides is essential to grow Bt cotton for a longer period (Ru et al. 2002). Resistance in insects to Bt can be dramatically reduced through the genetic engineering of chloroplasts in plants. Several copies of the Bt genes could be expressed per cell via the chloroplast genome as opposed to only two copies via the nuclear genome in a diploid cell. The Cry2Aa2 protoxin levels in chloroplast-transformed tobacco leaves are between 2 to 3% of total soluble protein, and are 20-to-30-fold higher than current commercial transgenic plants (Kota et al. 1999). If a toxin is consistently produced by a plant at a highly toxic concentration without having a negative effect on yield, and the toxin does not affect non-target organisms, then the constraints on high dose strategy would be quite low.

Another serious concern regarding the success of high dose strategy is that the hypothesis of resistance being recessive does not hold in different insect species. Inheritance of resistance showed incomplete dominance in *O. nubilalis* to a commercial preparation of Bt (Huang et al. 1999), and in *H. virescens* to Cry1Ab (Sims & Stone 1991). While, Tabashnik et al.(1998) demonstrated dominant resistance to Cry1Aa in a strain of *P. xylostella* having field-evolved Bt resistance.

CONTROLLED EXPRESSION of TOXINS Mono-cultivation of Bt transgenic crops is likely to select intensely for resistance because pests will be exposed to Bt even when they are not causing economic damage (Mallet & Porter 1992). The degree of yield reduction caused by a pest population is dependent on its density, as well as on when and where insects feed on the plants. Expression of toxin coding genes could be limited to vulnerable plant parts, and at times when toxicity is needed most. If a pest causes no damage when it feeds on mature leaves, but causes severe stunting when it feeds on buds and developing leaves, then toxin production only in buds would be useful. Having Bt expressed in plants so that the insect population is subjected to selection pressure for particular periods of time (e.g., through an inducible promoter) or in particular plant parts (e.g., through tissue-specific promoters) may provide larger refuges for susceptible alleles both within the field and within a region while at the same time minimizing the crop loss (Roush 1997b). This can be achieved by using gene constructs having a tissue specific promoter.

In *P. xylostella*, resistance to Bt declined when exposure to insecticide ceased (mean R = -0.19). In four other pests (*H. virescens*, *L. decemlineata*, *Musca domestica* and *P. interpunctella*), resistance to Bt declined slowly or not at all (mean R = -0.02) in the absence of exposure to Bt (Tabashnik et al. 1994). Similar loss of resistance in *O. nubilalis* was observed in the absence of selection pressure (Bolin et al. 1999). This can be exploited for formulating resistance management strategies by enforcing complete restriction on cultivation of certain Bt cultivars for a specified period.

Solutions to resistance management involve complex strategies. The track record of resistance management for chemical pesticides is not encouraging. The wisdom gained from previous pesticide failures should provide impetus for the proactive development and implementation of management strategies for transgenic crops. Keeping this in view, Cohen (2000) made four practical recommendations for promoting the sustainable use of Bt crops, based on existing knowledge of the principles of resistance management:

- Do not release Bt varieties that do not have a high dose of toxin. Toxin titers of 2 µg/g of leaf fresh weight or 0.2 % of soluble leaf protein have been shown to act as high doses against most insect pests of crops.

- Release only Bt cultivars that have two Bt toxin genes, which are not closely related to each other, and both should be expressed at a high dose.

- Do not release Bt-transformed versions of all popular crop varieties. Some popular non-Bt varieties should remain available to improve chances that some non-Bt fields (refuges) will exist.

- Implement resistance monitoring programs to serve as an early warning system for governments and farmers and provide valuable information for improved deployment of future pest-resistant cultivars.

However, the farm-level implementation of resistance management will face practical and social obstacles. A survey conducted by US maize growers has shown that in the year 2000, almost 30% of the farmers failed to comply with the refuge protocols designed to prevent or delay the emergence of insects resistant to Bt toxins (Dove 2001). Ensuring effective resistance management practices is a challenge that will require coordination from all sectors (public and private) concerned with crop protection, and will require the commitment of growers and advisers that current technology for crop protection is a precious resource vital to profitable production. There is a continuing need for interaction between ecologists, geneticists, and plant breeders in determining system-wide impacts and devising optimal ways of deploying insect-resistant crops. The current state of knowledge is not sufficient to support any single proven resistance management strategy that may be recommended as a general approach to avoid resistance to transgenic Bt plants, and demands thorough examination of the tritrophic interactions that occur between insecticidal proteins, the plant, and the insect.

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Generating Baseline Data for Insecticide Resistance Monitoring in Cotton Aphid, *Aphis gossypii* Glover

ABSTRACT The baseline susceptibility data were generated for the six commonly used insecticides viz., thiamethoxam, imidacloprid, dimethoate, methyl demeton, acephate, and monocrotophos in cotton ecosystems for the field population of *Aphis gossypii*. Populations were collected from the cotton fields of the Department of Cotton, Agricultural College and Research Institute, TNAU, Coimbatore, India. IRAC method No. 8 developed and recommended by Insecticide Resistance Action Committee (IRAC) with a slight modification was used for arriving the lethal concentrations. The base line susceptibility data were created for seven generations. The LC50 values varied from 0.3412 to 1.0414 for thiamethoxam, 0.4583 to 1.8055 for imidacloprid, 3.0096 to 10.6924 for dimethoate, 12.598 to 49.2606 for methyl demeton, 1.4615 to 5.3284 for acephate, and 1.1866 to 3.7057 for monocrotophos. The LC95 values varied from 10.8617 to 35.2153 for thiamethoxam, 17.9171 to 43.4310 for imidacloprid, 49.1667 to 629.6511 for dimethoate, 418.4538 to 1174.6270 for methyl demeton, 36.1800 to 130.4890 for acephate, and 24.9571 to 139.4943 for monocrotophos.

KEY WORDS: Insecticide resistance, *Aphis gossypii*, diagnostic doses

INTRODUCTION The importance of *Aphis gossypii* Glover as a cotton pest is increasing throughout the world (Leclant and Deguine, 1994). High aphid populations may stunt and retard cotton seedling growth and development as a result of its feeding. Late season populations can cause decreased fiber quality as the result of stickiness and the development of sooty mould associated with honeydew dropped onto cotton fibers (Isely, 1946). There has been a general decline in the effectiveness of several insecticides to control *A. gossypii*. The intensity of aphid infestations has increased over the last ten years and the use of insecticides to control aphids is questioned.

The pest problem is aggravated more rapidly due to control failures in many areas. Though control failure may be due to many factors, one of the major factors is the development of resistance to insecticides. The chief objective in resistance monitoring is to exaggerate the differences between susceptible and resistant individuals such that the frequency of misclassification is greatly reduced (Ffrench-constant and Roush, 1990). This is fulfilled by fixing the diagnostic doses.

Resistance to *A. gossypii* is in the initial stages of development and no systematic work has been done so far on monitoring of insecticide resistance in India as it has been done in *Amrasca devastans* (Distant) (Jaya Pradeepa and Regupathy, 2002), *Helicoverpa armigera* (Hub), *Plutella xylostella* (Linn.), and *Spodoptera litura* (Niranjan Kumar and Regupathy, 2001). Given the background, the present study was undertaken to determine the diagnostic doses for the commonly used insecticides in cotton for *A. gossypii*.

MATERIALS and METHODS The test insects were collected from the cotton field, Department of Cotton, Agricultural College and Research Institute, TNAU, Coimbatore, India. The population was maintained for seven continuous generations without exposure to pesticides under the laboratory conditions for generating the baseline data, i.e. fixing diagnostic doses.

The dilutions required were prepared from the commercial formulations of insecticides using distilled water. The dosages were attained after preliminary range finding studies for constructing log-concentration-probit-mortality (lcpm) lines (Regupathy and Dhamu, 2001).

The wingless adults aphids of ca 1.45mm size and weighing ca 0.19mg were taken from the culture maintained for the treatment. Each replication consisted of 10 aphids and there were three replications. Bioassays were conducted following the procedure based on the standard *Bemesia tabaci* Gennadius susceptibility test, IRAC method No.8 developed and recommended by the Insecticide Resistance Action Committee, with slight modification.

The experimental setup consisted of two disposable cups, one as an inner test chamber and the other as an outer water reservoir. The cup that served as the inner test chamber was taken and a hole was pierced in the centre of the bottom side of the cup.

The young green uncontaminated leaves were selected and the petiole was cut to a length of approximately four cm. The leaves were dipped in the concentrations for five seconds holding the leaf by the petiole with fine forceps. Care was taken to avoid the damage to the petiole. Then the leaves were left for

drying in the open air by placing the leaves on the filter paper (approximately five minutes). The petiole of the leaf was passed through the inner cup and the wingless aphids were released into the inner cup at the rate of 10 aphids per cup and the cup was covered with muslin cloth tightened with rubber band. A small amount of water was placed in a second cup and the test cup was placed inside that, so that it was supported by the protruding petiole. Observations on mortality of aphids were recorded after 48 h. The results were expressed as percentage mortality.

RESULTS and DISCUSSION The LC50 values varied from 0.3412 to 1.0414 for thiamethoxam, 0.4583 to 1.8055 for imidacloprid, 3.0096 to 10.6924 for dimethoate, 12.598 to 49.2606 for methyl demeton, 1.4615 to 5.3284 for acephate, and 1.1866 to 3.7057 for monocrotophos. Thiamethoxam was the most toxic pesticide. The acute toxicity of other insecticides based on LC50 was in the order of imidacloprid > monocrotophos > acephate > dimethoate > methyl demeton for all the seven generations tested.

The LC95 values varied from 10.8617 to 35.2153 for thiamethoxam, 17.9171 to 43.4310 for imidacloprid, 49.1667 to 629.6511 for dimethoate, 418.4538 to 1174.6270 for methyl demeton, 36.1800 to 130.4890 for acephate, and 24.9571 to 139.4943 for monocrotophos. The acute toxicity was in the order of thiamethoxam > imidacloprid > acephate > monocrotophos > dimethoate > methyl demeton for F1 and F2 generations, and thiamethoxam > imidacloprid > monocrotophos > acephate > dimethoate > methyl demeton for rest of the generations tested.

The susceptibility was gradually increased with the succeeding generation, which is evident from the decline in LC50 and LC95 values to all the insecticides tested. The extent of increase was greater for methyl demeton and dimethoate respectively. The susceptibility baseline data are not generated so far for these insecticides taken up for the study. Hence, the LC95s of the insecticides were considered as discriminating doses for monitoring the field populations for their resistance to these insecticides. From the acute toxicity studies conducted in our laboratory, the discriminating doses (ppm) fixed were 10 for thiamethoxam, 20 for imidacloprid, 50 for dimethoate, 400 for methyl demeton, 40 for acephate, and 20 for monocrotophos.

Based on the slope function and increased susceptibility, the discriminating dose screen was fixed for monitoring the level of insecticide resistance in future.

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Table 1. Susceptibility of *Aphis gossypii* to different insecticides.

Insecticide	Generation	Regression Equation	x^2	LC_{50} (ppm)	Fiducial Limits		LC_{95} (ppm)	Fiducial Limits	
					LL	UL		LL	UL
Thiamethoxam	1	Y = 1.7539 + 1.0757 x	0.4854	1.0414	0.6488	1.6715	35.2153	21.9405	56.522
	7	Y = 2.2275 + 1.0945 x	0.3026	0.3412	0.2159	0.5393	10.8617	6.873	17.1653
Imidacloprid	1	Y = 1.1220 + 1.1908 x	0.7636	1.8055	1.1698	2.7874	43.431	28.1449	67.0656
	7	Y = 2.2506 + 1.0332 x	0.8712	0.4583	0.2847	0.7379	17.9171	11.1285	28.847
Dimethoate	1	Y = 1.2557 + 0.9293 x	0.0833	10.6924	6.3045	18.1342	629.6511	371.2309	1067.802
	7	Y = 0.2836 + 1.3559 x	0.2866	3.0096	1.9986	4.532	49.1667	32.6499	74.039
Methyl demeton	1	Y = -0.6041 + 1.1943 x	0.3876	49.2606	32.2107	75.3529	1174.627	767.8917	1796.801
	7	Y = 0.0544 +1.2062 x	0.2635	12.598	7.8723	20.1589	418.4538	181.9038	465.8071
Acephate	1	Y = 0.5868 + 1.1843 x	0.3597	5.3284	3.4823	8.1533	130.489	85.2779	199.6692
	7	Y = 1.2647 + 1.1803 x	0.0085	1.4615	0.9166	2.3304	36.18	22.6902	57.6899
Monocrotophos	1	Y = 1.2744 + 1.0439 x	0.2594	3.7057	2.3047	5.9585	139.4943	86.7551	224.2944
	7	Y =1.1774 + 1.2434 x	0.389	1.1866	0.753	1.8699	24.9571	15.8373	39.3285

Baseline Susceptibility and Quantification of Resistance in *Plutella xylostella* (L.) to Spinosad

ABSTRACT Baseline susceptibility to spinosad in a *P. xylostella* population was determined by topical bioassay. Significant variations in the LC50 values ranged from 0.000250 in the 5th generation to 0.000299 in the 1st generation. The resistance ratio for the 7th generation as compared to the 1st generation

was 0.90, which indicated that resistance to spinosad did not develop in *P. xylostella* after continuous selection at least up to the 7th generation. Cross-resistance to commonly used insecticides was also studied. The toxicity of commonly used insecticides was also calculated against spinosad-selected *P. xylostella*. Comparisons of LC50 values indicated that monocrotophos (1.21278%) was the least toxic of all the insecticides whereas cypermethrin (0.0276%) was found to be the most toxic. The relative toxicity of cypermethrin, dichlorvos, malathion, endosulfan, and carbaryl reveal that these insecticides were 43.94, 20.80, 5.85, 4.24, and 4.17 times more toxic than monocrotophos.

INTRODUCTION The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae) is one of the major constraints in the profitable cultivation of cole crops. The pest occurs in endemic form with high population densities on early and late sown cauliflower. In case of severe infestation, the growing hearts are also damaged, affecting the production of marketable curds. The control of *P. xylostella* has depended primarily and extensively on the use of insecticides recommended for the last over forty years (Syed, 1992). However, the promiscuous use of a number of commercial insecticides lead to the development of resistance in this pest in most countries of Southeast Asia (Georghiou, 1981). Many factors like pronounced cultivation of early and late varieties of cauliflower, intensive use of conventional insecticides, and prospects of the higher value of the crop during off-season have been outlined for its extraordinary propensity to develop resistance to all classes of compounds. By and large one of the countermeasures suggested to combat the menace of resistance is the introduction of, and switching over to, newer, ecofriendly, and more potent insecticides.

Spinosad is a new class of polyketide-derived macrolide effective against a broad range of pests belonging to orders lepidoptera, diptera, and hymenoptera (Sparks et al., 2001). It contains a mixture of two very active principles: Spinosyn A and Spinosyn D, and is derived from a new species of soil bacterium *Saccharopolyspora spinosa* which acts both as a contact and a stomach poison. Electrophysiological evidences have demonstrated that it alters nicotinic currents in neuronal cell bodies and also disrupts the functions of GABA receptors of small neurons in the central nervous system (Salgado et al., 1997).

Keeping in view the introduction of this chemical in India and the potential of *P. xylostella* to develop resistance to all classes of compounds, a need was realized to generate base line susceptibility data and quantify resistance to spinosad as well as cross-resistance with other chemicals with the aim to detect shifts in susceptibility following its commercial use and to formulate future strategies to manage this pest.

MATERIALS and METHODS

Insect Rearing: The culture of *P. xylostella* was initiated by collecting about 200 larvae from farmers' cauliflower fields and brought to the laboratory for further multiplication at a regularly maintained temperature of 24±20C. The larvae were reared on cauliflower leaves till pupation. The adult moths were allowed to lay eggs in oven-dried glass jars having cauliflower leaves to serve as substrate for oviposition. They were provided with 10% honey solution fortified with multivitamins for feeding on a cotton swab. The neonates were provided with fresh cauliflower leaves to feed upon. At every successive instar, the larvae were shifted to clean jars containing fresh leaves. The whole stock was divided into two lots. One lot was named as parental stock and the other was used for exposure to spinosad.

Preparation of Insecticidal Concentration: The proprietary products of all the insecticides were used to prepare one percent stock solution in acetone from which further dilutions were prepared subsequently.

Bioassay and Laboratory Selection: FAO method No.21 (Busvine, 1980) for topical application of insecticide to the larvae with slight modification was followed. Instead of directly treating the larvae without any substrate, the larvae released on cut discs of cauliflower leaves of the size of a petri dish (2.5 cm diameter) were treated to simulate the application of insecticide in the field. To facilitate the movement of the larvae on both sides of the leaf disc, the leaves bearing slightly thick midribs and veins were selected for cutting the leaf discs. Before spraying, ten 3rd instar larvae were released on the upper side of the leaf disc as one replication. 1mL each of the insecticidal concentrations were sprayed on each side of the leaf disc with Potter's tower at 5lb/inch^2 pressure. All three replications were maintained for each concentration. After the treatment the petri dishes were shifted to BOD at 24±10C with 60-70% relative humidity. Larval mortality was recorded after every 72 hours of the exposure by counting the larvae as dead when they did not resume activity after repeated proddings. The survivals from the experiments, affording around 85 percent mortality, were reared to the next generation. The progeny of the first surviving lot was termed the F1 generation and the exposures and selections were conducted up to 7 generations. The parental strain was also maintained through without exposure.

Statistical Analysis: Data on mortality was subjected to Abbott's formula for correction wherever required

(Abbott, 1925). LC50 values of all insecticides were determined by Probit analysis (Finney, 1971).

Quantification of Insecticidal Resistance: The degree of development of resistance through different generations was determined by working out LC50 values in each generation and thus computing the resistance ratio by dividing the LC50 value for that generation with LC50 value of the F1.

Cross-resistance with Other Insecticides: The studies on cross-resistance of spinosad-selected strains of *P. xylostella* to commonly used insecticides viz. monocrotophos, malathion, endosulfan dichlorvos, cypermethrin, and carbaryl were also made as per the procedure described.

RESULTS and DISCUSSION Selection pressure of spinosad and responses produced in different generations has been presented in Table 1. Significant variations in the LC50 values of all seven generations indicate that there was no consistency in the toxicity of spinosad to *P. xylostella* larvae. The LC50 in the 2nd generation (0.000299%) decreased slightly compared to the 1st generation with a further decrease in the 3rd, 4th, and 5th generations. Thereafter, in the 6th and 7th generations, it increased slightly but never came at par with the F1. Thus, the decrease in LC50 values in the subsequent generations showed slightly increased susceptibility. The resistance ratios also showed a similar trend. The resistance ratio for the 7th generation as compared to the 1st generation was 0.90, which indicated that resistance to spinosad did not develop in *P. xylostella* after continuous selection at least up to 7 generations. From this, it can be construed that spinosad can be used commercially as an alternative to particularly those insecticides against which *P. xylostella* has developed resistance. As per the available literature, such studies have not been undertaken on the development of resistance to spinosad in *P. xylostella*. However, the bioefficacy of spinosad to 10 different strains of *Pseudoplusia includens* (Walker) revealed that LC50 values varied from 4.19 to 13.46 ppm (Mascarenhas and Boethal, 1997). The results of topical bioassays conducted by Sparks et al. (1998) with spinosyn A and D against *Heliothis virescens* larvae showed LC50 values ranging between 1.28 to 2.56 µg/g.

Table 1. Toxicity of Spinosad to third instar larvae of *Plutella xylostella* in different generations.

Generation	Heterogeneity X2 (n-2)	Regression Equation	Slope± S.E.	Fiducial Limits	LC_{50} (%)	Resistance Ratio
I	$X^2_{(4)}$=3.040	Y=2.13+1.16x	1.16± 0.03	0.000251 0.000355	0.000299	1
II	$X^2_{(5)}$=0.664	Y=1.58+1.38x	1.38± 0.04	0.000243 0.000365	0.000297	0.99
III	$X^2_{(5)}$=0.374	Y=2.06+1.20x	1.20± 0.03	0.000237 0.000328	0.000279	0.93
IV	$X^2_{(6)}$=5.796	Y=2.13+1.18x	1.18± 0.03	0.000223 0.000314	0.000265	0.88
V	$X^2_{(5)}$=0.465	Y=2.10+1.20x	1.20± 0.03	0.000211 0.000297	0.00025	0.83
VI	$X^2_{(5)}$=2.232	Y=2.03+1.22x	1.22± 0.05	0.000201 0.000339	0.000267	0.87
VII	$X^2_{(4)}$=0.166	Y=1.67+1.36x	1.36± 0.07	0.000191 0.000382	0.00027	0.9

Table 2. Cross resistance of spinosad-selected strain of *Plutella xylostella* to different insecticides.

Insecticide	Heterogeneity X2 (n-2)	Regression Equation	Slope± S.E.	Fiducial Limits	LC_{50} (%)	Resistance of SS over PS
Monocrotophos(SS)	$X^2_{(6)}$=1.380	Y=2.55+0.48x	0.48± 0.33	0.277-5.381	1.212	0.89
Monocrotophos(PS)	$X^2_{(5)}$=0.544	Y=3.17+0.44x	0.44± 0.34	0.580-6.580	1.37	
Malathion (SS)	$X^2_{(6)}$=0.805	Y=2.51+0.57x	0.57± 0.21	0.077-0.553	0.207	0.93
Malathion (PS)	$X^2_{(5)}$=0.556	Y=3.12+0.56x	0.56± 0.22	0.079-0.627	0.223	
Endosulfan (SS)	$X^2_{(6)}$=0.736	Y=3.88+0.46x	0.46± 0.26	0.008-0.095	0.285	0.57
Endosulfan (PS)	$X^2_{(5)}$=0.384	Y=3.17+0.49x	0.49± 0.30	0.128-1.326	0.502	
Dichlorvos (SS)	$X^2_{(6)}$=0.394	Y=2.08+0.77x	0.77± 0.17	0.026-0.130	0.058	1.34
Dichlorvos (PS)	$X^2_{(6)}$=0.293	Y=2.09+0.79x	0.79± 0.17	0.019-0.094	0.043	
Cypermethrin (SS)	$X^2_{(7)}$=4.147	Y=3.07+0.56x	0.56± 0.20	0.010-0.070	0.027	1.11
Cypermethrin (PS)	$X^2_{(7)}$=0.496	Y=2.84+0.63x	0.63± 0.20	0.010-0.061	0.025	
Carbaryl (SS)	$X^2_{(7)}$=1.102	Y=2.52+0.55x	0.55± 0.60	0.018-0.545	0.29	0.9
Carbaryl (PS)	$X^2_{(7)}$=1.079	Y=2.57+0.53x	0.53± 0.22	0.118-0.879	0.322	

SS= Spinosad-selected strain
PS= Parental strain

The toxicity of commonly used insecticides was also calculated against spinosad-selected *P. xylostella* (Table 2). The comparisons of LC50 values indicated that monocrotophos (1.21278%) was the least toxic of all the insecticides used against this strain whereas cypermethrin (0.0276%) was found to be the most toxic. Data pertaining to the relative toxicity of cypermethrin, dichlorvos, malathion, endosulfan, and carbaryl revealed that these insecticides were 43.94, 20.80, 5.85, 4.24, and 4.17 times more toxic than monocrotophos. It could be concluded that spinosad-selected strains of *P. xylostella* did not show any cross resistance to these insecticides. As per literature screened, no such studies have been carried out so far on the quantification of cross resistance to the spinosad-selected strain of *P. xylostella*. Since the mode of action of spinosad is altogether different from other insecticides, the chances of any cross-resistance in this case seem to be quite dim (Salgado et al.1997).

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Baseline Susceptibility of Diamondback Moth, *Plutella xylostella* (Linn.), to New Insecticides

ABSTRACT Toxicity of three new insecticides (fipronil, indoxacarb, and diafenthiuron) was assessed against 3rd instar larvae of diamondback moth (DBM) using a leaf residue technique. LC50 values of these insecticides were observed to be very low indicating the high toxicity and potential of these compounds against multi-resistant populations of DBM. Baseline susceptibility data will be useful to monitor the response of DBM to these compounds in future for early detection of resistance.

KEY WORDS Diamondback moth, cabbage, new insecticides, bioassay, insecticide resistance, baseline susceptibility

INTRODUCTION Large scale and indiscriminate use of insecticides for the control of insect pests, necessitated by ever increasing demand for quality food and better public health has resulted in a number of problems. One of the major problems that has arisen out of the abuse of insecticides is the development of resistance in insect pests to pest control chemicals. Diamondback moth (DBM), *Plutella xylostella* (Linn.), is a ubiquitous pest wherever crucifers are grown. It is the most destructive and is a regular pest of cabbage and cauliflower. The pest is known for its propensity towards quick development of resistance (Georghiou, 1990) and there have been instances when this pest has developed resistance to a new molecule within a few years of its introduction (Hama, 1989). The pest has developed resistance to almost all the recommended insecticides belonging to major groups in many parts of the world (Talekar and Shelton, 1993) and is becoming increasingly difficult to control. In India, resistance in *P. xylostella* to different insecticides has been reported from several states like Punjab, Haryana, Uttar Pradesh, Karnatka, Tamil Naidu, and Andhra Pradesh (Mehrotra and Phokela 2000). In Punjab, DBM has developed resistance to quinalphos, fenvalerate, cypermethrin, and several other insecticides (Chawla and Joia, 1992). The problem is acute in areas where vegetables are grown extensively in a staggered manner almost throughout the year, particularly around and near big cities (Joia et al. 1996). In view of the reports of field control failure and the development of resistance in the pest, it was considered appropriate to undertake studies to assess toxicity of new molecules towards multi-resistant populations of DBM. The objective was to identify potential compounds for insecticide resistance management of the pest and to establish baseline susceptibility of DBM to these insecticides.

MATERIALS and METHODS

Test Insects: Pupae and larvae of diamondback moth were collected from infested plants in various cabbage / cauliflower growing areas of Punjab during the period from September to October. The insects were reared in the laboratory on cabbage leaves obtained from unsprayed crops. Third instar larvae of uniform size and weight from F1or subsequent generations were used for bioassay.

Insecticides: Commercial formulations of test insecticides were procured from the manufacturers directly. The products were diluted with water to obtain a range of test concentrations, usually 6 to 7 for each of the test insecticides.

Bioassay Method: The leaf residue technique (Tabashnik et al., 1987) with slight modifications was used for exposing larvae to test insecticides. Discs (5 cm diameter) of cabbage leaves were dipped in test concentrations for 5 seconds and dried for 1 hour at room temperature. Distilled water was used as control. After drying, the discs were placed in glass containers. Around thirty 3rd instar larvae were released per two discs for each concentration and the mouth of the container was secured with muslin cloth tied with

rubber band. The test larvae were allowed to feed for 48 hours on treated discs. Thereafter, larval mortality was recorded and LC 50 worked out using a computer program based on Probit Analysis (Finney, 1971).

RESULTS and DISCUSSION

Fipronil: Fipronil, 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-(trifluorosulfoxide-1,2-pyrazole), is an insecticide of recent introduction. This insecticide was evaluated in the laboratory for its toxicity towards DBM larvae. Fipronil proved to be very toxic to test larvae. The LC50 values of fipronil against six populations of DBM ranged from 0.0001 to 0.0045% (Table 1). The population from Khanna was the most susceptible, and that from Amritsar was the least susceptible to this insecticide. Low LC50 values indicate high toxicity of fipronil to multi-resistant populations of DBM. Non-exposure of the pest populations to this compound and high inherent toxicity may be the possible reasons for low LC50 values. Low LC50 values (3.17 µg/ml) of fipronil have been reported in diet bioassay with DBM larvae (Argentine et al., 2002). The compound has been reported to be effective against DBM in China (Zhao et al. 1995) and Hawaii (Mau and Gusukuma-Minuta, 1999). It has also been registered for the pest on cauliflower in Western Australia (Lancaster and Burt, 2001).

Indoxacarb: Indoxacarb, (S)-methyl-7-chloro-2,5-dihydro-2 [[(methoxycarbonyl) [4-(trifluoromethoxy) phenyl] amino] carbonyl]-indeno{1,2-e} {1,3,4}oxadiazine-4a(3H)-carboxylate, is another new insecticide, which has been specifically introduced for the control of lepidopterous insect pests. Bioassay studies with indoxacarb were also conducted against multi-resistant populations of the pest from Jagraon, Samrala, Phagwara, and Khanna and the results are given in Table 2. This insecticide was found to be extremely toxic and the LC50 values of this compound ranged from 0.00003 to 0.00007%. Very high toxicity of this insecticide against DBM larvae indicates the high potential of indoxacarb. Field trials conducted at the University of Arizona have shown it to give good control of DBM infesting cabbage (Umeda et al. 2000). It has also been reported to exhibit synergism with granulosis virus against DBM (Krishnamoorthy 2002). Mau and Gusukuma-Minuta, (2002) have reported its use in insecticide resistance management of DBM Hawaii.

Diafenthiuron: Another introduction of recent times, diafenthiuron, 3-(2,6-diisopropyl-4-phenoxyphenyl)-1-tert-butyl-thiourea, is a new type of thiourea. It is a pro-insecticide. After application, it gets converted to carbodiimide, which is an inhibitor of mitochondrial ATPase. This compound was also tested for its toxicity to DBM larvae from four different populations and the LC50 values and other probit parameters are presented in Table 3. These data show that this insecticide also possesses fairly high toxicity to resistant DBM populations but it is not as toxic as previously described fipronil and indoxacarb. The LC50 values varied from 0.0051 to 0.011%. Zhao et al. (1995) have reported diafenthiuron to be effective against DBM in China. Solang and Sribhuddachart (2002) have reported that in addition to its high toxicity to DBM, the pest did not develop resistance after selection with 25 generations.

All of these compounds have been introduced recently and are still at the evaluation stage under Indian conditions. Apart from inherent toxicity of these insecticides, non-exposure of DBM populations to these chemicals is perhaps the other reason for very low LC50 values. After further testing, these insecticides may prove as alternate control measures for DBM in problematic areas. However, to fully realize their potential and to increase useful life for long-term use in effective IRM, these and similar new generation compounds will have to be used very judiciously. There must be some mechanism to

Table 1. Toxicity of fipronil against different populations of DBM.

Population	Probit analysis parameters			
	LC50 (%)	Fiducial limits (95%)	Chi-square (x^2)	Slope (b)
Jagraon	0.0012	0.0001 – 0.0045	4.22	1.67
Kotmanji Sahib	0.00132	0.0004 – 0.0050	2.8	0.86
Amritsar	0.0045	0.0017 – 0.0082	1.65	1.71
Samrala	0.00072	0.00030 – 0.00158	0.87	0.71
Phagwara	0.00013	0.00002 – 0.00032	2.51	0.68
Khanna	0.0001	0.00002 – 0.00025	0.94	0.75

Table 2. Toxicity of indoxacarb against different populations of DBM.

Population	Probit analysis parameters			
	LC50 (%)	Fiducial limits (95%)	Chi-square (x^2)	Slope (b)
Jagraon	0.00006	0.00003 – 0.00011	2.62	1.5
Samrala	0.00006	0.00002 –0- 0.00012	3.17	0.71
Phagwara	0.00007	0.00004 – 0.00014	0.79	1.03
Khanna	0.00003	0.00001 – 0.00006	3.81	0.79

Table 3. Toxicity of daifenthiuron against different populations of DBM.

Population	Probit analysis parameters			
	LC50 (%)	Fiducial limits (95%)	Chi-square (x^2)	Slope (b)
Amritsar	0.011	0.008 – 0.014	1.6	1.8
Jalandhar	0.0072	0.005 – 0.012	1.48	0.9
Phagwara	0.0082	0.005 – 0.0132	2.03	1.5
Khanna	0.0051	0.0039 – 0.0062	1.86	0.92

regulate the use of insecticides by farmers. Education of farmers and formulation of guidelines and strict compliance can prove very useful in this direction.

Based on bioassays of these compounds with DBM populations from different locations in the state, LC50s of 0.0001%, 0.00003%, and 0.0051%, for fipronil, indoxacarb, and diafenthiuron respectively, have been worked out. The baseline susceptibility data will be very useful for monitoring of susceptibility status of DBM to these insecticides in the future and may help in early detection of resistance development in the pest to these compounds.

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Arthropod Resistance

Resurgence of Spider Mite *Tetranychus ludeni* Zacher (Acarina: Tetranychidae) Against Acaricides and Botanical Pesticides on Cowpea

ABSTRACT During the summer months, spider mite (*Tetranychus ludeni* Zacher) is a detrimental pest of vegetable crops, especially on cowpea in the Varanasi region of India. Field experiments were conducted to find out the resurgence of this mite pest against some acaricides (viz., dicofol (18.5% EC), dicofol (5%WP), abamectin (1.9%EC), phosalone (35%EC), ethion (50%EC), and sulphur (80%WP)), and botanical pesticides (viz., pongamia oil (2%), N.S.K.E. (5%), neem oil (2%), azadirachtin (0.03%), mahua oil (2%), and PSKE (5%)) at their recommended doses on cowpea crops. The chemical solutions were prepared just before each spray, and were sprayed every two weeks. The mortality of spider mite was observed at different intervals including pre-spray and 1, 3, 7, and 14 days after spray. The last observation of the previous spray was counted as the pre-spray observation of the following spray, with the rest of the observations taken similarly. The results indicated that no resurgence was observed with dicofol (18.5%EC), dicofol (5%WP), abamectin (1.9%EC), and phosalone (35%EC), whereas some resurgence was observed in ethion (50%EC) and sulphur (35%EC) despite encouraging performances. Resurgence was shown in

mahua oil (*Madhuca indica*), PSKE, pungum oil (*Pomgamia pinata*), neem oil, azadirachtin, and NSKE. The maximum resurgence was observed with mahua oil, and the maximum negative resurgence was observed with dicofol (18.5 E.C.) in both sprays. Botanical pesticides had some resurgence but could be used as safe pesticides on vegetables, while also being ecologically friendly.

KEY WORDS: Resurgence, spider mite, acaricides, botanical pesticides.

INTRODUCTION In recent years spider mite, *Tetranychus ludeni* Zacher, has been observed as a serious pest to vegetable crops, especially on cowpea, a common vegetable of summer months in India. This has attracted the attention of growers and acarologists in India's Varanasi region. Heavy populations of mites and their profuse webs cover the plants. The regular monoculture of cowpea without crop rotation has aggravated the problem. It has been reported that regular use of dimethoate (Singh et.al 1989) has also resulted in outbreaks of this mite. It had been earlier reported that phosphamidon, fluvalinate, fenvalerate, and dimethoate showed resurgence of this mite (Kumar and Singh, 1999). For the economic harvest of the cowpea crop, the current effective treatment is to use acaricides that can manage this mite pest.

MATERALS and METHODS Field experiments were conducted to find out the resurgence of this mite pest against some acaricides and botanical pesticides at their recommended doses on cowpea crops. The trials were replicated four times at vegetable grower fields during summer months in RBD. The plot size was 3x5m and row-to-row spacing was maintained at 50 cm apart. Twelve formulations of acaricides and botanical pesticides were taken in this trial (Tables 1 and 2). The control was treated with water + sandovit spray. The amount of proprietary ingredient required was calculated by using the following formula:

$$\text{Amount of acaricide and botanical pesticide} = \frac{\text{(Desired concentration) x (Amount of spray fluid required)}}{\text{Percent toxicant in formulation}}$$

The observations were taken from five randomly selected tagged and numbered plants from each plot. Five leaves were plucked from the upper, middle, and lower portions of each plant and a total number of twenty-five leaves were collected from each plot for observation. The mite population was counted on the basis of 2cm squared leaf areas at four spots per leaf. The mortality of spider mite was observed in different intervals at pre-spray and at 1, 3, 7, and 14 days after. The fourteenth day observation of the first spray was treated as the pre-spray observation of the 2nd spray, with the rest of the observations taken similarly. The percent mortality was calculated by using following formula:

$$\text{Percent mortality} = \frac{\text{Average reduction in population}}{\text{Average pre-treatment population}} \text{ X } 100$$

The corrected percent mortality was calculated through Abbot's formula (1925), which is as follows:

$$P = \frac{P_1 - C}{100 - C} \text{ X } 100$$

Where:

- P = percent corrected mortality,
- P1 = percent observed mortality
- C = percent mortality in control.

The percent resurgence of mite population was calculated by Henderson and Tilton's formula as follows:

$$\text{Resurgence (\%)} = \{ \frac{(Ts \text{ x} CF)}{Cs \text{ x } TF} - 1 \} \text{ X } 100$$

Where:

- Ts = Number of live mite in post treatment count
- TF = Number of live mite in pre treatment count
- Cs = Number of mite in untreated check (Post-treatment)
- CF = Number of mite in untreated check (Pre-treatment)

RESULTS and DISCUSSION The results indicate that some botanical pesticides showed maximum positive resurgence viz. mahua oil (+ 38.68 %), neem oil (+ 12.26 %), PSKE (+ 10.36 %), NSKE (+ 5.22 %), and pongamia oil (+ 4.68 %) (Table 1). Some acaricides viz. sulphure (+ 6.22 %), ethion (+ 3.72 %), and botanical pesticides i.e. azadirachtin (+ 4.66 %) had encouraging performances but showed some resurgence, whereas resurgence was not noticed with dicofol 18.5% EC (- 26.28 %), dicofol 5 % WP (- 14.22 %), abamectin (- 12.22 %), and phosalone (- 10.68 %) in the first spray (Table 1). In the second spray, the trend of maximum resurgence of some botanical pesticides was changed, including mahua oil (+ 88.64 %), pongamia oil (+ 32.66 %), P.S.K.E. (+ 25.00 %), neem oil (+ 18.43 %), azadirachtin (+ 14.09 %), and NSKE (+ 8.32 %) (Kumar & Singh (1998) and Rai et al. (1993) supported these findings). There was no observed change in the trend of the encouraging performance of acaricides viz., sulphur (+ 8.32 %), and ethion (+ 7.99 %) (Table 2). The same trend was followed in the second spray. No resurgence was shown with some acaricides like dicofol 18.5%EC (- 74.30 %), dicofol 5% WP (- 21.65 %), abamectin (- 18.02 %), and phosalone (- 12.75 %) (Table 2).

Zhang and Sanderson (1990) earlier reported the relative toxicity of abamectin to *Phytoseiulus persimilis* Anthias-Henriot and the spider mite

Table 1. Resurgence of spider mites *Tetranychus ludeni* Zacher against acaricides and botanical pesticides (1st spray)

S. No.	Treatments	Conc.	TSM Population after days				Mean	% Increase / Decrease
			1	3	7	14		
1	Dicofol	0.04	1.25*	7.66	15.68	24.4	12.24	-26.28
	(18.5 % EC)		(1.32)**	-2.85	-4.02	-4.98	-3.57	
2	Dicofol	0.0125	1.16	8.38	21.22	26.85	14.4	-14.22
	(5 %WP)		-1.28	-2.97	-4.66	-5.22	-3.86	
3	Ethion	0.05	1.76	15.26	23.66	24.22	16.2	3.72
	(50 % EC)		-1.5	-3.96	-4.9	-5	-4.1	
4	Sulphur	0.1	6.86	25.26	68.82	70.77	43.92	6.22
	(80 % WP)		-2.71	-5.07	-8.32	-8.44	-6.62	
5	Phosalone	0.07	8.88	23.65	54.26	72.88	39.91	-10.68
	(35 % EC)		-3.06	-4.91	-7.4	-8.56	-6.35	
6	Abamectine	0.014	0.82	2.14	3.36	5.68	3	-12.22
	(1.9 % EC)		-1.14	-1.62	-1.96	-2.48	-1.87	
7	NSKE (5 %)	5	7.48	26.73	72.84	75.88	45.73	5.22
			-2.82	-5.21	-8.6	-8.73	-6.79	
8	Azadirachtin	5	5.84	24.22	32.66	56.96	29.92	4.66
	(0.03 % EC)		-2.51	-4.97	-5.75	-7.58	-5.51	
9	Neem oil	2	4.88	18.26	38.69	54.26	29.02	12.26
			-2.31	-4.33	-6.26	-7.4	-5.43	
10	Pongamia oil	2	6.96	26.62	42.55	50.88	31.75	4.68
			-2.73	-5.2	-6.56	-7.16	-5.67	
11	Mahua oil	2	3.22	10.45	18.54	32.38	15.37	38.68
			-1.92	-3.3	-4.36	-5.72	-3.98	
12	Pongamia Seed Kernal Extract	5	4.28	17.36	28.26	52.86	25.69	10.36
			-2.18	-4.22	-5.36	-7.3	-5.11	
13	Control (Water+Sandivit)		3.48	11.26	34.84	48.65	24.55	---
			-1.99	-3.42	-5.94	-6.97	-5	

* Mean percent mortality is an average of four replications

** Figures in parenthesis are the $\sqrt{X+0.5}$ transformed value, where X= mean percent mortality

Interaction: - CD at 5%

Between period = 0.6210681

Between period & pesticides = 2.018471

Table 2. Resurgence of spider mite, *Tetranychus ludeni* Zacher against acaricides and botanical pesticides (2nd spray)

S. No.	Treatments	Concentration	TSM Population after days				Mean	% Increase / Decrease
			1	3	7	14		
1	Dicofol	0.04	0.44*	4.66	5.22	6.76	4.27	-74.3
	(18.5 % EC)		(0.96)**	-2.27	-2.39	-2.69	-2.14	
2	Dicofol	0.0125	0.86	12.26	20.65	22.68	14.11	-21.65
	(5 %WP)		-1.16	-3.57	-4.59	-4.81	-3.82	
3	Ethion	0.05	1.16	8.6	16.45	28.2	13.6	7.99
	(50 % EC)		-1.28	-3.01	-4.11	-5.35	-3.75	
4	Sulphur	0.1	7.26	27.28	71.86	82.65	47.26	8.32
	(80 % WP)		-2.78	-5.27	-8.5	-9.11	-6.91	
5	Phosalone	0.07	3.96	22.28	62.77	68.55	39.39	-12.75
	(35 % EC)		-2.11	-4.77	-7.95	-8.3	-6.31	
6	Abamectine	0.014	0.24	2.62	4.12	5.02	3	-18.02
	(1.9 % EC)		-0.86	-1.76	-2.14	-2.34	-1.87	
7	NSKE (5 %)	5	8.56	28.22	72.65	88.36	49.44	8.32
			(3.000	-5.35	-8.55	-9.42	-7.06	
8	Azadirachtin	5	4.26	25.55	60.22	70.12	40.03	14.09
	(0.03 % EC)		-2.18	-5.1	-7.79	-8.4	-6.36	
9	Neem oil	2	5.76	24.78	58.27	69.28	39.52	18.43
			-2.5	-5.02	-7.66	-8.35	-6.32	
10	Pongamia oil	2	6.77	26.86	68.75	72.77	43.78	32.66
			-2.69	-5.23	-8.32	-8.55	-6.65	
11	Mahua oil	2	2.86	18.7	40.68	65.65	31.97	88.64
			-1.83	-4.38	-6.41	-8.13	-5.69	
12	Pongamia Seed Kernal Extract	5	5.86	25.66	54.68	71.24	39.36	25
			-2.52	-5.11	-7.42	-8.46	-6.31	
13	Control (Water+Sandivit)		3.5	14.28	32.86	52.45	28.27	---
			-2	-3.84	-5.77	-7.93	-5.36	

* Mean percent mortality is an average of four replications

** Figures in parenthesis are the $\sqrt{X+0.5}$ transformed value, where X= mean percent mortality

Interaction: CD at 5%

Between period = 0.111345

Between period & pesticides = 0.361871

Tetranychus urticae Koch. At 24 hrs after treatment, two spotted spider mites survived but most were immobilized at concentrations of 1, 4, and 8 ppm; at 16 ppm, two spotted mite mortality was >70% after treatment. Survival and mobility of two spotted spider mites were significantly affected at high concentrations of 4 and 8 ppm (almost all survivors were immobilized). Singh and Singh (1992) have reported the effectiveness of dicofol against this mite. Dicofol and phosalone were statistically on par with untreated controls but superior to the rest, indicating that they did not induce any mite resurgence but rather became ineffective against the mite.

Kumar and Singh (1999) had earlier reported that the menace of spider mite, *Tetranychus urticae* Koch, has been identified as major problem of okra during summer months. In this experiment, neem-based formulations and conventional acaricides were used for the study of resurgence. The results indicated that phosphamidon, fenvalerate, and dimethoate showed resurgence of spider mites. The neem-based formulation (azadirachtin) showed encouraging performance but showed resurgence.

Kumar et al. (2001) reported that some pesticides show resurgence of two spotted mite viz., phosphamidon + 128.51% and dimethoate + 118.18%, while azadirachtin shows encouraging performances + 14.89 % resurgence. Resurgence was not noticed by acaricides viz., dicofol - 67.78 % and phosalone - 16.06 %.

Kumar et al. (2002) reported that no resurgence was observed with dicofol (EC), dicofol (WP), abamectin, and phosalone, whereas encouraging performance but some resurgence was noticed with ethion (50%EC) and sulphur (80%WP). However, resurgence in mahua oil, PSKE, pungum oil, neem oil, azadirachtin, and NSKE did not show any effectiveness against this pest.

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Insecticide Usage Patterns in South Indian Cotton Ecosystems to Control Cotton Bollworm, *Helicoverpa armigera*

Cotton, as an important commercial crop of India, has boosted the economic conditions of farmers especially when introduced under irrigation. The traditional cotton ecosystem of South India is spread over Maharastra, Andhra Pradesh, Karnataka, and Tamil Nadu where the well-known block cotton soils have prevailed, both under assured irrigation and rainfed situations. A complex of insect pests in India has damaged cotton and pulse crops. The cotton bollworm, *Helicoverpa armigera*, has been a major pest with severe economic consequences and has inflicted huge crop losses ranging from 47-90%. As a result, cotton crops grown over only 5% of the total cultivated area have consumed more than 55% of the total amount of pesticides used in India (1). The cotton bollworm has attained the status of a national pest owing to its devastative nature on cotton and other crops in India and elsewhere. Frequent outbreaks of *Helicoverpa armigera* in India on cotton crops have lead to severe social disturbances, with several reports of suicide by farmers (2). To combat the unprecedented *H. armigera* pressure, farmers in the region have applied synthetic pyrethroids or organophosphate insecticides - sometimes as mixtures at 2-3 day intervals - during critical periods, resulting in over 30 sprays (against the recommended 8-10 sprays). This has led to the development of high levels of insecticide resistance in the cotton ecosystem (1). During 1992-1997, crop failure in many states of the South Indian cotton ecosystem, particularly Andhra Pradesh and Karnataka, was followed by the suicide of several

farmers, which has been traced to insecticide resistance in *H. armigera* (3).

The consumption pattern of different insecticides belonging to different groups varies across the geographic locations primarily based on the dealer recommendations, intensity of pests and diseases, influence of peer groups, efficacy of particular insecticides, knowledge level of the farmer, availability of a particular insecticide, and socioeconomic conditions of the farmer (4). Though a number of studies were conducted on knowledge and pesticide use, the changing scenario warrants more studies. In view of this, to determine the relative predominance of individual insecticide molecules and their relative usage over the South Indian cotton ecosystem, a survey was undertaken during the 2000-2001 cropping season.

We selected four South Indian states viz., Maharastra, Andhra Pradesh, Karnataka, and Tamil Nadu, which comprise more than 95 percent of the cotton cultivation in South India. Looking into the distribution of cotton cultivation, three sampling locations in each state were selected to collect the data. The locations selected included: Nagpur, Parbhani Nanded (Maharastra), Guntur, Madhira, Kovilpatti (Andhra Pradesh), Raichur, Dharwad, Mysore (Karnataka), Coimbatore, Madurai, and Kovilpatti (Tamil Nadu). During the cropping season, each location was visited and interacted with by at least 25 farmers with a schedule on various aspects of insect pest control including insecticide usage pattern. Aspects concerning insecticides being used, dosage per application, number of insecticide applications per crop, number of times a particular insecticide was used, and the relative efficacy in farmers' perception etc., were collected. Wherever possible, fields were visited and actual prevalence of cotton bollworm was studied. Later, information on number of insecticides, relative usage in terms of number of sprays, concentration, farmer perception, and attitude, etc., were computed for each location and overall cotton ecosystem of South India.

Overall, in the South Indian cotton ecosystem, as many as 15 different insecticides: monocrotophos, quinolphos, chlorpyriphos, cypermethrin, decamethrin, acephate, endosulfan, fenvelarate, polytrin, sumicidine, carbaryl, permethrin, avaunt, *Bacillus thurengenesis* (Bt), and spinosad were used with the specific objective of controlling cotton bollworm. Among chemical insecticides, monocrotophos was the most extensively used insecticide with a share of 26.8 percent of all the insecticides, followed by chlorpyriphos (19.9%), quinolphos (18.8%), cypermethrin (14.93%), and endosulfan (12.53%) (Figure 1). Bt was the only biological agent encountered in the whole ecosystem and accounted for 0.76% overall. However, it was used only in the Raichur region of Karnataka state where it forms 9.09% of all the insecticides used in the region. Similarly, spinosad (a recent chemical in South India which is not yet recommended and commercially available in market), permethrin, and polytrin comprised 0.36%, 0.46%, and 0.76% respectively overall in the ecosystem but were used only in Coimbatore (4.34%), Nalagonda (5.55%), and Nanded (9.09%) respectively (Table 1). The number of insecticides being used to control bollworm varied across locations in South India. A maximum of 8 insecticides including 1biological agent were recorded in the Raichur region followed by 7 in Nalagonda, Coimbatore, and Kovilpatti. Farmers in the Mysore region used 4 different insecticides, which was the least number in the overall ecosystem. Raichur is known historically for being the cotton city of India and for its high intensity use of insecticides in Asia. Apart from using the maximum number of insecticides, Raichur recorded up to 25 sprays to control the bollworm in the present study. Most of the cotton regions of northern states of South India used between 18-20 sprays, while Dharwad and Mysore used fewer (8-12 and 10-12) numbers of applications of insecticides in the region (Table 1). In all of the locations the usage of insecticides was erratic and indiscriminate. Overall, 60-75% of the farmers applied the insecticides as mixture of 3 to 6 in an interval of 2-3 days during the critical period. Armes et al (5) reported similar insecticide usage patterns in Karnataka and Andhra Pradesh for the control of *H. armigera*. Generally, only the first two sprays were not mixtures of insecticides, and monocrotophos was always used throughout South India without exception. However, only 21.23% of cotton farmers of the Raichur region used the insecticides as a mixture. It was interesting to note that 78.77% of the farmers in this area used a definite schedule of insecticides for the control of cotton bollworm. Most of the farmers in this region rotated the insecticides. Further, this was only region where

Table 1. Number of insecticides and applications for the control of cotton bollworm in South India.

Location	# of Insecticides Used	# of Applications
Nagpur	5	18-20
Parbhani	5	18-20
Nanded	6	18-20
Guntur	6	18-20
Nalgonda	7	18-20
Madhira	6	16-18
Raichur	8	20-25
Dharwad	5	8-12
Mysore	4	10-12
Coimbatore	7	12-15
Madurai	6	10-12
Kovilpatti	7	15-16

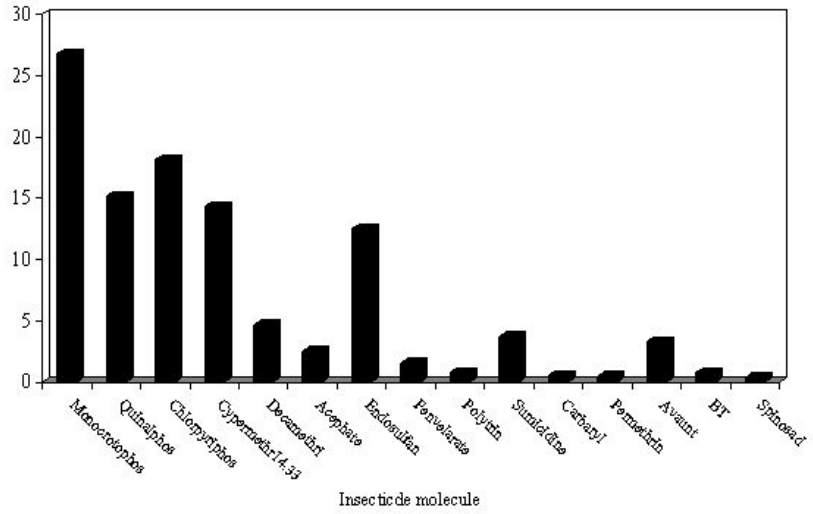

Figure 1. Percent prevalence of different insecticides in South Indian cotton ecosystems.

credit. The dealers profited from using the illiteracy of the farmers, and the lack of a good extension services in many of the remote rural areas also aggravated the problem. Prolonged use of the same insecticides will definitely elevate the problem of insecticide resistance, as had happened in Andhra Pradesh and Karnataka during late 1980s and early 1990s (1).

Identification of insecticide usage patterns allows for the rotation of insecticides and the rational use of pesticides for the better management of the pest and for insecticide resistance (4, 7). This study brings to light that farmer knowledge about insecticide usage is very poor and as such, insecticides are being used indiscriminately by the

Table 1. Relative usage (%) of different insecticides in Indian cotton ecosystems.

	Nagpur	Parbhani	Nanded	Guntur	Nalgonda	Madhira	Raichur	Dharwad	Mysore	Coimbatore	Madurai	Kovilpatti
Monocrotophos	34.78	27.27	36.36	37.50	5.55	23.80	36.36	30.76	30.77	21.73	18.18	19.00
Quinalphos	21.73	18.18	-	6.25	5.55	23.80	9.09	15.38	30.77	21.73	22.72	6.25
Chlorpyriphos	4.34	-	-	-	16.67	14.28	9.09	-	15.38	13.04	18.18	6.25
Cypermethrin	-	18.18	9.09	12.50	-	23.80	9.09	30.76	-	21.73	22.72	31.25
Decamethrin	4.34	-	-	-	16.67	4.76	-	-	-	8.69	9.09	12.50
Acephate	-	9.09	-	-	5.55	-	-	-	-	-	-	15.50
Endosulfan	30.40	18.18	18.18	25.00	11.11	-	9.09	15.38	23.07	-	-	-
Fenvelarate	-	-	9.09	-	-	-	9.09	-	-	-	-	-
Polytrin	-	-	9.09	-	-	-	-	-	-	-	-	-
Sumicidine	-	-	18.18	12.50	-	-	-	7.70	-	-	-	6.25
Carbaryl	-	-	-	6.25	-	-	-	-	-	-	-	-
Permethrin	-	-	-	-	5.55	-	-	-	-	-	-	-
Avaunt	-	-	-	-	-	9.52	9.09	-	-	8.69	9.09	-
Bt	-	-	-	-	-	-	9.09	-	-	-	-	-
Spinosad	-	-	-	-	-	-	-	-	-	4.34	-	-

farmers were aware of and used Bt for the control of bollworm on cotton.

Monocrotophos was the single most common insecticide used in all of the locations of South India for the control of cotton bollworm. In all of the regions except Kovilpatti and Nalagonda, monocrotophos and quinolphos were the primary choices, by more than 30 percent, of insecticides for use in controlling bollworm. Both the relative prevalence and the use of insecticides varied across the geographical locations of South India (Table 2).

It was very clear from the survey that the majority of the farmers were greatly influenced by the dealers. Beside the fact that the pesticide dealers had such an impact on the pesticide use pattern among farmers, the farmers tended to be more loyal to those dealers who also provided technical advice in all aspects of plant protection. These results were in line with those of Rakila and Padmanaban (6). The main reason for this dependence appeared to be that most farmers were economically poor and depended on the dealers for farmers of South Indian cotton ecosystems. The exceptions are the regions of Raichur and a few regions of Andhra Pradesh, which suffered severe outbreaks in the past. Perhaps the extensive extension efforts with respect to insecticide usage in these regions are responsible for the better knowledge of the farmers (7). In order to rationalize the pesticide use on the farms, it is imperative to stress the importance of economic threshold levels in the application of pesticides and to follow the integrated pest management practices to bring down the expenditure and to increase the effectiveness of plant protection measures in cotton. Further, the outcome of the survey clearly indicates the need for genetic investigations of the geographic populations of bollworm and the formulation of population specific integrated pest management (IPM) modules. Based on the genetic similarity and the insecticide composition patter by different geographic populations, we need to force the rotation of modules for the better management of cotton bollworm and insecticide resistance. Concomitantly, there is a greater

need for educating the farmers about the pests, insecticides, and their uses to avoid indiscriminate usage and to prevent a chain of problems that effect nature and human health.

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Recent Advances in Host Plant Resistance to Whiteflies in Cassava

INTRODUCTION Whiteflies are considered one of the world's major agricultural pest groups, attacking a wide range of plant hosts and causing considerable crop loss. There are nearly 1200 whitefly species with a host range that includes legumes, vegetables, fruit trees, ornamentals, and root crops. As direct feeding pests and virus vectors, whiteflies cause major damage in agroecosystems based on cassava (Euphorbiaceae; *Manihot esculenta* Crantz) in the Americas, Africa, and to a lesser extent Asia. The most damaging species on cassava in northern South America is *Aleurotrachelus socialis*. Typical damage symptoms include curling of apical leaves, yellowing and necrosis of basal leaves, and plant retardation (Fig. 1). Adult whiteflies are most frequently observed on the underside of apical leaves where they feed on plant fluids and oviposit. The "honeydew" excreted is a substrate for a sooty-mold fungus that interferes with photosynthesis (Fig. 1C). The combination of direct feeding and impaired photosynthetic rate reduces root yield by 4% to 79% depending on the duration of attack (Bellotti, 2002).

More than 5,000 cassava genotypes have been

Fig.1. **A**: Nymphal Stages of *A. socialis*, on a cassava leaf. **B**: Leaf curling an a cassava plant with high populations of *A. socialis*. **C**: Presence of sooty mold fungus on a cassava leaves attacked by *A. socialis*. **D**: Resistant genotype MEcu-72 and a susceptible genotype.

evaluated at CIAT and CORPOICA for whitefly resistance. At present, the major source of host resistance in cassava is the genotype MEcu-72 (Bellotti and Arias, 2001) (Fig. 1D). When feeding on MEcu-72 *A. socialis* had less oviposition, longer development periods, reduced size, and higher mortality than when feeding on the susceptible genotype (Fig. 2). Due to the importance of whiteflies as a pest and virus vector, it is important to understand the nature of genes that confer resistance in the resistant genotype, MEcu-72. To study the genetics of this resistance, a cross was made between MEcu-72 (resistance genotype) x MCol-2246 (a very susceptible genotype), to evaluate F1 segregation, using molecular markers. This will accelerate the selection of whitefly resistant germplasm and isolate resistant genes.

MATERIALS and METHODS

Plant Material: For the present work we have used the cross MEcu-72 (as the resistant parent) x MCol-2246 (as the susceptible parent). A total F1 offspring of 286 genotypes (family CM8996) was produced from this cross. These materials were sowed and evaluated in the field during May 2001, March and August 2002 at two different locations: Espinal-Tolima, Colombia (CORPOICA-NATAIMA) at 350 m.a.s.l. and Santander de Quilichao, Cauca, Colombia, at 990 m.a.s.l. With this evaluation we will identify gene segregation in the offspring and we will be able to select the resistant and susceptible materials. The evaluation was performed in the field using population and damage scales ranging between 1-6, where 1 is an absence of damage and population and 6 is high population and damage (curling, chlorosis, sooty mold fungus, etc.) (Table 1). Three evaluations were performed in 2002 using the highest damage and population data for information processing.

Molecular Analysis: We are using Simple Sequences Repeat (SSR) to find markers associated with resistance for mapping the resistant gene(s). As part of a collaborative project with Clemson University funded by USAID a BAC library for cassava using the clone MEcu 72 was constructed. The library contains 73,728 clones with an average insert size of 93 kb. Based on a genome size of 760 Mb, library coverage is approximately 10 haploid genome equivalents. The whitefly resistance will be target for map-based cloning using the BAC libraries as tools. We are using silver staining to visualize the allelic segregation of the markers.

RESULTS

Field Evaluation: The whitefly resistant variety CG 489-31 (Fig. 2), a progeny from the MEcu 72 x MBra 12 cross, has been released to cassava farmers by CORPOICA, Colombia under the name Nataima-31 (Fig. 3).

Initial field evaluations showed that these materials (family CM 8996) had low levels of the pest, because test plants (materials very susceptible to *A. socialis*) did not present high levels of damage and populations (scale of 4 to 6 Table 1). The harvest evaluation showed that the root yield was between 4.5 and 86.5 ton/ha, and many materials presented desirable characteristics (high percentage of dry matter, palatability, etc.). Currently, the family is under a second sowing cycle at the same locality from Tolima, and high pressure exerted by the pest has been detected since test materials have high degrees of damage (from 4 to 5).

Preliminary evaluations have demonstrated that some materials from the family present low levels of damage and population (up to 2) (Fig. 4 and 5).

Molecular Analysis: Both parents MEcu-72 and MCol-2246 were evaluated with 343 cassava SSR markers (Mba et al, 2001) including 156 cDNA SSRs developed (Mba et al, by submitted). Approximately 155 of the SSRs were polymorphic in the parentals and

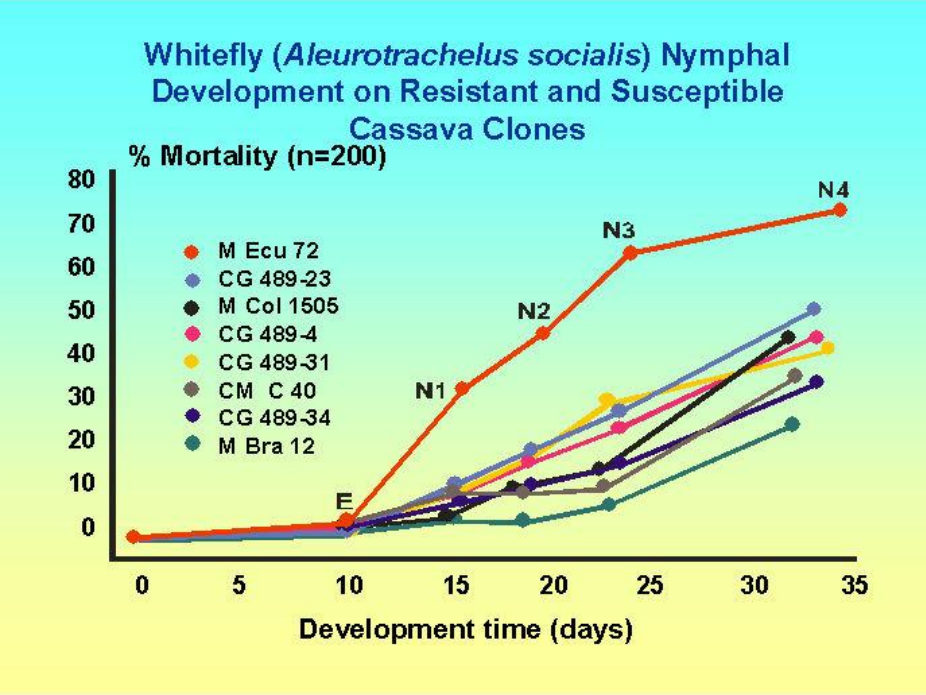

Fig. 2. A: Whitefly (*A. socialis*) nymphal mortality on resistant (R), tolerant (T) and susceptible (S) cassava clones.

were evaluated in the F1 (Fig. 6).

Association between Molecular Markers and Resistance: The molecular data are being analyzed using QTL packages (QTL cartographer Qgene) to determine linkages between the markers and the phenotypic characterization. As preliminary analysis X 2 at the 5% level was done using SAS. Putative associations were found between 43 SSRs markers and the field phenotypic characterization (score 1.0 to 2.0 of the levels of damage and populations).

CONCLUSIONS and ONGOING WORK

- The field evaluations in the family CM 8996 and their parental show the resistance of the genotype MEcu-72 and the high susceptibility of the parental MCol-2246.
- Using SSR markers, putative association with the resistant lines was found. A linkage map is being constructed using the SSR data and the field phenotypic characterization.
- Based on going QTL analysis, the marker linked to the resistant gene(s) will be used as part of large scale screening of breeding lines and to accelerate the breeding cycle for whiteflies resistance. Fine mapping of the genes involved will be carried as a first step toward the cloning of the resistant genes and the study of their expression.

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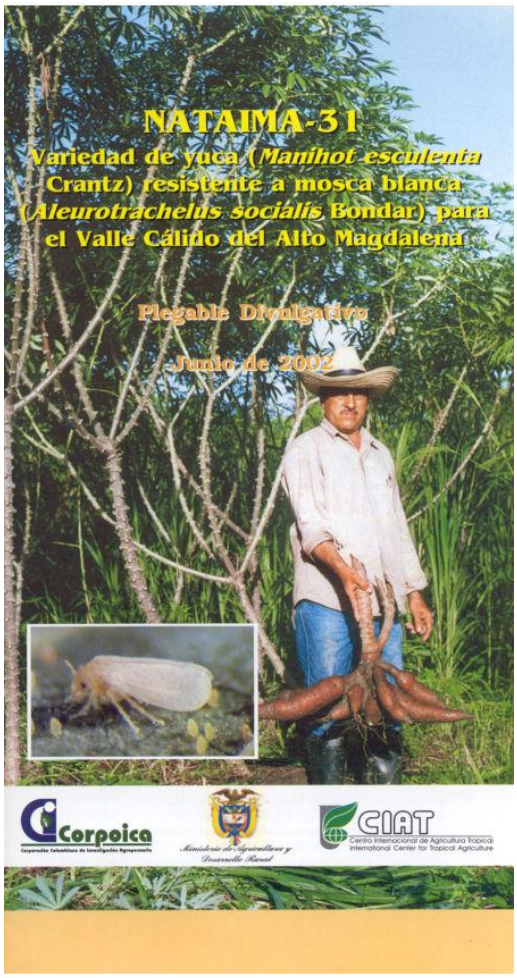

Fig. 3 CORPOICA (MADR) pamphlet announcing the official release of the cassava variety "Nataima-31," developed for resistance to whiteflies.

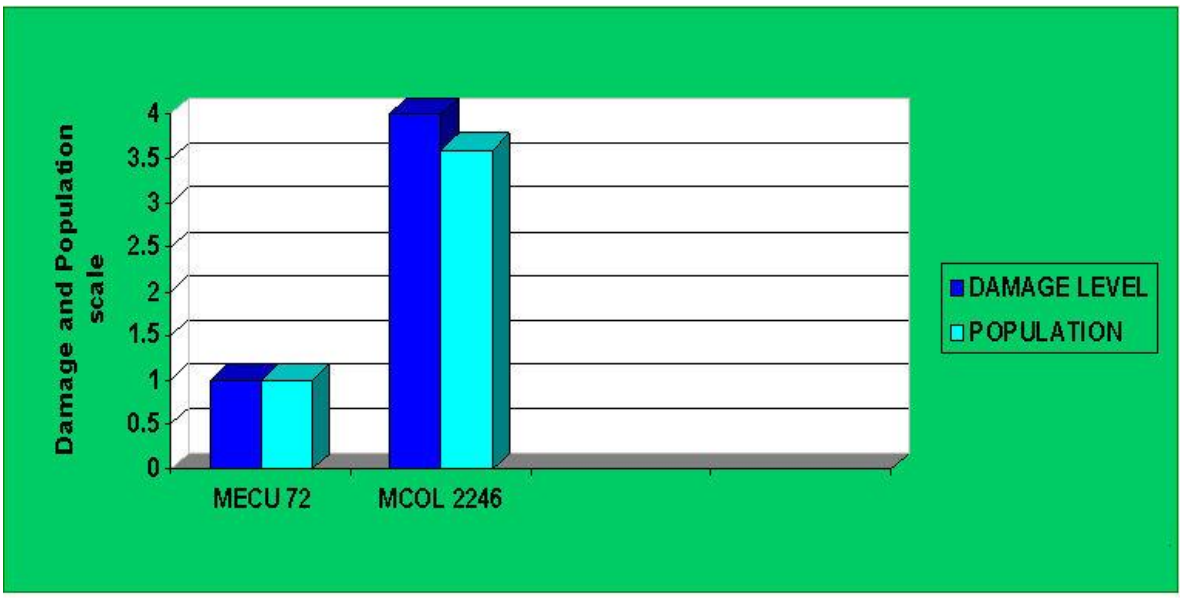

Fig. 4. Cassava damage and whitefly population ratings due to *A. socialis* feeding on parental genotypes MEcu-72 and MCol-2246 at CORPOICA, Nataima (Tolima, Colombia).

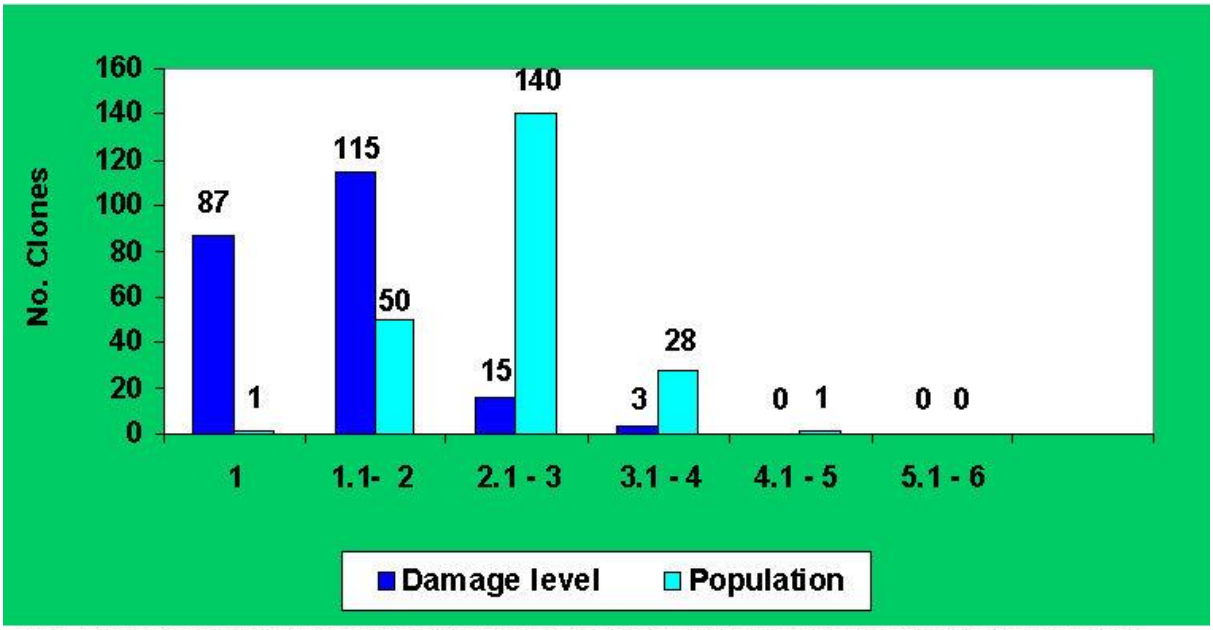

Fig. 5. Cassava damage and whitefly population ratings due to *A. socialis* feeding on clones from the family CM 8996 (MEcu-72 x MCol-2246) at CORPOICA, Nataima (Tolima, Colombia).

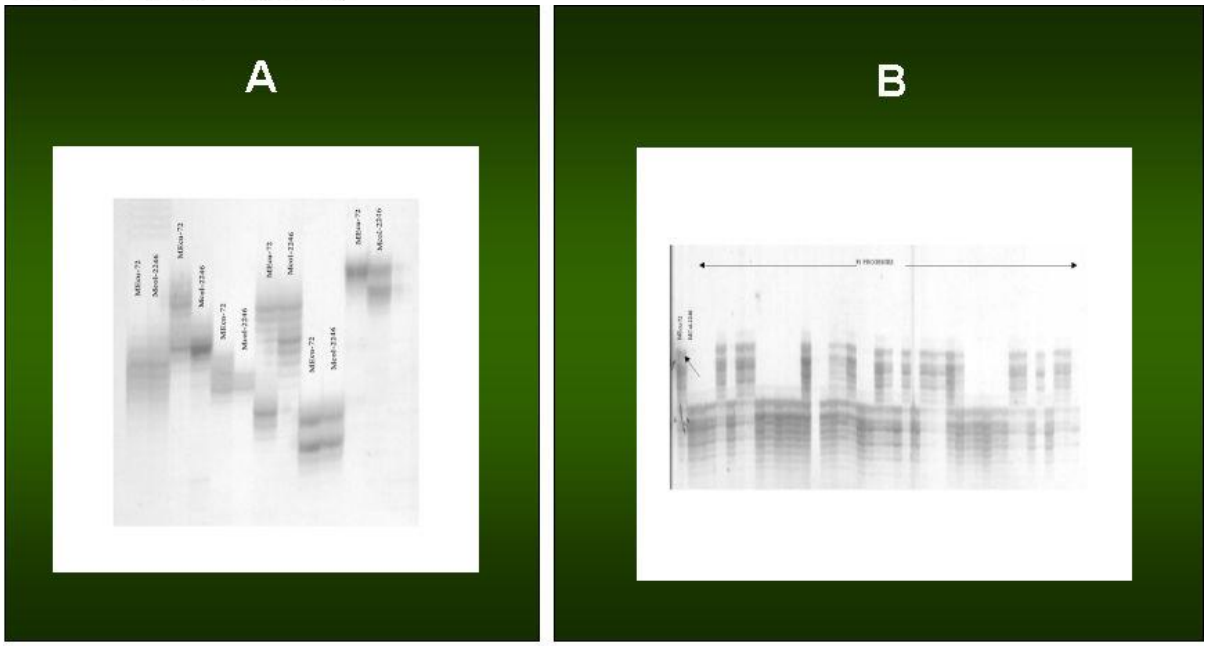

Fig. 6. Silver staining polyacrylamide gel showing: A: the parentals MEcu-72 and MCol-2246 evaluated with six cassava SSRs. B: unique allele in MEcu-72 of cassava SSR 234. Forty-one F1 progenies show the inheritance of this allele.

Table 1. Population and damage scales for evaluation cassava germplasm for resistance to whiteflies.*

Population scale (nymphs & pupae)
1= no whitefly stages present
2= 1-200 individuals per cassava leaf
3= 201-500 per leaf
4= 501-2000 per leaf
5= 2001-4000 per leaf
6= >4000 per leaf
Damage scale
1= no leaf damage
2= young leaves still green but slightly flaccid
3= some twisting of young leaves, slight leaf curling
4= apical leaves curled & twisted; yellow-green mottled appearance
5= same as 4, but with sooty mold & yellowing of leaves
6= considerable leaf necrosis & defoliation, sooty mold on mid & lower leaves and young stems

*Extracted of Bellotti & Arias, 2001 Crop Protection. 813-823.

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Evidence for Multiple Mechanisms of Resistance to Cry1Ac and Cry2A Toxins from *Bacillus thuringiensis* in *Heliothis virescens*

Bacillus thuringiensis (Bt) is a common spore-forming bacterium that produces insecticidal proteins called Cry toxins (from *Cry*stal). The commercialization of transgenic plants producing Cry toxins has greatly affected insect control methods due to their environmental safety and increased crop yield. In 1996, transgenic cotton plants producing Cry1Ac toxin were commercialized to control *Heliothis virescens* (tobacco budworm). This insect is one of the most important pests of cotton, among other crops. As with any insect control method, development of resistance to Bt toxins is one of the main concerns on the wide use of transgenic Bt plants. Although no *H. virescens* resistance episodes to Bt cotton have been reported in the field so far, laboratory resistance selection of *H. virescens* has demonstrated that the genetic potential for resistance development exists (Gould et al., 1992, 1995). The study of resistance in these laboratory-selected insect strains has helped to identify potential resistance mechanisms and strategies aimed to manage and delay the onset of resistance.

Disruption of any step in the mode of action of Bt toxins can result in resistance to these toxins. The general mode of action of Bt toxins includes ingestion by the susceptible insect, solubilization and activation to toxic forms by insect midgut enzymes, binding and insertion into the membrane of the midgut epithelium, and midgut cell lysis by osmotic shock (Knowles, 1994). Although several mechanisms of resistance to Bt toxins in laboratory-selected insects have been proposed, alteration of toxin binding to midgut receptors is the best studied (Ferré and Van Rie, 2002).

Since an insect is less likely to develop resistance to two toxins with distinct modes of action, one of the proposed methods to delay the onset of resistance to Bt plants in the field is the generation of transgenic lines expressing different Bt toxins in combination (Gould, 1998). To assure the efficacy of this approach the toxins selected for expression should not share common binding sites and must have distinct modes of action.

In brush border epithelium membrane vesicles (BBMV) from *H. virescens*, Cry1Aa, Cry1Ab, Cry1Ac, Cry1Fa, and Cry1Ja toxins share a common binding site (receptor A). Cry1Ab and Cry1Ac have an additional binding site (receptor B) and Cry1Ac is the only toxin that can recognize a third binding site (receptor C) (Van Rie et al., 1989; Jurat-Fuentes and Adang, 2001). According to this model of binding sites, alteration of receptor A would potentially lead to reduced binding and possibly resistance to all Cry1A, Cry1Fa and Cry1Ja toxins. This mechanism was proposed to occur in the Cry1Ac-selected YHD2 strain of *H. virescens* (Lee et al., 1995).

One of the most important toxin candidates to be used in combination with Cry1Ac in Bt cotton to control *H. virescens* is Cry2A. This toxin does not share binding sites with Cry1A toxins (Jurat-Fuentes and Adang, 2001) and has a distinct mode of action (English et al., 1994; Morse et al., 2001). Transgenic Bt cotton plants expressing both Cry1Ac and Cry2A have been shown to enhance control of *H. virescens* (Stewart et al., 2001). Interestingly, the Cry1Ac laboratory selected CP73-3 and KCB *H. virescens* strains developed cross-resistance to Cry2A, among other toxins (Gould et al., 1992; Forcada et al., 1999). These strains were backcrossed to susceptible insects and the offspring were selected with Cry2A to increase resistance to this toxin. This selection regime led to the generation of the CXC (derived from CP73-3) and KCBhyb (derived from KCB) strains, which showed increased Cry2A and Cry1Ac resistance levels when compared to their parental strains (Kota et al., 1999). Both strains were also cross-resistant to Cry1Aa, Cry1Ab, and Cry1Fa toxins (F. Gould, unpublished observation).

To study the mechanism of resistance in the CXC and KCBhyb strains, we performed toxin-binding assays with radio labeled Cry1A toxins. BBMV from YDK (susceptible control strain), CXC and KCBHyb insects were isolated and incubated with increasing concentrations of labeled Cry1A toxins to generate a binding saturation curve for each Cry1A toxin.

Saturation curves were analyzed and the binding affinities of each toxin for the CXC, KCBhyb, and control susceptible BBMV were calculated. No changes in either toxin affinity or concentration of receptors were detected in BBMV from the CXC strain when compared to susceptible vesicles. On the other hand, binding of Cry1Aa was greatly reduced in vesicles from KCBhyb, while Cry1Ab and Cry1Ac binding was as in BBMV from susceptible insects.

These results are evidence that resistance in the CXC strain is not due to changes in toxin binding to midgut receptors. Resistance in this strain should be the result of a change in a common step of the Cry1Ac and Cry2A toxin mode of action. Since these toxins seem to recognize different receptors in *H. virescens*, one possibility is alteration of steps prior to receptor binding in this strain. Such a change in the solubilization or processing of the Cry toxins in midguts of CXC insects would lead to resistance to both Cry1Ac and Cry2A. The existence of such a mechanism would be consistent with the decreased levels of susceptibility to other Bt toxins, as is the case for Cry1Aa, Cry1Ab, and Cry1Fa.

Since Cry1Aa and Cry1Fa share a common binding site, we used biotinylated Cry1Fa (since iodination inactivates this toxin) to study binding of this toxin to BBMV from KCBhyb. No differences in Cry1Fa toxin binding were observed between YDK and KCBhyb, suggesting that binding of this toxin is not altered in KCBHyb larvae. Or at least, Cry1Fa binding is not altered to a degree detectable by the binding assay. Since Cry1Aa shares its only BBMV binding site with Cry1Ab, Cry1Ac, and Cry1Fa, the change that is preventing Cry1Aa binding in KCBhyb BBMV is probably also responsible for resistance to all these toxins. This hypothesis was also proposed for the Cry1Ac resistant YHD2 strain of *H. virescens* (Lee et al., 1995) after obtaining the same qualitative toxin binding results we observed in KCBHyb BBMV. Additionally, since Cry1Aa and Cry2A do not share binding sites in *H. virescens* BBMV, cross-resistance to Cry2A cannot be explained by alteration of Cry1Aa binding. In this case, a second mechanism of resistance that would affect both Cry1Ac and Cry2A mode of action needs to be present. As outlined for the CXC strain such a mechanism is may be related to alteration of toxin solubilization and/or processing conditions in the midguts of CXC and KCBhyb midguts.

In conclusion, our results indicate the presence of at least two resistance mechanisms in larvae from the KCBHyb strain. One of the mechanisms would be related to Cry1A receptor alteration, and possibly the second mechanism related to toxin solubilization and/or processing in the larval midgut. Similar conclusions have been presented for resistant *Plodia interpunctella* (Indianmeal moth) (Herrero et al., 2001). Alteration of toxin solubilization and/or processing seems to be the main mechanism of resistance in larvae from the CXC strain. Interestingly, high levels of Cry2A expression in chloroplasts of tobacco plants overcome resistance in CXC larvae (Kota et al., 1999), indicating a possible solution to this resistance mechanism. Nevertheless, our conclusions raise questions as to how *H. virescens* in the field will respond to transgenic cotton producing Cry1Ac and Cry2A proteins. Our results are also evidence of the array of resistance mechanisms to Bt toxins that *H. virescens* can develop after selection with a single Cry toxin. This information is extremely important when designing and implementing strategies aimed at delaying resistance and cross-resistance to Bt transgenic crops.

Experiments in our laboratory are presently aimed at elucidating the molecular mechanism by which decreased toxin binding is achieved in the KCBhyb resistant insects, as well as the molecular nature of the resistance mechanism in CXC larvae.

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Monitoring Onion Thrips Resistance to Pyrethroids in New York

The onion thrips, *Thrips tabaci* Lindeman, is a pest of onions and related *Allium* species, as well as dozens of other plant families, throughout the world. The major control strategy for onion thrips on onions is the frequent use of insecticides and growers may apply treatments weekly, especially in hot, dry years. There is concern that such intensive treatments may result in the development of resistance. The results reported in this paper include studying the susceptibility of onion thrips populations in New York to l-Cyhalothrin (Warrior T) in 2001 and in 2002 using a newly developed assay system and conducting a simulated field test to compare the results from the laboratory assays to field performance.

MATERIALS and METHODS

Evaluating Onion Thrips Populations against Warrior:

We used the Thrips Insecticide Bioassay System (TIBS) developed by Rueda and Shelton (2003). This system allows thrips to be collected directly from onion plants into a 0.5 ml microcentrifuge tube previously treated with an insecticide. Thrips mortality is assessed at 24 h with the help of a dissecting stereoscope and then data are analyzed to calculate the mean lethal concentration that would kill 50% of the population (LC50). During the 2001 growing season, thrips populations were collected from 16 different sites encompassing the major growing areas of New York. In 2002, populations were collected from 10 different sites at two different time periods (mid-season and late-season). The reason for the two collections was to determine whether there are changes in susceptibility over time. For each of these collections we only tested the populations against a single dose, the field dose of 100 ppm. This decision was based on evidence collected in 2001 that fields in which it was difficult to control thrips with Warrior had LC50 values > 100 ppm.

Determine the Relationship between Lab Assays and Simulated Field Performance:

From our collections of onion thrips in 2002, we selected four populations that varied significantly in their susceptibility to Warrior, based on our laboratory assay using TIBS. We introduced these populations onto onion plants treated with the field rate of Warrior using a carbon dioxide backpack sprayer. After spraying, the insecticide was allowed to dry for 24 hours and then 20 thrips larvae were introduced into a cage containing a single plant. Thrips were allowed to feed and then mortality was assessed after 5 days. There were eight cages (replicates) for each of the four populations of thrips used in this study.

RESULTS and DISCUSSION

Evaluating Onion Thrips Populations against Warrior:

Of the 16 populations examined in 2001, seven had LC50 values greater than the field dose of 100 ppm indicating a potential for poor field performance (Figure 1). In the figure the fields are grouped by region (e.g. Orange County denoted by OR 1-3)) and there was considerable variation within each region with some fields having populations of thrips with LC50 values much higher or lower than the field rate of 100 ppm. This indicates that individual grower

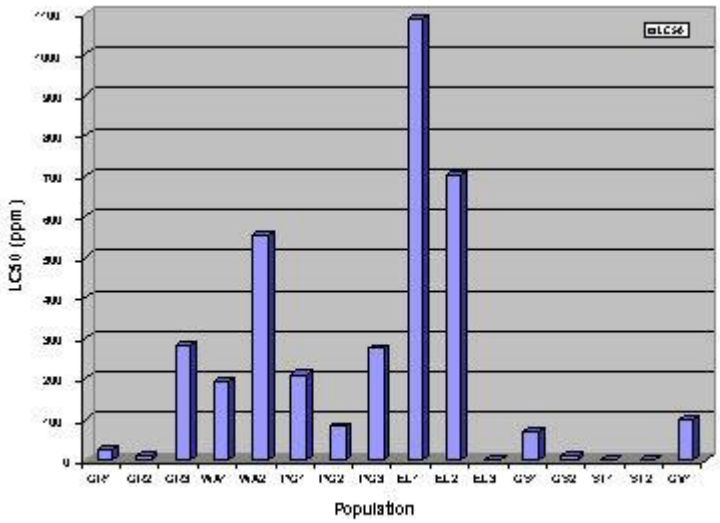

Figure 1. LC50 values for Warrior for onion thrips populations.

practices probably dictate the development of resistance.

In 2002, we assessed the susceptibility of thrips populations based on the % mortality at the field rate of 100 ppm (Figure 2). For the first collection (mid-season), there was considerable variation in the % control with half the populations having >50% mortality at 100 ppm. For the late-season collection, there was again considerable variation in the % control with half the populations having > 50% mortality at 100 ppm. However, only two of the populations that were susceptible (>50% mortality at 100 ppm) in the early part of the season were susceptible in the later part of the season. We suspect that changes in insecticide use caused these changes, but need to examine spray records to determine if this is the case.

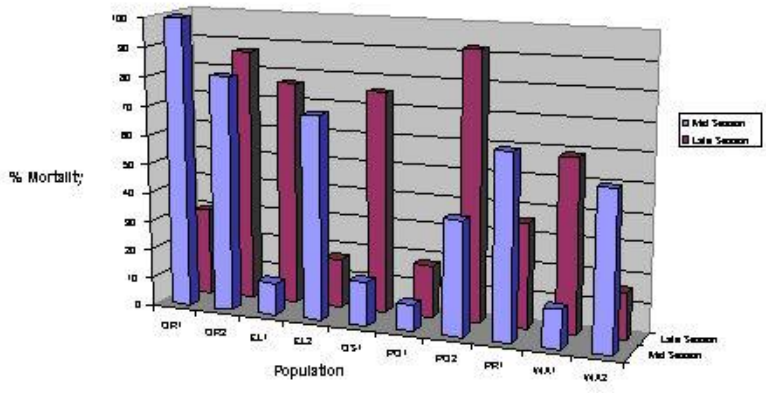

Figure 2. Mortality of onion thrips populations to 100 ppm Warrior.

Determine the Relationship between Lab Assays and Simulated Field Performance:

As we had suspected, there was an excellent relationship (r2= 0.97) between the mortality at 100 ppm using the TIBS method and the level of control that was seen when onion plants were sprayed with the field rate of Warrior (100 ppm). This indicates that one could sample a field of onions using TIBS and then determine whether acceptable control would be achieved if the field were sprayed (assuming good spray coverage).

CONCLUSIONS and NEXT STEPS These studies indicate that resistance to at least one insecticide, Warrior T, has occurred in some onion growing regions of New York, and that resistance appears to have developed because of practices in individual fields. Furthermore, susceptibility to Warrior in an individual field can change dramatically within a single season. This points to the need to monitor fields routinely before choosing the right insecticide. We are developing a TIBS system to determine susceptibility to other classes of insecticides. If susceptibility to a particular class of insecticide can be determined quickly, as with TIBS, then it will be possible to develop an insecticide resistance management strategy for onion thrips on onions.

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Pyrethroid Susceptibility of Tobacco Budworm, *Heliothis virescens* (F.), and Bollworm, *Helicoverpa zea* (Boddie), in Louisiana

ABSTRACT Louisiana has maintained a statewide pyrethroid susceptibility monitoring survey for tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie), since 1986 and 1988, respectively. Adult males of both species were captured with pheromone baited wire cone traps and exposed to cypermethrin in an insecticide residual on glass (AVT) bioassay. During 1987 to 2002, annual survival of tobacco budworm adults exposed to 10 µg/vial of cypermethrin using the AVT increased from 15% to 60%. The percentage of parishes in which greater than or equal to 50% survival of tobacco budworm was recorded increased from 8% in 1990 to >70% during 2002. Survival of bollworm adults exposed to 5 µg/vial of cypermethrin in the AVT increased from 2% in 1988 to 34% in 2002. Prior to 1990, bollworm survival exceeded 11% in only one parish. During 2002, survival exceeded 31% in six of nine parishes. No control failures of bollworms treated with pyrethroids have been observed in Louisiana. In 1995, pyrethroids no longer were recommended by the Louisiana Cooperative Extension Service for control of tobacco budworm.

KEYWORDS Insecticide resistance, tobacco budworm, *Heliothis virescens* (F.), bollworm, *Helicoverpa zea* (Boddie), pyrethroids

INTRODUCTION Insecticide resistance in key insect pests is an important issue for the cotton industry. Two of the most important cotton pests in the mid-south and southeastern United States are the tobacco budworm, *Heliothis virescens* (F.), and the bollworm, *Helicoverpa zea* (Boddie). Resistance to organochlorines, DDT, organophosphates (Sparks 1981), and carbamates (Elzen et al. 1992) has been reported with both species. Resistance in tobacco budworm populations to pyrethroid insecticides, as well as isolated field control failures were observed in Arkansas (Plapp et al. 1987), Louisiana (Leonard et al. 1987), Mississippi (Roush and Luttrell 1987), and Texas (Allen et al. 1987, Plapp et al. 1987) during 1986. In response, pyrethroid resistance management plans were implemented in those states to maintain the effectiveness of pyrethroids for control of tobacco budworm (Anonymous 1986). An important component of these plans was pyrethroid susceptibility monitoring for tobacco budworm. Resistance to DDT and organochlorine insecticides has been reported in bollworm (Sparks 1981). In 1998, field control failures (Walker et al. 1998) resulting from pyrethroid-resistant populations of bollworm (Brown et al. 1998) were reported in South Carolina. Monitoring of the susceptibility of bollworm populations in Louisiana to pyrethroids was initiated during 1988. To date no field control failures of bollworm infestations treated with pyrethroids have been observed in Louisiana.

MATERIALS and METHODS Adult vial bioassays (AVT) similar to those described by Plapp et al. (1987, 1990) were used to monitor the susceptibility of field collected tobacco budworm and bollworm moths to cypermethrin. Stock solutions of cypermethrin were developed by dissolving technical grade (98%) insecticide in acetone. Serial dilutions from each stock solution yielded the desired concentrations. The interior surface of 20 ml glass scintillation vials was coated with cypermethrin by pipetting 0.5 ml of the appropriate solution into vials. These vials were rotated on a modified hot dog roller (heating element disconnected) until all of the acetone had evaporated. Vials were stored in a dark environment until used in bioassays.

Male tobacco budworm and bollworm moths were collected using wire cone traps (Hartstack et al. 1979) baited with synthetic sex pheromone lures (Hendricks et al. 1987) from May through September. Moths were collected from parishes (tobacco budworm populations surveyed in 2 to 17 parishes during 1986 to 2002, bollworm populations surveyed in 8 to 20 parishes during 1988 to 2002) throughout the cotton production regions of Louisiana (Figure 1). The insecticide concentrations used in these surveys included 10µg/vial cypermethrin for tobacco budworm and 5µg/vial cypermethrin for bollworm. Moths were placed into insecticide-treated and control (non-treated) vials (one moth/vial) and mortality was determined after 24-h of exposure (HAE). Moths were considered dead if they were incapable of sustained flight for ca. 1 minute. Data were corrected for control mortality using Abbott's (1925) formula.

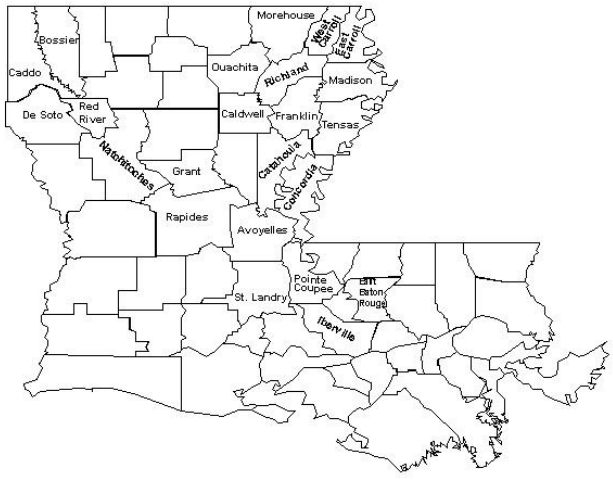

Figure 1. Parishes included in tobacco budworm and bollworm pyrethroid susceptibility surveys.

RESULTS and DISCUSSION In response to field control failures of tobacco budworm with pyrethroids a monitoring program was initiated in Louisiana and continued through the present year (Graves et al 1994, Bagwell et al. 2001, Cook et al. 2003). Limited surveys (two parishes) of tobacco budworm susceptibility to pyrethroids were conducted during 1986, and concentrated on fields associated with unsatisfactory control. Mean survival of tobacco budworm moths from Bossier and Tensas parishes was 33% and 41%, respectively (Figure 2). In 1987, more extensive monitoring efforts were conducted across Louisiana. In 1988, only one parish had tobacco budworm survival >30% (East Carroll), while tobacco budworm survival between 11% and 30% was observed in six parishes (Figure 3). During 1990, tobacco budworm survival in Catahoula parish was 51% (Figure 4). Tobacco budworm survival between 31% and 50% was observed in seven parishes. During 1992, tobacco budworm survival >50% was observed in two parishes (Figure 5). Survival in Madison Parish was approximately 60%. Tobacco budworm survival between 31% and 50% was observed in ten parishes. Survival in excess of 50% was not observed in any parish during 1994 (Figure 6). However, the number of parishes in which survival between 31% and 50% increased. During 1996, tobacco budworm survival between 31% and 50% was observed in 11 parishes (Figure 7). Survival >50% was observed in two parishes with 66% tobacco budworm survival observed

in East Carroll parish. During 1998, tobacco budworm survival >50% was observed in seven parishes, with 78% survival recorded in Morehouse parish (Figure 8). Also, survival >30% was observed in eight parishes. Tobacco budworm survival >50% was observed in seven parishes during 2000 (Figure 9) representing ca. 54% of the parishes in which monitoring was conducted. Tobacco budworm survival >50% was observed in 86% of the parishes during 2002 (Figure 10). In Catahoula parish during 2002, 83% survival of tobacco budworm adults exposed to cypermethrin in the AVT was observed.

Pyrethroid susceptibility surveys of Louisiana bollworm were initiated during 1988. Bollworm survival was <10% for all parishes monitored during 1988 and 1990, with the exception of Morehouse parish during 1988 (Figure 11, Figure 12). Bollworm survival exceeded 10% in two parishes of twelve during 1992 (Figure 13). During 1994, bollworm survival in one parish was ca. 13%, while survival in 11 other parishes was less than or equal to 10% (Figure 14). Survival >10% was observed in three parishes of 16 parishes during 1996 (Figure 15). During 1998, survival >10% was observed in 15 of 20 parishes with survival in one parish exceeding 30% (Figure 16). Bollworm survival >10% was recorded in nine parishes during 2000 (Figure 17). During 2002, bollworm survival exceeded 10% in seven of nine parishes and in five parishes survival was >30%, with survival of 42% observed in one parish (Figure 18). Pyrethroid resistance in tobacco budworm gradually increased from 15% statewide during the late 1980's to ca. 40% during the mid 1990's (Figure 19). Resistance management (IRM) plans extended the useful life of pyrethroids for tobacco budworm control until ca. 1995 when they were removed for the Louisiana Cooperative Extension Service Recommendations for control of tobacco budworm in cotton, but they continued to be used for bollworm control. Alternative insecticides for tobacco budworm control became available in 1995, as well as, Bollgard cotton varieties. During 1995 to 2002, tobacco budworm survival has steadily increased even though pyrethroids are not applied to control tobacco budworm. This continued increase is probably the result of inadvertent selection pressure from pyrethroid applications targeting bollworm and other insect pests of cotton.

No field control failures of bollworm treated with pyrethroids have been observed in Louisiana. However, bollworm survival in the AVT has increased since 1996 (Figure 20). The highest annual survival was observed during the 2002 season (ca. 34%). Presently, pyrethroids are used to control bollworm in non-Bollgard and Bollgard cotton varieties. In addition, these products are also used against other cotton insect pests as well as bollworm in other crop hosts including corn, grain sorghum, and soybean.

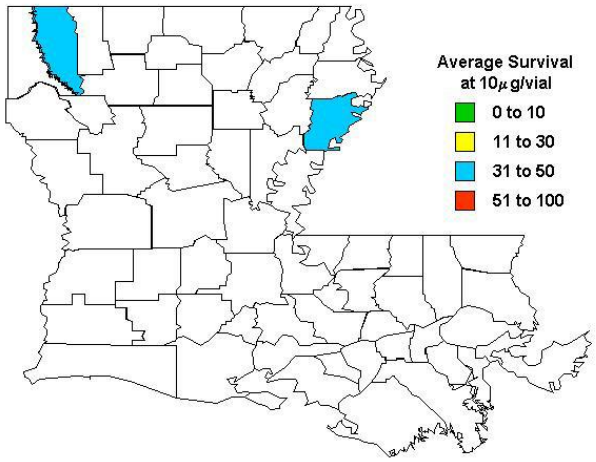

Figure 2. Annual tobacco budworm survival during 1986.

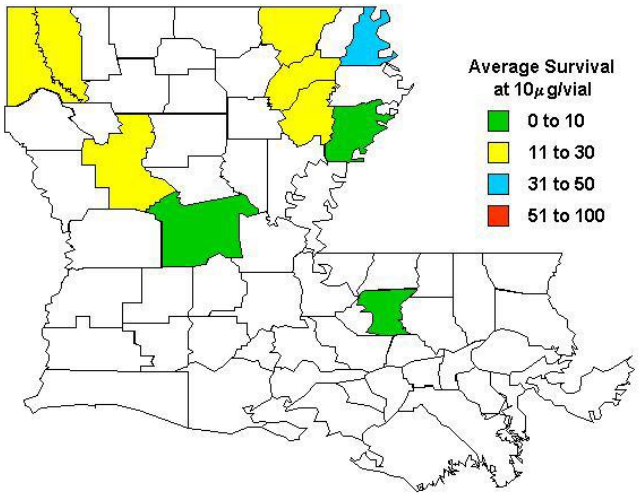

Figure 3. Annual tobacco budworm survival during 1988.

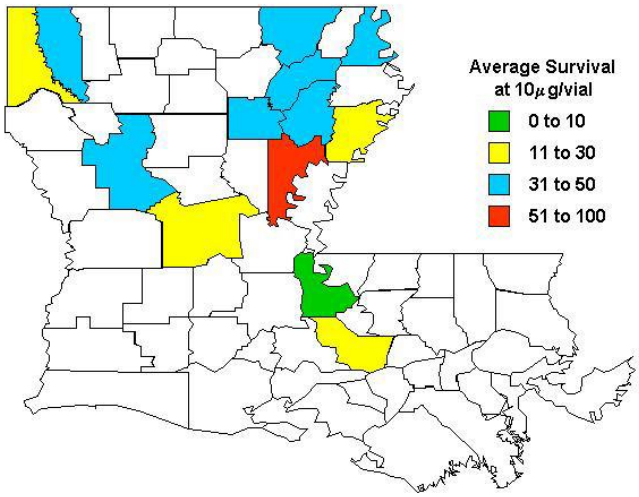

Figure 4. Annual tobacco budworm survival during 1990.

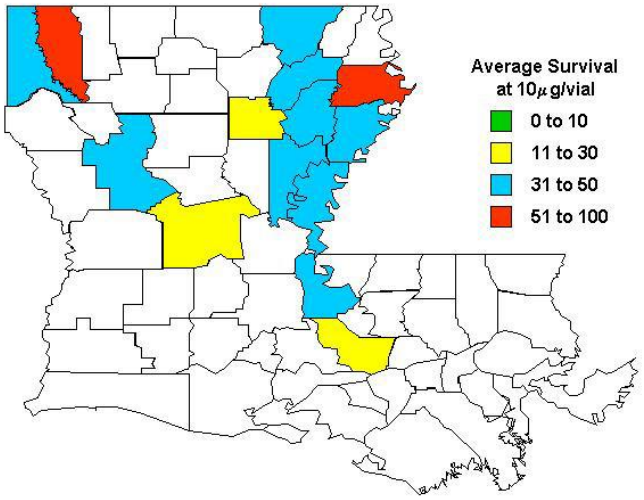

Figure 5. Annual tobacco budworm survival during 1992.

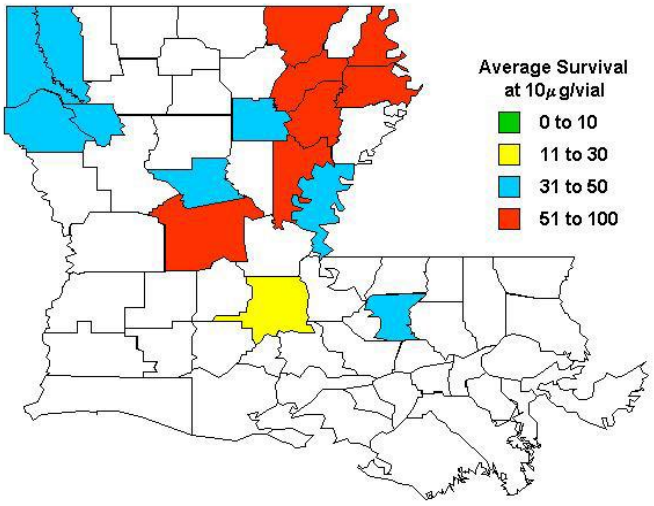

Figure 8. Annual tobacco budworm survival during 1998.

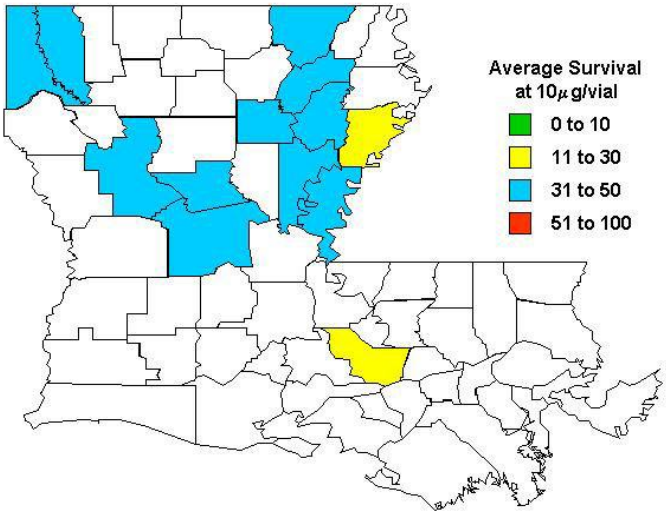

Figure 6. Annual tobacco budworm survival during 1994.

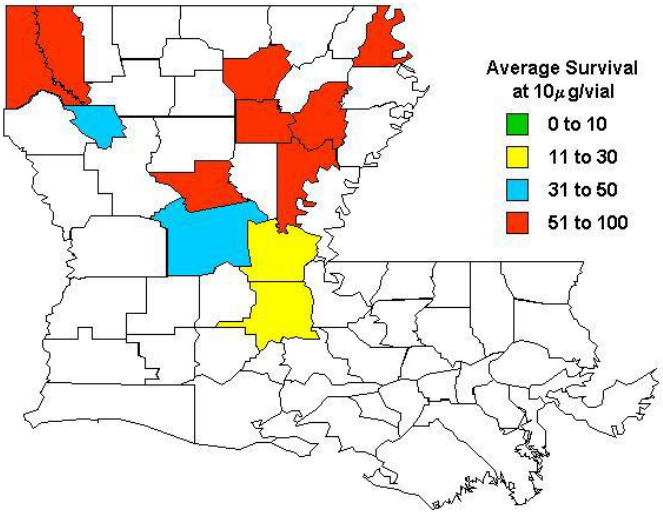

Figure 9. Annual tobacco budworm survival during 2000.

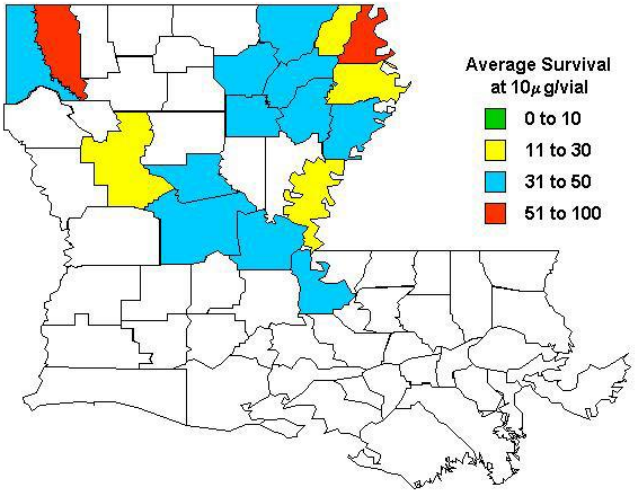

Figure 7. Annual tobacco budworm survival during 1996.

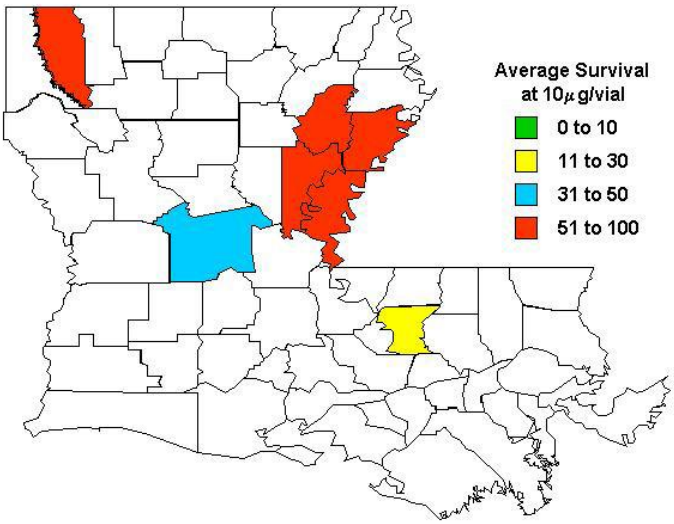

Figure 10. Annual tobacco budworm survival during 2002.

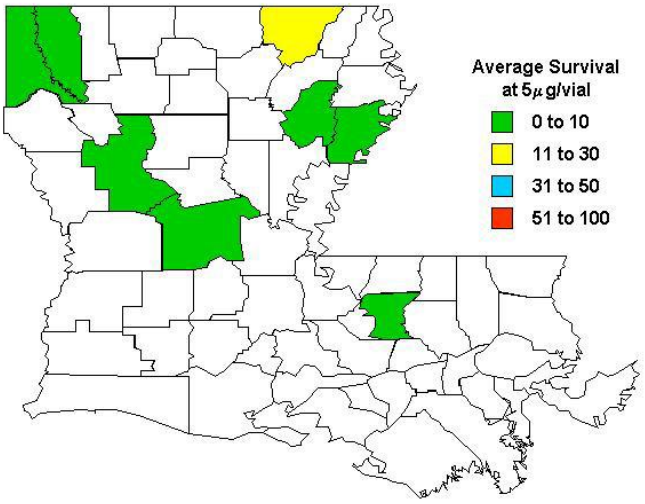

Figure 11. Annual bollworm survival during 1988.

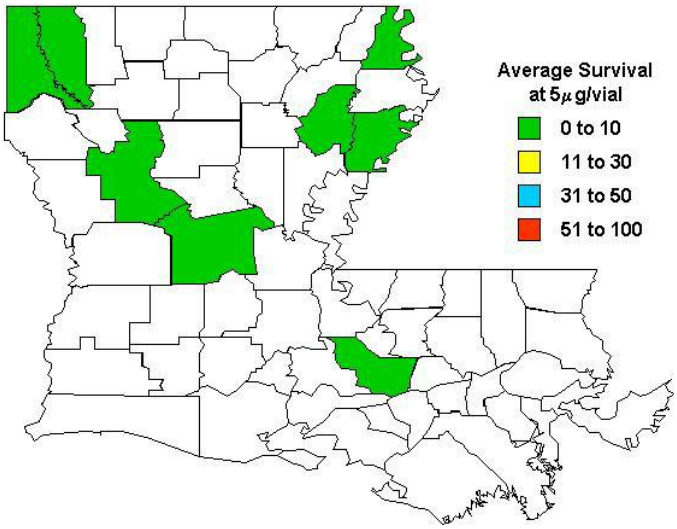

Figure 12. Annual bollworm survival during 1990.

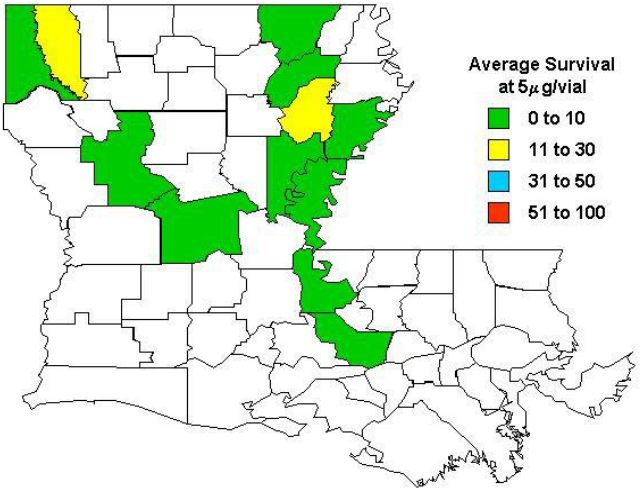

Figure 13. Annual bollworm survival during 1992.

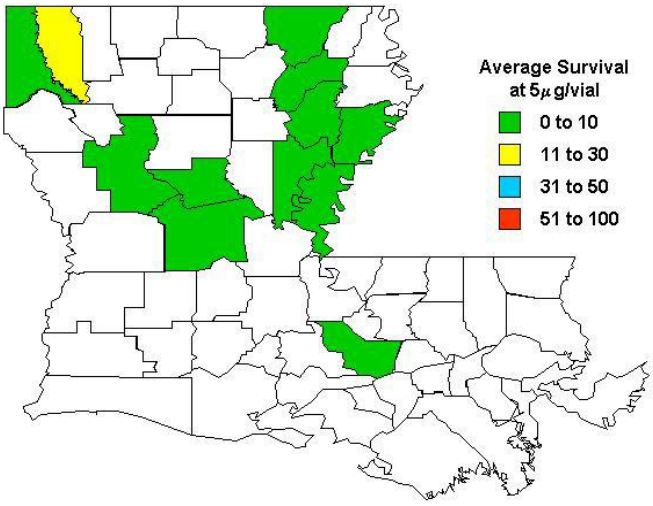

Figure 14. Annual bollworm survival during 1994.

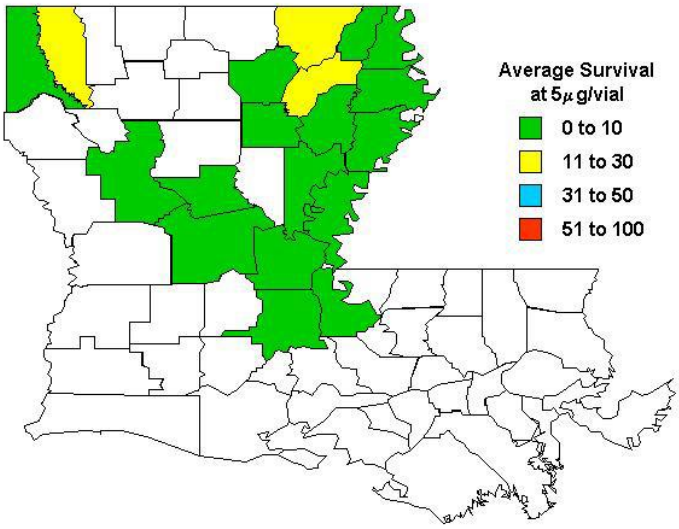

Figure 15. Annual bollworm survival during 1996.

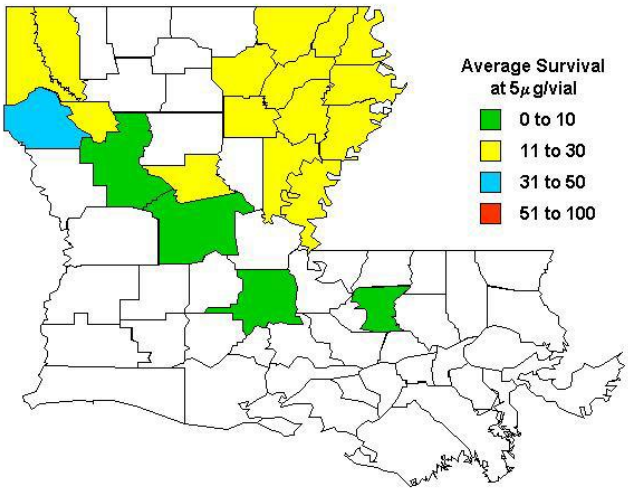

Figure 16. Annual bollworm survival during 1998.

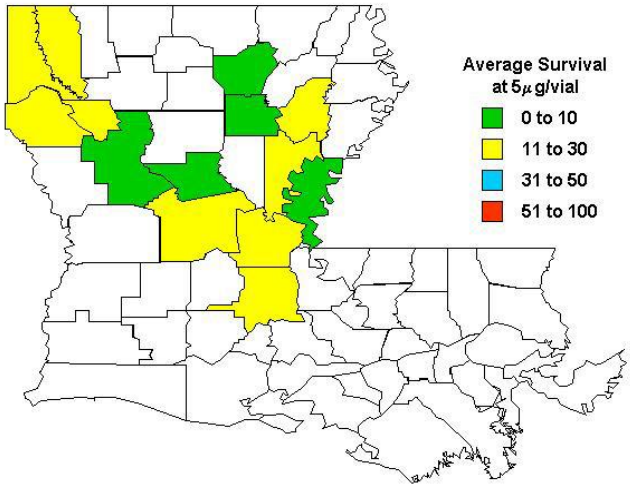

Figure 17. Annual bollworm survival during 2000.

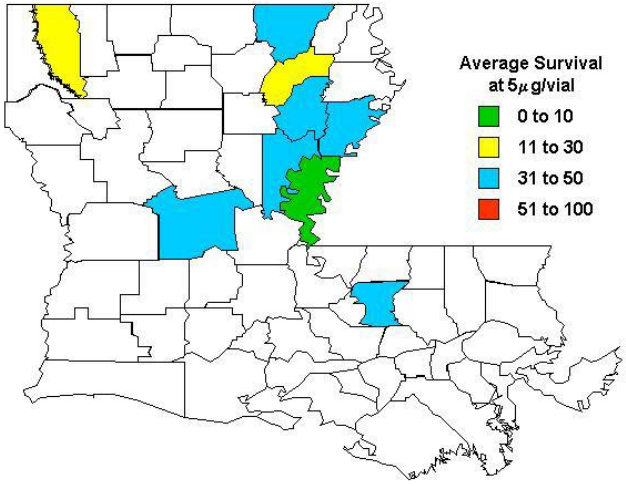

Figure 18. Annual bollworm survival during 2002.

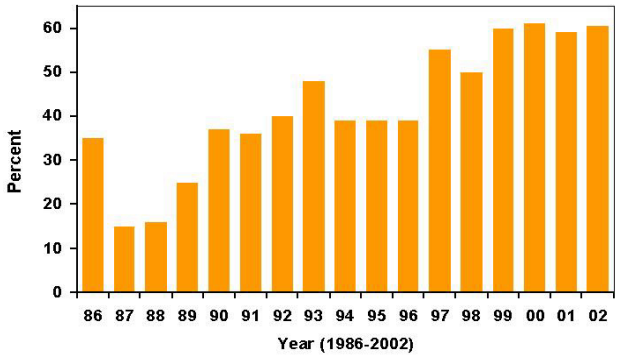

Figure 19. Mean statewide tobacco budworm survival at 10 µg cypermethrin/vial 1986 to 2002.

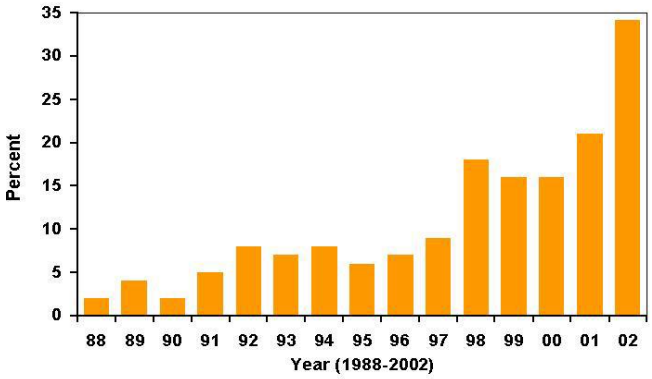

Figure 20. Mean statewide bollworm survival at 5 µg cypermethrin/vial 1986 to 2002.

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Monitoring of Insecticide Resistance in *Helicoverpa armigera* (Hübner) from 1998 to 2002 in Côte d'Ivoire, West Africa

Helicoverpa armigera (Hübner) is the major insect pest of the cotton crop in West Africa. Populations recently developed resistance to pyrethroids via the overproduction of oxidases. To control this pest, a resistance management strategy was applied in the major West African cotton growing countries from 1999 onwards. In Côte d'Ivoire insecticide resistance of *H. armigera* was monitored in field strains from 1998 to 2002 using vial tests and topical applications in third-instar larvae. Vial tests with discriminating doses of cypermethrin were used directly on field-collected larvae at the end of the cotton season. The percentage of resistant larvae varied around 67% with 6 µg/vial and around 13% with 30 µg/vial according to year and place.

Topical applications were used in the laboratory on the first generation of populations collected from cotton (*Gossypium hirsutum*), tomato (*Lycopersicum esculentum*), or a strongly infested ornamental flower (*Antirrhinum majus*) with various insecticides. The resistance factors calculated from dose-mortality regressions varied from 5 to 38 with deltamethrin. They were always higher for strains collected from cotton in the Bouaké area at the end of the season. Concerning the pyrethroid alternatives currently used in Côte d'Ivoire, no lack of susceptibility was detected with endosulfan and profenofos in the cotton field strains showing their interest in the resistance management strategy. All these results suggest a relative stability of the pyrethroid resistance in *H. armigera* for 1998 to 2002 and confirm the success of the resistance management strategy.

KEY WORDS *Helicoverpa armigera*, insecticide resistance, cotton.

INTRODUCTION More than two million small-scale farmers are cultivating cotton in West Africa on an average of 1 ha plots. Cotton is one of the most important cash crops in the region and provides more than 50% of the cash to the agricultural populations. It contributes largely to the struggle against poverty in the countries of the African Cotton Belt. Cotton crops are damaged by a large number of insect species, the most harmful being the cotton bollworm *Helicoverpa armigera* (Hübner). To control the whole cotton pest complex, available and profitable approaches include chemicals associated with the use of hairy cultivar and appropriate cultural practices. As a result, this crop receives the largest amount of insecticide among the crops cultivated in the area. Since the early eighties, pyrethroids have been extensively used, because they are very efficient against bollworms at low doses and because their toxicity to mammals is low. However, since 1996 high infestations of *H. armigera* were observed in some areas suggesting control failure due to the development of insecticide resistance (Vassal et al., 1997; Vaissayre et al., 1998; Martin et al., 2000). In 1998, pest infestations extended to several countries in West Africa despite an increase in spaying intensity. This event became highly threatening since similar resistance observed in India and Pakistan resulted in dramatic losses of cotton production, pushing some farmers to suicide (McCaffery et al., 1988 and 1989). Facing this problem, West African countries put

together efforts to better understand the phenomenon, studying the resistance mechanism. It appears that resistance was due to greater degradation of pyrethroids in resistant insects involving oxidases from the cytochrome P450 family (Martin et al., 2002). A network group, named PR-PRAO (Prevention and Management of Pyrethroid Resistance in *H. armigera*) was implemented, involving CIRAD, IRAC, and all the actors of cotton protection in West Africa from research to extension services and advisory services to initiate management strategies and monitor programs to survey the resistance level of insecticides used.

The control of *H. armigera* on the West African cotton regions has been the focus of an insecticide resistance management strategy (IRM) restricting the use of pyrethroids in cotton (Ochou et al., 1998, Ochou and Martin, 2002). This strategy has been generally successful in all West African cotton-growing regions since 1999. As no difference in oxidases activity has been described for resistance to endosulfan, we concluded that pyrethroid resistance would not cross. As this insecticide already proved to be very efficient against the bollworm before the introduction of pyrethroids, endosulfan was chosen to replace pyrethroids in the beginning of the cotton season.

In order to assure a sustainable resistance management strategy, it was essential to survey the pyrethroid resistance levels in each cotton-growing region because of the migrating habits of *H. armigera* (Nibouche, 1994). In the present investigation we monitored the pyrethroid resistance level in *H. armigera* from 1998 to 2002 in the cotton-growing area of Côte d'Ivoire. Populations were collected in cotton (*Gossypium hirsutum*), in tomato (*Lycopersicum esculentum*), and in a strongly infested ornamental flower (*Antirrhinum majus*). The susceptibility of pyrethroid alternatives such as endosulfan and profenofos has also been surveyed.

EXPERIMENTAL PROCEDURE

Insects. A susceptible *H. armigera* strain (BK77) was originally collected in Côte d'Ivoire in 1977 and reared in CIRAD Entomological Laboratory from Montpellier, France. Larvae were reared on artificial diet at 25°C, 75% humidity and at 12h/12h photoperiod in the laboratory as previously described (Couilloud and Giret, 1980).

Field samples of different stages of *H. armigera* were collected from 1998 to 2002. Samples were obtained from strongly infested crops in identified farmers' fields from the cotton-growing area. The strains were named according to the nearest large town (BK: Bouaké; SAR: Sarhala; OGL: Ouangolo; NIO: Niofoin; MKN: Mankono; BOU: Boundiali) with the collection date (year/month) and the crop name: 'c' for cotton, 't' for tomato, 'g' for gumbo, and 'f' for the ornamental flower. A minimum of 50 larvae were collected in each field and reared in the laboratory of the Centre National de Recherche Agronomique (CNRA) in Bouaké on an artificial diet for one generation at 25°C. The adults were placed in cages and fed on a 5% honey solution. Their eggs were collected on sterilised gauze and washed with 1% bleach.

Insecticides. The insecticides used were all technical grade materials. Deltamethrin (99%) and endosulfan (99%) were obtained from Aventis CropScience, France. Cypermethrin (93.2%) was obtained from FMC, USA. Profenofos (95%) was provided by Syngenta, Abidjan, Côte d'Ivoire.

Topical application. Standard third-instar larvae topical bioassays were used to determine insecticide toxicity. Five serially diluted concentrations were prepared. For each concentration, 10 third-instar larvae (35-45 mg) were treated with 1 µl of solution applied by microapplicator to the thorax. Each test was replicated 3 times and included acetone treated controls. Mortality in the controls was less than 10%. After dosage, the test larvae were held individually at 25°C and 75% humidity. Mortality was assessed 72h after treatment. Larvae were considered dead if unable to move in a co-coordinated way when prodded with a needle. LD50 was determined by using the Finney method (1961). Transformations and regression lines were automatically calculated by DL50 1.1 software of CIRAD.

Vial tests. Vials were impregnated with technical cypermethrin in acetone. Two discriminating doses were chosen: 5 µg/vial which killed 100% of the susceptible larvae from BK77 strain, and 30 µg/vial which killed 100% of the susceptible larvae and 60 to 80% of a resistant population collected in Benin in 1997 (Vaissayre et al., 2002). The tubes were kept in darkness at ambient temperature. Extension service agents conducted vial tests for four years in October during the strong infestation at the end of the cotton season. The three first years they worked in the areas of Bouaflé, Bouaké, Boundiali, Ferké, or Tortiya. In 2001, vial tests were conducted in twelve areas spread over the Center, West North, and North of the country. Larvae of *H. armigera* measuring 1 to 1.5 cm were collected from farmer cotton fields at least seven days after the last treatment. Two replications were conducted in different location. Each larva was placed in a vial without any food. The vials were kept horizontal and protected from heat. Larval mortality was assessed at 24 h. Larvae were considered dead if unable to move in a coordinated manner.

RESULTS

Vial tests. The vial test method was directly used in cotton field of farmers to confirm and survey the pyrethroid resistance of *H. armigera* in the whole West African cotton-growing region. Because of low infestations since the application of the IRM strategy for 1999, vial tests could be used only at the end of the cotton season. The results obtained in Côte d'Ivoire with two discriminating doses of cypermethrin are illustrated in Figure 1.

The first discriminating dose (6 µg/vial) showed high percentages of resisting larvae varying from 40 to 90%. With the second discriminating dose (30 µg/vial) the percentage of resisting larvae varied from 1 to 35%. On average, there was less than 20% of resisting larvae with 30 µg/vial of cypermethrin; this threshold corresponding with control failures in field (Vaissayre et al., 2002). Moreover this vial test method applied for the same period in Mali, Burkina Faso, Benin, and Togo, showed that *H. armigera* populations from Côte d'Ivoire have lower percentages of resisting larvae.

Despite of the low level of infestation during the survey, these results showed:

1. the presence of pyrethroid resisting larvae in all populations tested and
2. high variations between the populations tested.

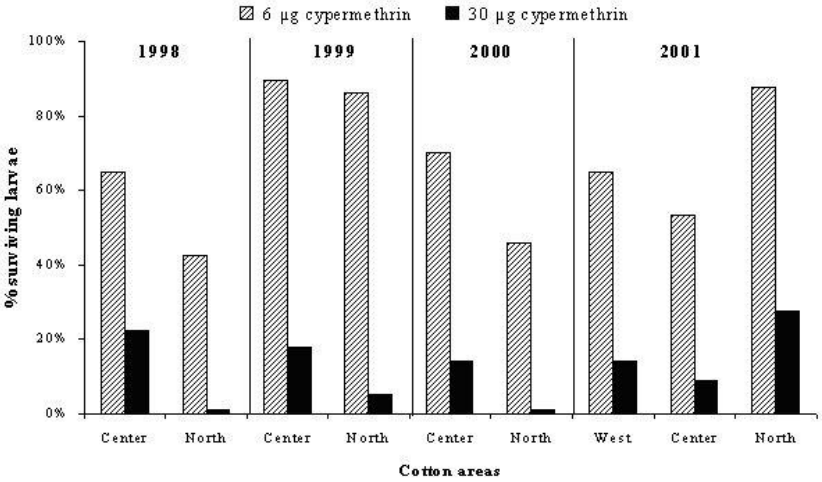

Figure 1: Vials test with 6 and 30 µg of cypermethrin in third-instar larvae of H. armigera populations collected in various cotton area from 1998 to 2001.

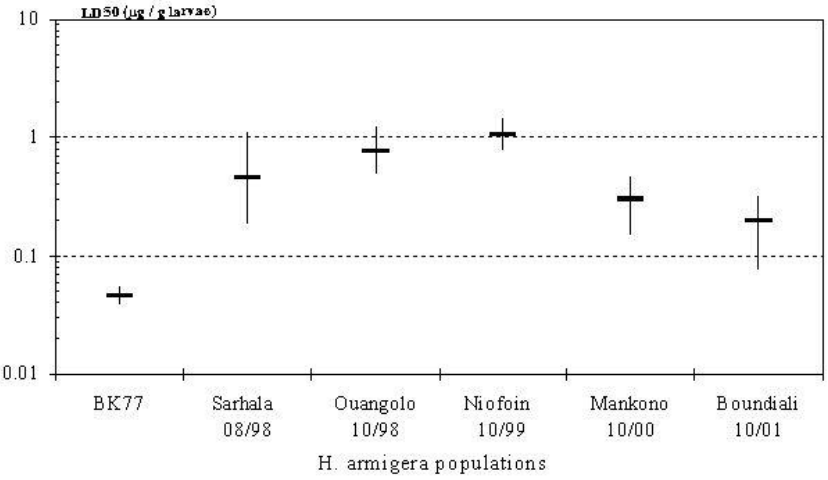

Figure 2: LD50 of deltamethrin in H. armigera populations collected in various cotton areas from 1998 to 2001.

Bioassays. In the same period, bioassays with topical applications were used to give results on the annual evolution of the pyrethroid resistance level in field populations. Dose-mortality regression lines were done with deltamethrin in the first generation of field populations collected from different locations from 1998 to 2001. LD50s varied from 0.30 to 1.05 µg/g indicating a low resistance level (maximum RF=20) (Fig.2).

To know the seasonal evolution of the pyrethroid resistance level in a location, dose-mortality regression lines were done in the first generation of all *H. armigera* populations collected each year from various host plants in Bouaké area. Data showed that deltamethrin LD50s in *H. armigera* varied from 0.4 to 2 µg/g (Fig. 3). Therefore, the resistance factor (RF) for field populations varied from 10 to 38 fold compared to the susceptible strain BK77. It was very difficult to find *H. armigera* in vegetables because of the sparse small plots and the high number of treatments. The ornamental flower *A. majus*, cultivated without any treatment in small plots near the Cotton Research Station of Bouaké, appeared to be very attractive for *H. armigera* and could be very useful in the future to collect populations throughout the dry season. The highest resistance level was annually observed in populations collected from cotton in October corresponding to the last period of treatments. The resistance level slightly decreased in the dry season as shown by the resistance level of the populations collected from ornamental flowers. This result was observed as well in *H. armigera* populations collected in Benin (Djinto et al., to be published) suggesting a fitness cost of the pyrethroid resistance. *H. armigera* populations collected in October from cotton in Bouaké seemed to be always the more resistant among field populations. This result can be explained by the high number of treatments in variety multiplication plots of the Cotton Research Station of Bouaké. Interestingly, the deltamethrin toxicity of

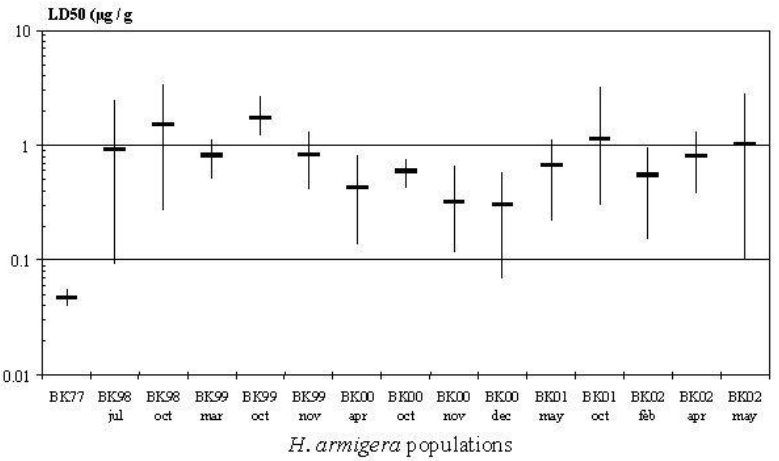

Figure 3: LD50 of deltamethrin in H. armigera populations collected in Bouaké area from 1998 to 2002 at various period in the year compared to LD50 of the susceptible strain (BK77).

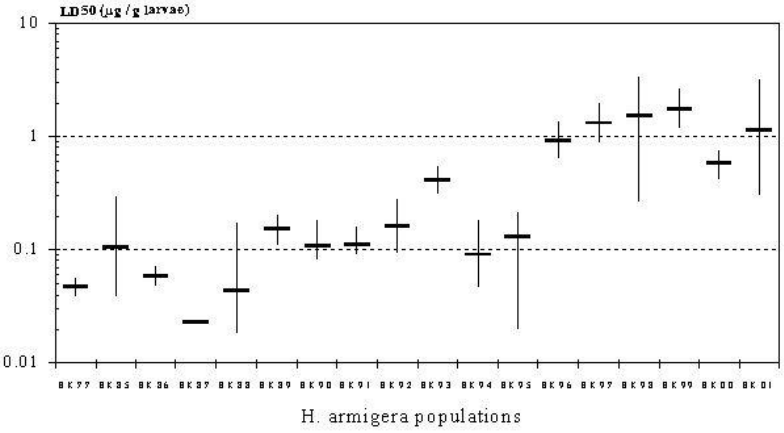

Figure 4: LD50 of deltamethrin (with 95% confidence intervals) in susceptible strain BK77 and annual Bouaké cotton field populations of H. armigera collected in October from 1985 (BK85) to 2001 (BK01).

these populations has been surveyed in each year since 1985 (Fig. 4). Three plateaux appeared showing schematically the apparition of pyrethroid resistance in *H. armigera* populations from Bouaké: susceptibility (1985-1988), decrease of susceptibility (1989-1995) and resistance (1996-2001).

Concerning the survey of pyrethroid alternatives, dose-mortality regression lines for endosulfan and profenofos have been done annually since 1999 in Bouaké populations. Endosulfan LD50s of field populations were at the same level than BK77 (Fig. 5). Thus endosulfan did not show any resistance in *H. armigera* cotton field populations. This cyclodiene is used from the 1970's in mixtures with DDT and methyl-parathion. It was replaced by pyrethroids in 1984. But from the development of pyrethroid resistance, endosulfan was reused alone with success (Ochou and Martin, 2002). From this result, it appears that this success partially originates from the absence of cross-resistance with pyrethroids (Martin et al., 2002). It is the same for the profenofos that did not show any resistance in *H. armigera* field populations (Fig. 6). This organophosphorus compound was used since 1977 in mixtures with pyrethroids to control the mites. It was used alone for 2000 as pyrethroids alternative. No cross-resistance was detected with pyrethroids (Martin et al., 2002).

DISCUSSION Results obtained on larvae with the application of discriminating doses by vial test method confirmed the presence of resistant *H. armigera* in all the field populations collected in Côte d'Ivoire. The larva vial test was not an accurate method. However, results obtained directly in the field were indicators of the pyrethroid resistance and could be confirmed with the bioassay method. Bioassays results showed that the deltamethrin resistance level in *H. armigera* populations could be considered as low in Côte d'Ivoire (in average RF<20) whatever the host plant, the collecting date, and the location of the population. The deltamethrin resistance level was generally highest in populations collected at the end of cotton treatments and decreased during the dry season. Therefore the pyrethroid resistance appeared globally stable from 1998 to 2002. This result may be an indicator of the success of the resistance management program.

That no resistance was detected for endosulfan and profenofos in field populations indicated the success of these pyrethroid alternatives. Therefore, endosulfan and profenofos resistance was showed in *H. armigera* from Pakistan (Ahmad et al., 1995) and Australia (Forrester et al., 1993; Gunning et al., 1995) indicating the risk of introduction of these genotypes in West Africa and their further selection. Moreover, cotton insecticides were frequently used in vegetable crops during the dry season to control *H. armigera*. So monitoring of insecticides resistance must be occurring and new molecules such as indoxacarb and spinosad, efficient to control *H. armigera* (Ochou and Martin, 2002), have to be used in mosaic with endosulfan and profenofos to limit the risk of selection of new cases of resistance.

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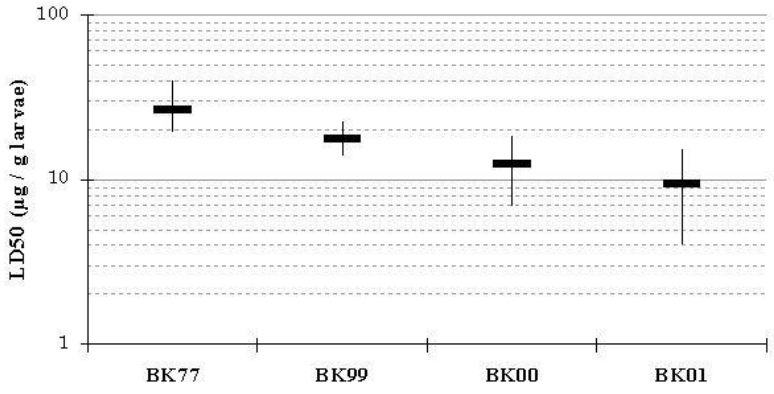

Figure 5: LD50 of endosulfan in susceptible strain BK77 and in three field populations collected from cotton in Bouaké in 1999 (BK99), 2000 (BK00) and 2001 (BK01).

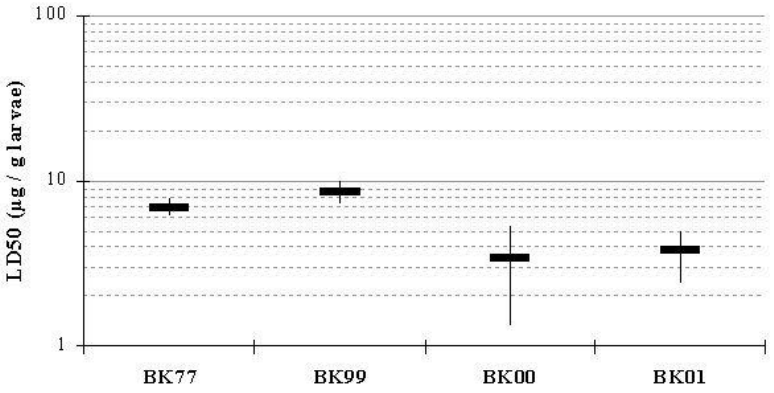

Figure 6: LD50 of profenofos in susceptible strain BK77 and in three field populations collected from cotton in Bouaké in 1999 (BK99), 2000 (BK00) and 2001 (BK01)

Vassal, J. M., Vaissayre, M. and Martin, T. (1997). Decrease in the susceptibility of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) to pyrethroid insecticides in Côte d'Ivoire. Resistance Pest Management 9, 2, 14-15.

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Susceptibility Level of Colorado Potato Beetle (*Leptinotarsa decemlineata* Say) to some Pyrethroids and Nereistoxin Derivative (Bensultap) Insecticides in Poland in 2002

INTRODUCTION Poland is a major producer of potatoes (*Solanum tuberosum*). This plant is cultivated on about 1,000,000 ha (2002) in this country but the average crop is very low - 17.2 t/ha. Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say) is the most serious potato pest in Poland and considered to be the Polish pest with the highest likelihood of developing insecticide resistance (Wegorek et al. 2001) All classes of insecticide have been widely used to control CPB in Poland. A 50 year period of intensive selection pressure has lead to CPB resistance to five major chemical classes of insecticide: chlorinated hydrocarbons, organophosphates, carbamates, nereistoxin analoques, and pyrethroids (Pruszynski et al. 1988, Wegorek et al. 2001).

For many years pyrethroids (deltamethrin, cypermethrin, alpha-cypermethrin, zeta-cypermethrin, lambda cyhalothrin, esfenvalerate, fenvalerate, and ethofenprox) and bensultap have held the primary place in CPB control in Poland. Over a period of 26 years for pyrethroids and 17 years for bensultap, these two classes of insecticides have been most commonly used for controlling CPB in Poland (Szczesna et al. 1990, Przybysz et al. 1996, Pawinska M. 2000, Mrowczynski M. 2000). Nowadays the use of pyrethroids and the nereistoxin derivative (bensultap) is systematically decreasing. The cases of observed CPB resistance for both classes under practical conditions have increased.

Bioassays of determined pyrethroids and nereistoxin derivative (bensultap) for resistance monitoring in CPB were performed in western, central, and northern regions of Poland (Institute of Plant Protection in Poznan, Plant Breeding and Acclimatization Institute in Bonin, and Institute of Organic Industry in Warsaw). Project P06r 126 21 is supported by the Polish State Committee For Scientific Research.

METHODS

Laboratory Tests: In laboratory tests the standard method recommended by Insecticide Resistance Action Committee (IRAC method nr.7) was used. Fourteen insect populations were used from three regions of Poland: 5 populations from the western region - Winna Gora, Rogalinek, Plewiska, Skoki, and Bolewice; 3 populations from northern region - Czarnoszyce, Bonin, and Zamarte; and 6 populations from the central region - Bielawy, Rudka, Radachowek, Gorzno, Chobot, and Rabiez. A representative sample of CPB larvae (2-st-instar) in selected field populations and sufficient non-infested, untreated leaves were collected for testing.

Chemicals:

Pyrethroids -

- alpha-cypermethrin (commercially-available product Fastac 100 EC) 20, 10, 5 ppm concentrations were tested (recommended concentration in Poland: 20-33 ppm)
- lambda-cyhalothrin (commercially-available product Karate 025 EC) 10, 5, 2,5 ppm concentrations were tested (recommended concentration in Poland: 15-25 ppm)
- deltamethrin (commercially-available product Decis 2,5 EC) 20, 10, 5 ppm concentrations were tested (recommended concentration in Poland: 15-25 ppm)

Nereistoxin analogues -

- bensultap (commercially-available product Bancol 50 WP) 20, 10 ppm concentrations were tested (recommended concentration in Poland: 500- 667 ppm)

Accurate dilutions of the tested compound from commercially available products were used in determined doses.

Leaves were dipped in water for untreated control and other leaves in tested insecticide concentration liquids for about five seconds and placed on paper towel to dry. Untreated and treated dry leaves were placed into 10 cm diameter Petri dishes with 10 cm diameter filter paper and 10 larvae were placed in each dish. 3-5 replicates were conducted for each concentration and control.

A final assessment of the lethal effects of the pyrethroides insecticides were determined after 72 hours and assessment of the nereistoixin analogue (bensultap) was determined after 120 hours after application and expressed as percent mortality at each dose, correcting for untreated (control) mortalities using Abbott's formula (Abbot 1925). Untreated mortalities were quoted.

At each assessment, larvae were classed as either: (a) unaffected, giving a normal response (such as taking a co-ordinated step) when gently stimulated by touch, or (b) dead or affected, the latter giving an abnormal response to stimulation. Corrected Mortality = 100 x (P-C/100-C) where P = % mortality in treatment, C = % mortality in controls.

Tests were performed in the laboratory with conditions of 22-24 degrees C and a photoperiod of 16:8 (L;D).

RESULTS and DISCUSSION

Populations from all three provinces demonstrated some level of resistance to one or more pyrethroids insecticides (deltamethrin, alpha-cypermethrin, lambda-cyhalothrin). In laboratory studies (2002) the pyrethroids insecticides were less effective in controlling CPB larvae. Survival at 20ppm concentration in the case of alpha-cypermethrine and deltamethrin and 10ppm concentration in the case of lambda-cyhalothrin indicated the occurence of strong field resistance in tested populations.

The populations from the central and northern regions of Poland were more resistant to tested pyrethroids than populations from the western region. CPB strains tested in 2002 were not tolerant to nereistoxin analogue (bensultap). The LC50 data for bensultap from 1998 (western populations) ranged from 11.86 to 14.59 (Wegorek et al. 1999) were not significantly different than data in 2002. The results indicated that populations tolerant for pyrethroids were not cross-resistant to bensultap. The widespread use of pyrethroids in Poland can lead to control failure. Understanding the conditions that favour the development, the causes, and the mechanisms of resistance are the crucial challenges for the future of pyrethroid use to CPB control in Poland.

The constant monitoring of CPB susceptibility levels to insecticides used in Poland and studies on mechanisms of CPB resistance to those insectivides will allow for the development of the best strategy for delaying CPB resistance. At present the general principles of the management strategy involve the rational application of all recommended insecticides

Table 1. Susceptibility of Colorado potato beetle larvae L2 to alpha-cypermethrine in 2002. (western populations (W), northern (N), central (C)) (recommended concentration in Poland: 20 - 33 ppm).

Populations	alpha-cypermethrine		
	20 ppm - % mortality	10 ppm - % mortality	5 ppm - % mortality
Winna Gora (W)	100	97	88
Rogalinek (W)	98	96	90
Plewiska (W)	100	95	90
Skoki (W)	100	98	90
Bolewice (W)	98	96	91
Czarnoszyce (N)	84	86	64
Bonin (N)	88	78	82
Zamarte (N)	94	82	66
Bielawy (C)	92	84	52
Rudka (C)	100	92	88
Radachowek (C)	80	70	60
Gorzno (C)	96	73	66
Chobot (C)	90	86	72
Rabiez (C)	94	97	89

Table 2. Susceptibility of Colorado potato beetle larvae L2 to deltamethrine in 2002. (western populations (W), northern (N), central (C)) (recommended concentration in Poland: 15 - 25 ppm).

Populations	deltamethrine		
	20 ppm - % mortality	10 ppm - % mortality	5 ppm - % mortality
Winna Gora (W)	99	94	89
Rogalinek (W)	98	95	88
Plewiska (W)	97	96	90
Skoki (W)	95	90	90
Bolewice (W)	95	90	88
Czarnoszyce (N)	92	90	86
Bonin (N)	72	70	70
Zamarte (N)	64	52	48
Bielawy (C)	88	82	74
Rudka (C)	98	70	72
Radachowek (C)	96	72	84
Gorzno (C)	92	92	76
Chobot (C)	91	74	55
Rabiez (C)	92	No data	92

Table 3. Susceptibility of Colorado potato beetle larvae L2 to lambda-cyhalothrine in 2002. (western populations (W), northern (N), central (C)) (recommended concentration in Poland: 15 - 25 ppm).

Populations	lambda-cyhalothrine		
	10 ppm - % mortality	5 ppm - % mortality	2.5 ppm - % mortality
Winna Gora (W)	99	94	88
Rogalinek (W)	95	93	82
Plewiska (W)	95	90	77
Skoki (W)	95	90	68
Bolewice (W)	94	90	59
Czarnoszyce (N)	50	28	16
Bonin (N)	28	10	10
Zamarte (N)	50	42	40
Bielawy (C)	50	10	0
Rudka (C)	60	14	2
Radachowek (C)	-no data	-no data	-no data
Gorzno (C)	94	90	84
Chobot (C)	43	0	0
Rabiez (C)	-no data	no data	-no data

Table 4. Susceptibility of Colorado potato beetle larvae L2 to bensultap in 2002. (western populations (W), northern (N), central (C)) (recommended concentration in Poland: 500 - 667 ppm).

Populations	Bensultap	
	20 ppm - % mortality	10 ppm - % mortality
Winna Gora (W)	90	56
Rogalinek (W)	90	75
Plewiska (W)	89	70
Skoki (W)	90	65
Bolewice (W)	80	75
Czarnoszyce (N)	82	80
Bonin (N)	84	74
Zamarte (N)	100	86
Bielawy (C)	79	39
Rudka (C)	32	60
Radachowek (C)	33	34
Gorzno (C)	86	67
Chobot (C)	49	36
Rabiez (C)	85	42

and their rotation including different modes of their toxic action (Wegorek et al. 1998).

To conserve as long as possible the high insecticidal potency of all chemical classes of insecticides in Poland it is necessery to follow the general resistance management guidelines, which were elaborated by the Institute of Plant Protection in Poznan with help of IRAC (Wegorek et al. 2002). These guidelines could be adopted in all areas of potato insecticidal protection in Poland. For this reason simple field tests are recommended.

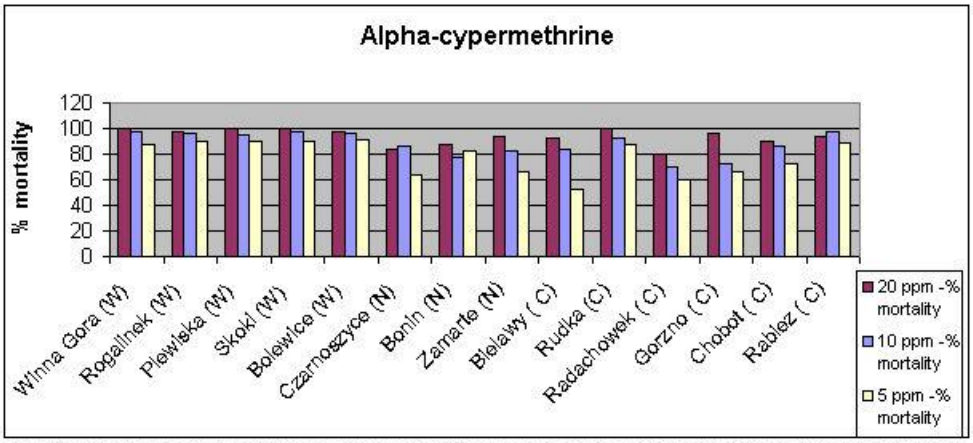

Fig 1. Comparison of CPB (larvae L2) mortality among different population from Poland using discriminating concentrations of 20, 10 and 5 ppm of alpha-cypermethrine.

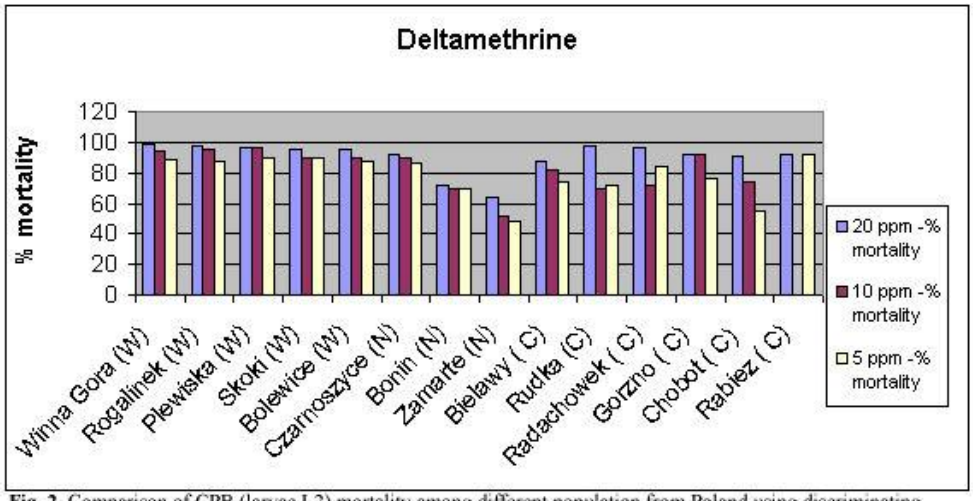

Fig. 2. Comparison of CPB (larvae L2) mortality among different population from Poland using discriminating concentrations of 20, 10 and 5 ppm of deltamethrine.

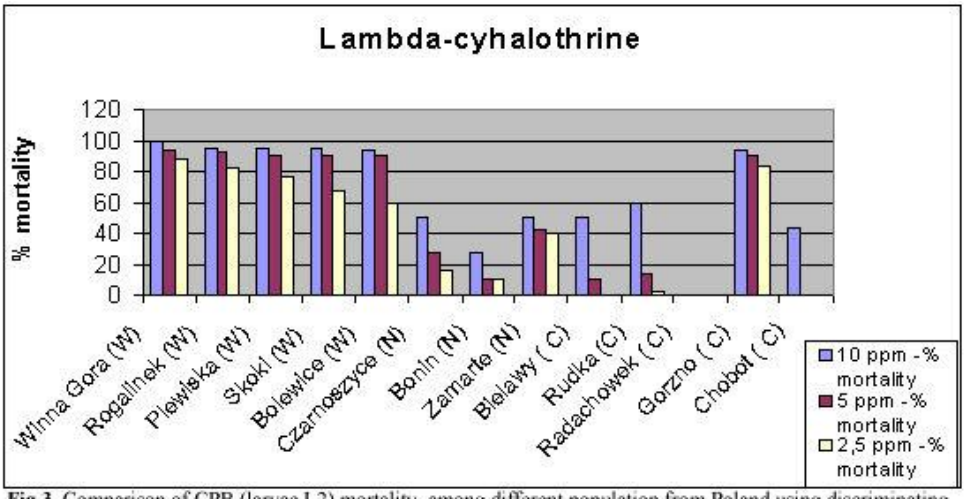

Fig 3. Comparison of CPB (larvae L2) mortality among different population from Poland using discriminating concentrations of 20, 10 and 5 ppm of lambda-cyhalothrine.

The Insecticide Susceptibility Test Method for CPB Resistance Detection

Plant protection advisors and farmers in Poland should consider using the simple IRAC method nr.7 before "high-risk" CPB population treatment to detect the field efficacy of insecticides for CPB control.

1. Collect a representative sample (300 - 400) of CPB larvae L2 (or L3) stage in the different places of the field.
2. Collect sufficient non-infested, untreated leaves to perform the test.
3. Wearing solvent-proof gloves, syringe or pipette and prepare accurate recommended (field concentration) water dilutions from commercially available products.
4. Dip leaves in water for untreated control and other leaves in the tested liquid for 5 seconds and place on paper towel to dry.
5. Place the untreated and treated dry leaves in containers, which must be suitable for keeping enough leaf material in good condition for up 2 -3 days.
6. Add equal numbers of L2 (or L3) CPB larvae to each container (one container should be use for

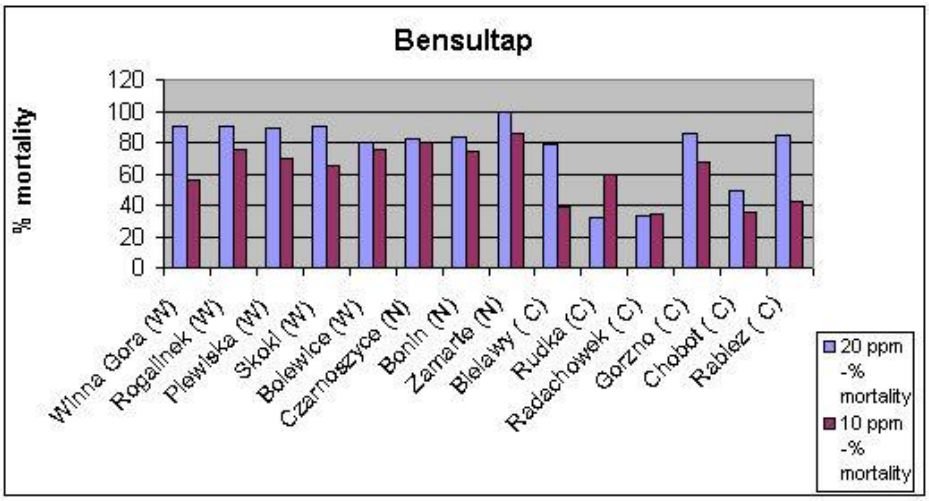

Fig 4. Comparison of CPB (larvae L2) mortality among different population from Poland using discriminating concentrations of 20, 10 ppm of bensultap.

untreated control) but no more than 20 larvae/container and store the containers in the area where they are not exposed to direct sunlight or extremes temperature (a mean temperature of 22-240 C is preferred).

7. For rapidly acting insecticides (pyrethroids, chloronicotinyls, phenylpyrazoles, organophosphores, and carbamates) a final assessment of larval mortalities is made after 48 h. For slowly acting insecticides (bensultap, *Bacillus thuringiensis*, etc.) assess after 120 h.

Larval mortality in control container should be less than 10%. Larval mortality in treatment should to be 100%. If 1 or more larvae survive the treatment test the product should not be recommended to control the tested population.

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Susceptibility Level of Colorado Potato Beetle (*Leptinotarsa decemlineata* Say) to Phenylpyrazole and Chloronicotinyl Insecticides in Poland in 2002

INTRODUCTION Insecticides from the phenylpyrazole and chloronicotinyl (neonicotinoid) classes are relatively new for the control of CPB Poland (fipronil - 1996, acetamiprid - 1996, imidacloprid - 1998, thiamethoxam - 1999, thiacloprid 2002) (Pawinska et al. 1995,1996, Mrowczynski et al.1997, Wegorek et al. 2001). The importance of both new classes of insecticide for CPB control in Poland will be systematically increased and it may be assumed that the CPB resistance will occur in these new classes too. The use of bensultap, the inhibitor of the acetylocholine receptor, the target of chloronicotinyl insecticides (Yamamoto et al.1995) was widespread in Poland during 17 last years.

The constant monitoring of CPB susceptibility level to these classes of insecticides in Poland and

studies on mechanisms of CPB resistance to them will allow the development of an enterprising strategy for delaying CPB resistance. Bioassays of phenylpyrazoles and chloronicotinyls for resistance monitoring in CPB have been performed at the Institute of Plant Protection in Poznan since 2001(Wegorek et al. 2001)

MATERIALS and METHODS

Laboratory Tests Method: In laboratory tests the standard method recommended by the Insecticide Resistance Action Committee (IRAC method nr.7) was used. A representative sample of CPB insects (larvae 2-st-instar or second generation adults) in selected field populations and on sufficient non-infested, untreated leaves was collected for testing.

CPB Populations: The 6 insect populations originated from three regions of Poland (Winna Gora, Rogalinek, Skoki, and Bolewice from western Poland, Rarwino from northern Poland, and Badrzychowice from central Poland).

Accurate dilutions of the tested compound from commercially available product were used in determined doses.

Chemicals:

Chloronicotinyls

- imidacloprid (commercially-available product Confidor 200 Sl) LC50 and LC 95 were calculated (recommended field concentration 30-50 ppm)
- acetamiprid (commercially-available product Stonkat 160 SL) LC50 and LC 95 were calculated (recommended field concentration 30-50 ppm))
- thiamethoxam (commercially-available product Actara 25 WG)) LC50 and LC 95 were calculated (recommended field concentration 50-67 ppm)

Phenylpyrazoles

- fipronil (commercially-available product Regent 200 S.C.) LC50 and LC 95 were calculated (recommended field concentration 50- 67 ppm)

Leaves were dipped in water for untreated control and other leaves in tested insecticides liquid for about five seconds and placed on paper towel to dry. Untreated and treated dry leaves were placed into 10 cm diameter Petri dishes with 10 cm diameter filter paper, and 10 larvae or adults insects were placed in each dish. 5 or 10 replicates were conducted for each concentration and control.

A final assessment of the lethal effects of the phenylpyrazoles and chloronicotinyls insecticide was determined 72 hours after application and expressed as percent mortality at each dose, correcting for untreated (control) mortalities using Abbott's formula (Abbot 1925). Untreated mortality was quoted. The lethal concentration at which > 50% of insects are killed -LC 50 (ppm) and lethal concentration at which > 95% of insects are killed -LC 95 (ppm) were calculated using the probit analysis Finney method (Finney 1952).

At each assessment, larvae or beetles were classed as either (a) unaffected, giving a normal response (such as taking a co-ordinated step) when gently stimulated by touch, or (b) dead or affected, the latter giving an abnormal response to stimulation or showing abnormal growth which should be described. Corrected Mortality = 100 x (P-C/100-C) where P = % mortality in treatment, C = % mortality in controls.

Tests were performed in the laboratory with conditions of 22-24 degrees C and a photoperiod of 16:8 (L;D).

RESULTS and DISCUSSION Laboratory investigations gave no indication of resistance elicited by tested populations of CPB to insecticides from phenylpirazole and chloronicotinyl groups (fpronil, thiamethoxam, imidacloprid, and acetamiprid). No significant differences in LC50 and LC95 values and no evidence

Table 1. Susceptibility of Colorado potato betle to imidacloprid - 2002. (western populations (W), northern (N), central (C)) (recommended concentration in Poland 50 ppm).

Population	beetles		larvae	
	LC 50	LC95	LC 50	LC 95
1-Badrzychowice (S)	3.8	27.4	0.9	2.9
2-Bolewice (W)	3.7	14.4	0.8	2.9
3-Dębki (N)	3.3	19.8	0.6	3.3
4-Rarwino (N)	4.7	16.7	0.7	3.9
5-Rogalinek(W)	2.9	11.9	0.4	2.9
6-Skoki (W)	3.9	13.4	0.6	2.0
7-Winna-Góra (W)	3.5	15.7	0.6	2.8

Table 2. Susceptibility of Colorado potato betle to acetamiprid - 2002. (western populations (W), northern (N), central (C)) (recommended concentration in Poland 50 ppm).

Population	beetles		larvae	
	LC 50	LC95	LC 50	LC 95
1-Badrzychowice (C)	2.2	21.9	0.6	2.5
2-Bolewice (W)	1.8	10.8	0.2	2.6
3-Dębki (N)	1.9	12.2	0.4	3.3
4-Rarwino (N)	2.0	10.8	0.6	4.3
5-Rogalinek(W)	1.8	17.9	0.1	2.1
6-Skoki (W)	1.8	11.2	0.5	1.8
7-Winna-Góra (W)	2.2	6.7	0.1	1.9

Table 3. Susceptibility of Colorado potato beetle to thiamethoxam - 2002. (western populations (W), northern (N), central (C)) (recommended concentration in Poland 67 ppm).

Population	beetles		larvae	
	LC 50	LC95	LC 50	LC 95
1-Badrzychowice (C)	3.3	6.1	0.4	2.10
2-Bolewice (W)	3.8	5.2	0.4	1.15
3-Dębki (N)	3.1	2.3	0.6	1.20
4-Rarwino (N)	2.8	8.3	0.3	1.05
5-Rogalinek(W)	2.2	4.9	0.4	1.10
6-Skoki (W)	3.2	6.8	0.6	2.10
7-Winna-Góra (W)	2.4	4.4	0.2	1.80

of cross-resistance to pyrethroid insecticides were detected. The LC50 and LC95 values were very similar with those from 2001 (Wegorek et al. 2001), disclosing no increased tendency for any population to tolerate higher concentrations of the tested insecticides. The bioassay method reported is considered well suited for monitoring the response of CPB to phenylpirazole and chloronicotinyl insecticides. Since in one growing season CPB usually produces only one complete generation, and on average 1.5 applications are used on it, there is small risk of a fast development of high resistance to a given biologically active ingredient or a chemical group over a few seasons. However, a continual selection pressure with similar products must be avoided, and insecticides from different chemical classes and with different mechanisms of action should be used.

Using phenylpirazoles and chloronicotinyls in Poland we must remember that CPB is multiply and cross-resistant to five major groups of insecticides in United States (Forgash 1985), inclding resistance to *Bacillus thuringiensis* (Whalon 1997), abamectine (Argentine, Clark 1990), and imidacloprid (Grafius and Bishop 1995)) and tolerance to fipronil (Colliot et all 1992).

Table 4. Susceptibility of Colorado potato betle to fipronil - 2002. (western populations (W), northern (N), central (C)) (recommended fconcentration in Poland 67 ppm).

Population	beetles		larvae	
	LC 50	LC95	LC 50	LC 95
1-Badrzychowice (C)	0.75	1.90	0.11	0.40
2-Bolewice (W)	0.70	2.20	0.12	0.70
3-Dębki (N)	0.30	1.70	0.11	0.20
4-Rarwino (N)	0.60	1.90	0.07	0.20
5-Rogalinek(W)	0.45	1.50	0.09	0.30
6-Skoki (W)	0.25	1.20	0.10	0.40
7-Winna-Góra (W)	0.30	1.25	0.06	0.70

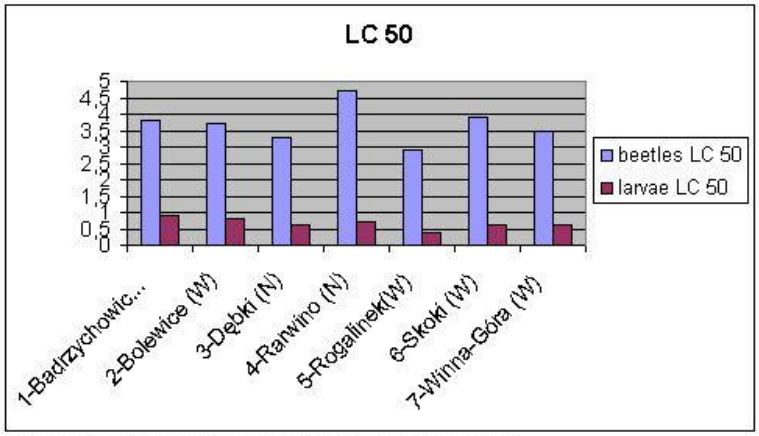

Fig. 1. LC50 value of imidacloprid for CPB beetles and larvae (L2).

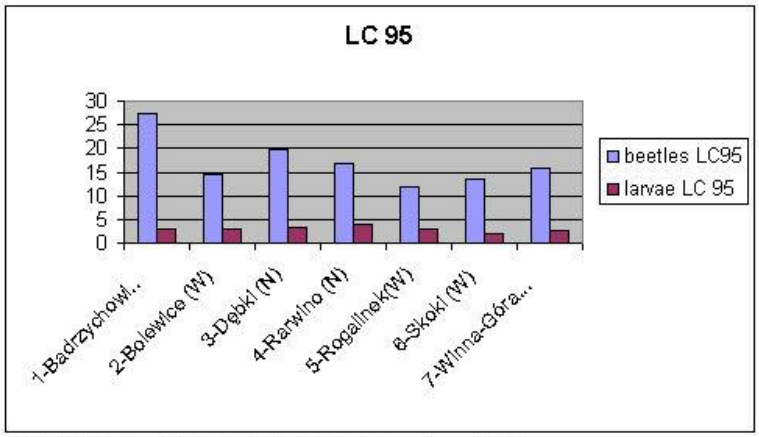

Fig. 2. LC95 value of imidacloprid for CPB beetles and larvae (L2).

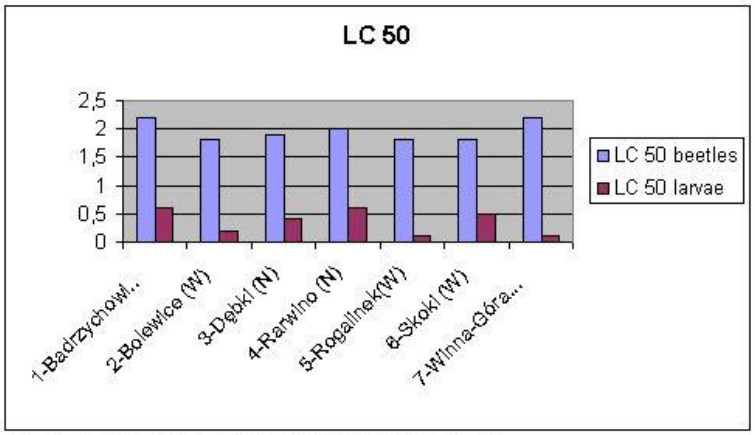

Fig. 3. LC50 value of acetamipridfor CPB beetles and larvae (L2).

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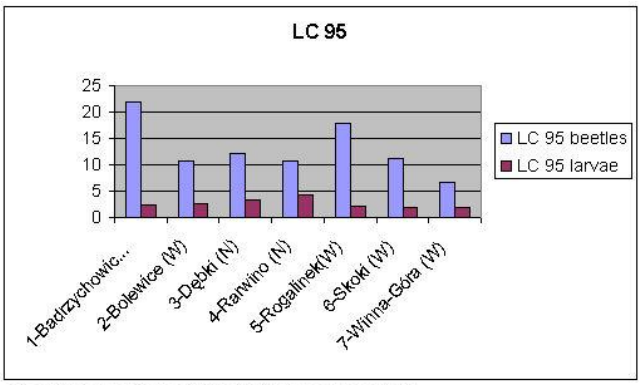

Fig. 4. LC95 value of acetamiprid for CPB beetles and larvae (L2).

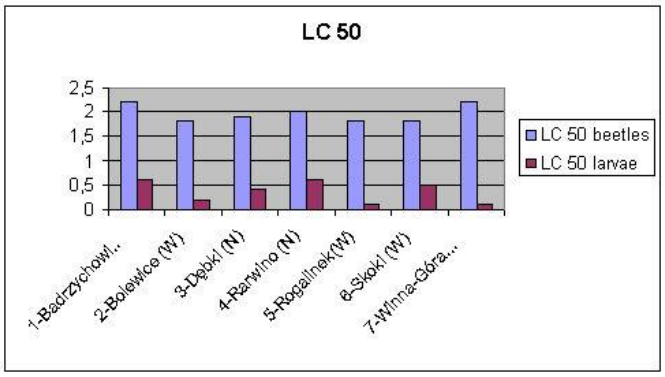

Fig. 5. LC50 value of thiametoxam for CPB beetles and larvae (L2).

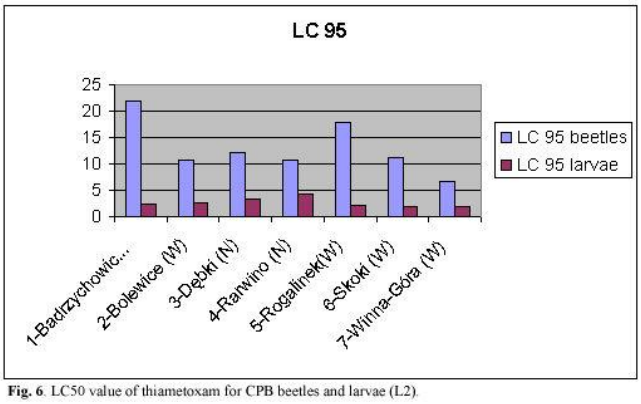

Fig. 6. LC50 value of thiametoxam for CPB beetles and larvae (L2).

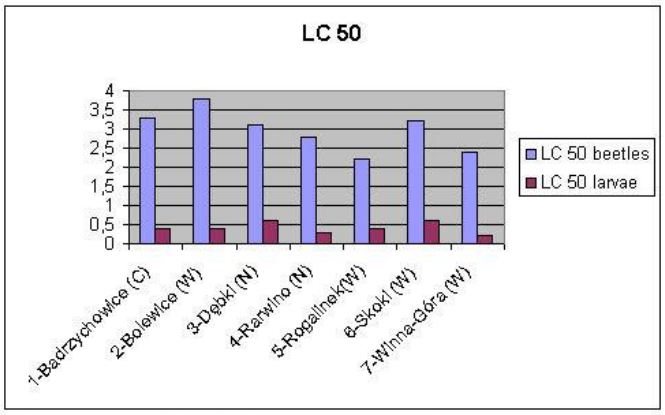

Fig. 7. LC50 value of fipronil for CPB beetles and larvae (L2).

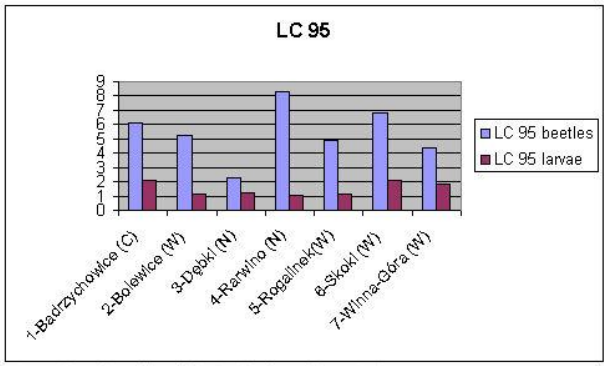

Fig. 8. LC95 value of fipronil for CPB beetles and larvae (L2).

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Relative Resistance in Open and Greenhouse Populations of *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) on Rose to Dimethoate and Acephate

ABSTRACT LC50 values for two commonly used insecticides viz., dimethoate and acephate in open field and greenhouse populations of *Scirtothrips dorsalis* Hood on rose collected from Bangalore, India were calculated. The LC50 values varied from 0.1072 - 0.0253 % in the case of dimethoate and 0.0309 - 0.1455 % in the case of acephate. Greenhouse populations of *S. dorsalis* have developed 1.5 and 4.7 fold resistance to dimethoate and acephate respectively in comparison to open field populations.

KEY WORDS: *Scirtothrips dorsalis*, insecticide resistance, rose

INTRODUCTION The chilli thrips, *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae) is one of the most devastating pests of several agricultural and

horticultural crops worldwide. Roses are being grown both under open and greenhouse conditions and *S. dorsalis* is a major pest of this crop, especially under greenhouse conditions. Dimethoate and acephate, apart from the new compounds ethofenprox and impadacloprid, are recommended for control of this pest (Jhansi Rani and Eswara Reddy, 2001; Nair et al., 1991). Growers have repeatedly observed that dimethoate and acephate have not given satisfactory control against this pest in and around Bangalore, particularly under greenhouse conditions. Reddy et al. (1992) have documented the relative resistance in thrips, *S. dorsalis*, to different conventional insecticides on chilli from Andhra Pradesh in India. However, information regarding relative susceptibility of *S. dorsalis* with regard to open and greenhouse populations from India is not available. Therefore, the present trial was taken up with the objective to determine the relative susceptibility of two populations of *S. dorsalis* on rose, from open fields and greenhouses, to dimethoate and acephate.

MATERIALS and METHODS Populations of *S. dorsalis* were collected both from the field and from greenhouses from the Indian Institute of Horticultural Research (IIHR), Bangalore. Two insecticides viz., dimethoate 30 EC (Rogor) and acephate 75 SP (Starthane) as commercial formulations were used for the study. The assay procedure followed was modified from Reddy et al (1992). Tender rose leaves were dipped in suspensions of different concentrations for each insecticide, air dried for 30 minutes, and introduced into glass vials. Using a fine brush, ten nymphs from homogeneous populations of *S. dorsalis* were carefully transferred to each concentration of insecticide treated leaves (open and greenhouse populations in two separate sets). The vials were sealed with parafilm and minute holes were made for ventilation. Leaves dipped in water alone were used as control. Each concentration of insecticide treatment and control were replicated thrice. Mortality counts were taken 24 hours after the release of the test insects. Based on the number of insects that responded to the different concentrations of insecticides, a probit analysis was carried out for arriving at LC50 values for both open field and greenhouse populations using a computer aided MSTATC package. The resistance index (RI) was computed according to the formula suggested by FAO (1979) as, RI = LC50 of resistant strain/LC50 of susceptible strain.

RESULTS and DISCUSSION Data on the LC50 values of the two insecticides between two populations of thrips, *S. dorsalis*, revealed that LC50 values varied from 0.1072 - 0.0253 % in the case of dimethoate and from 0.0309 - 0.1455 % in the case of acephate (Table 1). Greenhouse populations were less susceptible when compared to field populations in cases where both insecticides were tested. When the resistance index was taken into consideration, greenhouse populations of *S. dorsalis* had developed 1.5 and 4.7 fold resistance to dimethoate and acephate, respectively when compared to open field populations (Table 1). The differential response of thrips to the two insecticides in the study in terms of LC50 values was attributed to the development of resistance by *S. dorsalis* in greenhouse populations when compared to open field populations. Relatively less susceptibility of *S. dorsalis* populations in greenhouses may be attributed to their frequent exposure to different insecticides (nearly at fortnightly intervals) and the quick elimination of susceptible populations/genes in comparison to open populations. However, in the case of open populations, there is relatively more chance of passing of susceptible genes in successive generations by mating with resistant populations, resulting in more susceptible populations when compared to greenhouse populations.

Earlier reports have indicated that citrus thrips, *Scirtothrips citri* (Moulton) developed resistance in DDT, dimethoate, acephate, bendiocarb, and formentanate (Morse and Brawner, 1986 and Immaraju et al., 1989). *S. dorsalis*, a dominant species of thrips on roses, may have undergone selection for a number of insecticides in the past which might have led to cross-resistance to related compounds that are widely used. Hence, there is an urgent need to curb indiscriminate insecticide use on roses particularly in greenhouses. The result also suggests that future control programmes for *S. dorsalis* on rose in greenhouses need to incorporate a resistance management strategy as a major component.

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Table 1. Toxicity of insecticides to *S. dorsalis* populations from rose open fields and greenhouses.

Insecticide	Thrips populations from rose (greenhouse/open field)	No. of Insects	Slope	Variance of Slope	LC_{50} (% Conc.)	95% Confidence limits (Fiducial limits)	Relative resistance (Greenhouse to open field)
Dimethoate	Greenhouse	240	1.6371	0.17287	0.0253	0.0198-0.0308	1.5
	Open field	180	2.5186	0.19067	0.0172	0.0148-0.0197	
Acephate	Greenhouse	210	0.1595	0.15953	0.1455	0.1167-0.1741	4.7
	Open field	210	3.1922	0.21913	0.0309	0.0275-0.0344	

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Status of Pyrethroid Resistance in *Helicoverpa armigera* in India

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is charismatic and one of the most dreaded insect pests in agriculture, accounting for the consumption of over 30% of the total insecticide use worldwide. The frequent and rapid changes in cropping patterns and agro-ecosystems, the polyphagus nature of the pest, and its cosmopolitan abundance have accentuated the problem globally. The problems of this pest are magnified due to its direct attack on fruiting structures, its voracious feeding habits, its high mobility and fecundity, its multivoltine, overlapping generations with facultative diapause, its nocturnal behaviour, migration, and host selection by learning, and a propensity for acquiring resistance against insecticides (Satpute and Sarode, 1995; Sarode, 1999).

This pest has been recorded feeding on 182 plant species across 47 families in the Indian subcontinent, of which 56 are heavily damaged and 126 are rarely affected (Pawar et al., 1986). Losses due solely to this pest of up to Rs.10,000 million have been reported in crops like cotton, pigeonpea, chickpea, groundnut, sorghum, pearl millet, tomato, and other crops of economic importance (Raheja, 1996).

In India, *Helicoverpa* is represented by three species, with *H. armigera* constituting 99.2%, *H. peltigera* at 0.6%, and *H. assulta* at 0.2% of the total population (Pawar, 1998). In recent years, *H. armigera* has assumed such serious proportions in the country that for the past decade, farmers and plant protection agencies of central and state governments have virtually become perplexed regarding its control which ultimately has lead to an array of social, economical, and political problems. Of these, a primary problem concerns the development of resistance in this pest to a number of insecticides including pyrethroids.

Resistance to pyrethroids in *H. armigera* had been reported from a number of countries through out the world including India. The control failures of synthetic pyrethroids were first detected on pigeonpea against *H. armigera* at Lam farm, Guntur, A.P. in 1986. From 1987-88 to 1989-90, continuous monitoring and evaluation of the *H. armigera* population revealed that the resistance levels were low during 1988-89 in Andhra Pradesh, decreased by a factor of 10 (Table 1). During the cotton season 1989-90, it increased nearly 2-fold greater than that encountered in 1988-89. It may also be elucidated that the resistance response in Northern India, i.e. the Delhi and Karnal strains, remained constant. It was also shown that the resistance was not restricted to one or the other pyrethroid, but had extended to all the three pyrethroids used in the country viz., cypermethrin, fenvelerate, and deltamethrin (Mehrotra, 1991). The subsequent studies confirmed the major cause of crop failure in A.P. was resistance to synthetic pyrethroids in this pest (Srivastava, 1995). Although, the pyrethroid resistance in *H. armigera* was found to be restricted to an approximately 75km wide and 200km long belt comprising three districts of A.P. viz. Prakasham, Guntur, and parts of Krishna (Dhingra et al., 1988), the presence of increased tolerance to cypermethrin in the two strains collected in Tamil Nadu from cotton (1989) and groundnut (1991) suggested that resistance to pyrethroids was wide spread and could be featured throughout South India (Armes et al., 1992). In Coimbatore, resistance to cypermethrin was found to

Table 1. Relative resistance of various strains of *Helicoverpa* (3rd instar larvae) during 1987-88, 1988-89, and 1989-90 cotton seasons.

Location	Date of Testing	LC50 (%)	Fiducial Limits	RF
Delhi (lab strain)	01.02.88	0.024	0.0383 0.0145	1
Delhi (field strain)	02.05.88	0.064	1.1015 0.04	2.7
Delhi (field strain)	03.02.89	0.045	0.0763 0.0265	1.9
Delhi (field strain)	10.05.90	0.039	0.084 0.0161	1.6
Karnal	18.02.88	0.089	0.2228 0.0337	3.7
Karnal	08.05.89	0.089	0.1745 0.0453	3.7
Hisar	28.03.89	0.069	0.1235 0.0382	2.9
Guntur	25.01.88	3.875	4.91 3.057	161.5
Guntur	04.01.89	0.044	0.0813 0.0243	1.8
Guntur(Y)	04.01.89	0.0379	0.5192 0.276	15.8
ICRISAT 28.8	04.12.89 16.01.90	3 0.6914	- 1.136 0.409	125
Ongole	18.02.90	0.1509	0.3394 0.0673	6.3
Juzurru	23.01.88	7	-	290

be 25- to140-fold during 1992 and 1993 (Armes et al., 1996), but despite a reduction in the use of pyrethroids in the state over the past few years, resistance levels increased 64- to 207-fold (Kranthi et al., 2001).

The pyrethroid resistance in *H. armigera* has substantially increased in certain regions of Central and Northren India too. Although, the pyrethroid use in the districts Akola and Amravati of Central India is not as high as in the Warangal or Guntur districts of A.P., the highest level of pyrethroid resistance was recorded in these districts during the *H. armigera* outbreak of 1997-1998. This is in sharp contrast to resistance levels reported from the Bhatinda district of Punjab where pyrethroid use was reasonably high (Kranthi et al., 2001). In the Varanasi area in Uttar Pradesh, pyrethroid resistance was recorded in *H. armigera* larvae collected from early pigeonpea in November 1991 and from chickpea in March 1992 (Armes et al., 1992). These details reveal that the pyrethroid resistance has already moved from South India to other parts of India.

A survey of insecticide resistance in *H. armigera* on the Indian sub-continent during 1991-95 revealed that pyrethroid resistance levels were highest in the intensive cotton and pulse growing regions of Central and Southern India where excessive application of insecticides is common (Armes, 1996). However, it has been observed that several regions of the country where insecticides are used in a very low quantity, resistance in this pest can be expected over space and time (Tripathy and Singh, 1999). Among the several possibilities in this regard, many workers suspected it to be the resultant of immigration of resistant moths in a windward direction either from the North Indian states of Punjab and Haryana where to control this pest, pyrethroid use was ever increasing (Pedegley et al., 1987) or from Central India (Vaishampayan and Singh, 1995).

Thus, the possibility of dispersal or migration of *H. armigera*, that may occur at particular times during or after cropping seasons eventually influence the resistance patterns across the country. Hence, revised insecticide resistance management (IRM) strategies are urgently required if further widespread failures to control this pest are to be avoided.

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Integrated Resistant Management of Codling Moth *Cydia pomonella* L. in Italy

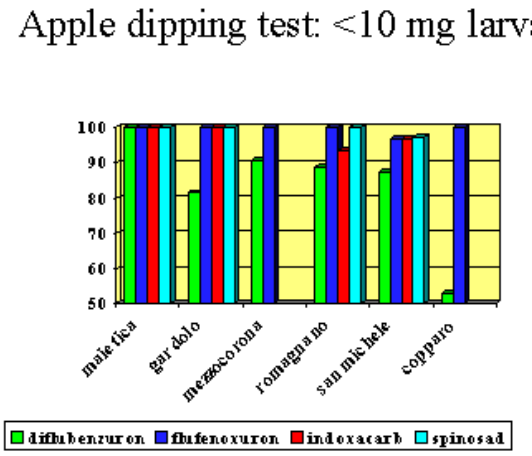
INTRODUCTION Trentino and Emilia-Romagna are two of the major apple-growing regions in Italy, with a yearly apple production of 450,000 and 210,000 tonnes a year, respectively. Emilia-Romagna is also the most important area for pear production, with 630,000 tonnes of pears grown annually. The codling moth (*Cydia pomonella*) is the key pest affecting Italian apple and pear orchards; those pest populations have until now been primarily managed by insect growth regulators (IGRs) and organo-phosphate applications (OP). Subsequent to the increase in the damage caused by the codling moth at the end of the 1990s, a monitoring programme was started in 1998 in order to detect resistance, and field trials were carried out in order to define the most appropriate IRM strategies (Ioriatti and Bouvier, 2000).

MONITORING of the RESISTANCE Resistance monitoring was carried out by using two methods: the attracticide susceptibility test was used to evaluate the azinphos-methyl activity on the feral male moths, while resistance to the IGR diflubenzuron was evaluated by treating the over-wintering larvae collected directly in the field (Ioriatti et al., 2003).

According to the results, codling moth resistance was spread differently across the two regions:

- in Emilia-Romagna half of the samples collected, when exposed to the resistance-monitoring test for azinphos methyl and diflubenzuron, showed a significant reduction in mortality in comparison to the susceptible strain;
- in Trentino the situation was less serious: the samples analysed proved to be less susceptible than the reference strain, although the difference was not statistically significant. A significant decrease in mortality was only detected in a small area.

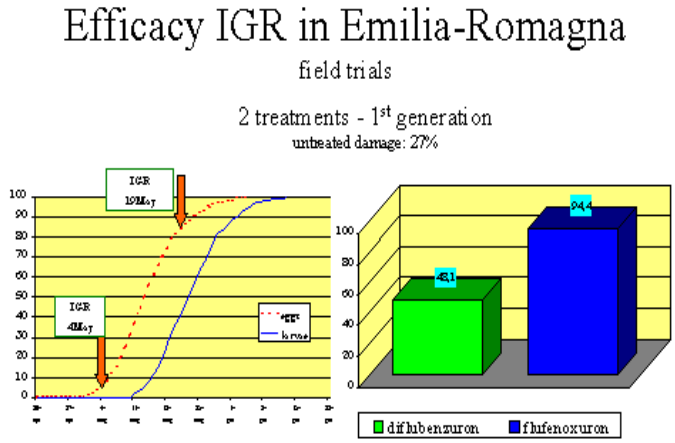
Recently, the apple-dipping test has been used to detect resistance directly on the larvae collected from the infested fruits in Trentino and Emilia-Romagna. According to the dose-response line evaluated for different insecticides by Charmillot (in press) on a susceptible laboratory strain, a discriminating dose was chosen for diflubenzuron, flufenoxuron, indoxacarb, and spinosad. The collected larvae were divided into four classes according to weight. The results obtained have shown that the mortality caused by the discriminating concentration was closely linked to the weight of the field-collected larvae, even in the susceptible strain. Only the first class of larvae, less than 10 mg in weight, responded to the treatment, as was expected from the previously used monitoring tests. The preliminary results confirmed that there was a large difference in mortality between susceptible and resistant (i.e.Copparo) strains when treated with diflubenzuron, while the tested codling moth strains showed only a slight reduction when treated with the other three insecticides (Fig.1).



EFFICACY of DIFFERENT INSECTICIDES upon SENSITIVE and RESISTANT POPULATIONS of *CYDIA POMONELLA* During the monitoring on the territory of the resistant CM populations, a series of field tests have been planned in the two regions with a view to assessing, on the one hand, which active substances were capable of constituting a valid alternative for the containment of damage within acceptable limits, and on the other, defining new strategies based on less toxic control measures.

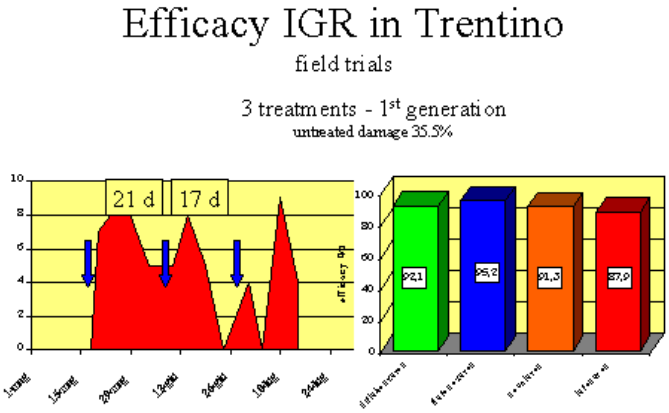
IGR: As a result of their environmentally friendly characteristics, insect growth regulators are still the most suitable insecticides for Integrated Fruit Production, and their use for the codling moth control is preferable when they prove to be effective. The field efficacy of IGR insecticides has been evaluated in these two fruit-growing regions. In Emilia-Romagna the trials were performed in orchards that had over the past few years recorded a rise in damage in spite of the increase in the number of insecticide treatments. On the other hand, in Trentino, where the level of the CM population is generally lower, the tests were carried out on fruit-farms where CM proved to be less susceptible than the reference strain, although the difference was not statistically significant.

Emilia-Romagna: The efficacy of two IGRs has been tested (Boselli et al, 2001) in a pear-growing fruit-farm with a population of resistant CM. Two treatments of flufenoxuron were applied against the first generation of carpocapsa, in comparision with two diflubenzuron treatments. The first treatment for each of the products was applied at the beginning of egg-laying. For both products the second application was repeated 15 days after the start of the treatment. Under a severe infestation (27% of the fruits attacked in the untreated plots) diflubenzuron cannot ensure an adequate crop protection (48.1% efficacy), while flufenoxuron achieved a good efficacy rate (94.4% efficacy) (Fig.2). These results confirm that flufenoxuron loses its ovicidal effect when used against a resistant population, while maintaining its efficacy upon the larvae (Sauphanor et al., 1998).

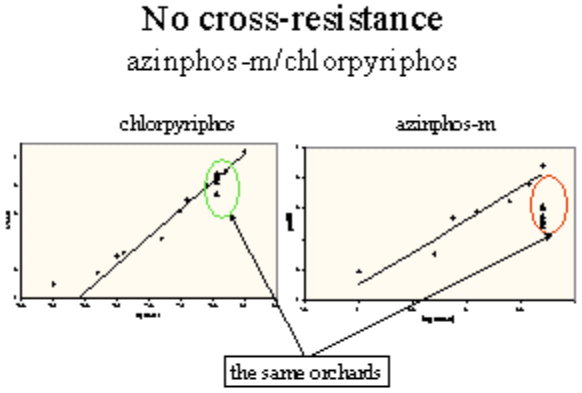


Trentino: Several IGRs (flufenoxuron, diflubenzuron, novaluron, lufenuron) were compared on an apple orchard against the first generation of CM. The first generation lasts longer than in the southern regions and three insecticide applications were needed to cover the entire egg-laying period. At the end of the first generation fruit damage in the untreated plot was

35%. The efficacy of the 4 IGRs did not differ greatly and ranged from 90 to 95% (Fig.3).



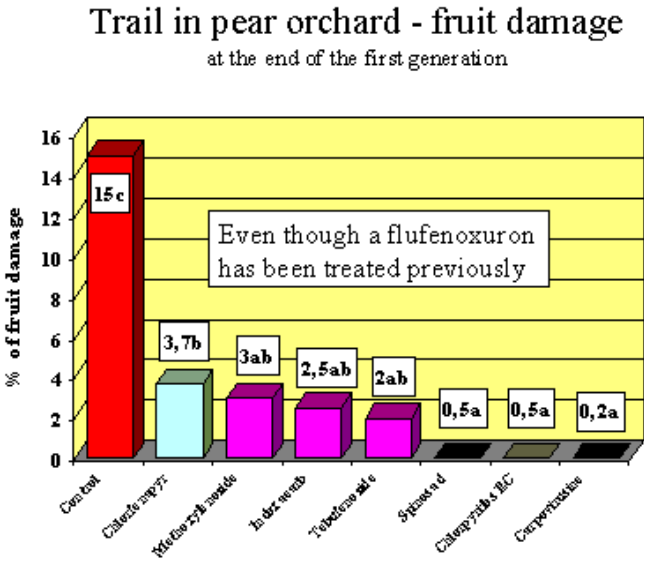
Chlorpyriphos: It is used in Emilia-Romagna and Trentino for pest-control in orchards affected by a high CM density and where resistance to either azinphos-m or diflubenzuron was detected. The choice was made according to the results that demonstrated no cross-resistance with azinphos methyl. As a matter of fact, the attracticide susceptibility test carried out on male moths showed that there was not a reduction in mortality when applied on the azinphos-resistant strain of codling moth (Fig. 4).



In Emilia-Romagna field tests were again carried out on the first generation. Such tests comprised the two applications of chlorpyriphos targeted to the new-born larvae, preceded by an action with different IGRs (diflubenzuron, flufenoxuron, esaflumuron teflubenzuron, methoxyfenozide, triflumuron, lufenuron) applied at the beginning of the egg-laying period. As a further treatment two applications of chlorpyriphos were applied. All the treatments were statistically differentiated from the untreated plot, but not among themselves. Indeed, just two applications of chlorpyrifos provided the same efficacy as those strategies where an IGR was applied at the beginning of the season.

The EVALUATION of NEW STRATEGIES BASED on LESS TOXIC CONTROL ACTIONS As chlorpyriphos does not have a good profile in terms of toxicity for humans and beneficial organisms, new strategies have been evaluated based on less toxic control measures with a view to replacing chlorpyrifos in the IRM.

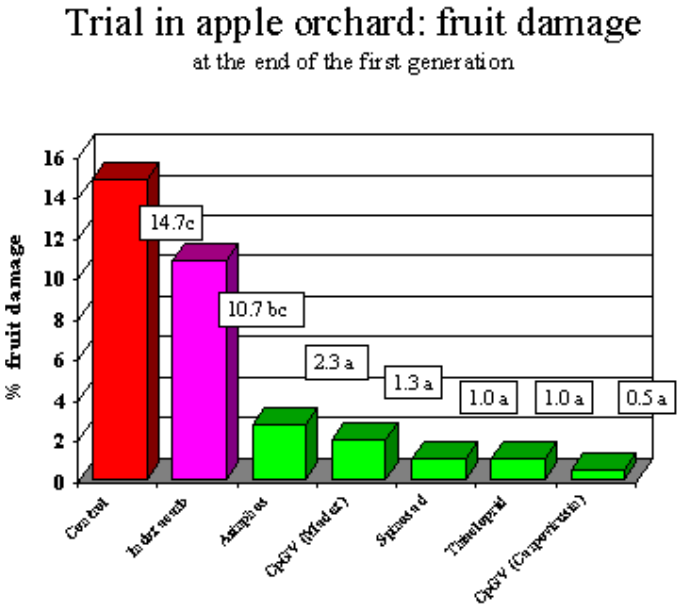
In Emilia-Romagna, field trials were conducted in a pear orchard where the previous year's harvest fruit damage had been 85%, in spite of the application of as many as 12 treatments. The efficacy of different insecticides was assessed against the first generation of carpocapsa (Boselli et al, 2001). All the treatments were based on one application of flufenoxuron, at the start of egg-laying, followed by two larvacide treatments with different active ingredients (carpovirusine, chlorpyriphos, chlorfenapyr, methoxyfenozide, indoxacarb, tebufenozide, and spinosad) against the new-born larvae. The results at the end of the first generation show that there were no significant differences between six of the seven insecticides compared. In this case there is a suspicion that the flufenoxuron applied at the start of the season had actually contributed to significantly limiting the final damage in all the experimental regimes, thus reducing the differences between the various products being tested (Fig. 5). Only the fruit damage in the chlorfenapyr plots resulted significantly higher than the



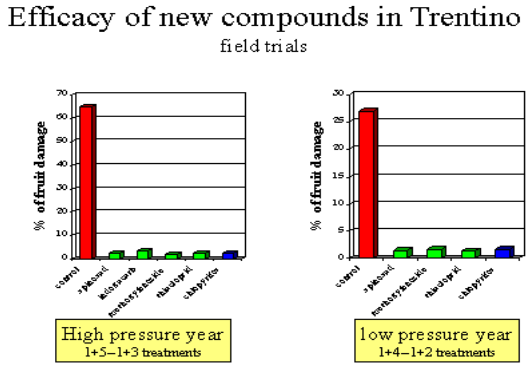
others.

New field trials were organised in orchards where the presence of resistant CM populations had been demonstrated, in order to evaluate the efficacy of the insecticides when used alone, with no IGR as the initial treatment. The products used were the granulosis virus and new products like indoxacarb, spinosad, and thiacloprid; azinphos-methyl was used as a standard of reference. The treatments were applied at the start of egg hatching and repeated eight days later (Boselli et al, 2001).

The results support the excellent local larvicide activity of the virus-based products, spinosad and thiacloprid, whose efficacy was superior, even if not in a statistically significant way, to azinphos-methyl. Instead, the average damage recorded in the plots treated by indoxacarb was greater than with the compared insecticides, thus not ensuring sufficient protection (10.7% damage) (Fig. 6).



In Trentino the field experiments were carried out in different years and with different CM population densities. The insecticides evaluated were spinosad, methoxyfenozide, thiacloprid, and indoxacarb as compared with chlorpyriphos. Each experimental regime comprised a treatment with an IGR at the start of egg-laying. Different insecticides were applied according to the intensity of egg-laying recorded by scouting in the orchard. Only chlorpirifos was applied curatively, 3 times in high-pressure years and 2 times in low-pressure years. In this context, spinosad and thiacloprid resulted to be as effective as methoxyfenozide and indoxacarb (Fig. 7).



CONCLUSIONS Technical recommendation IRM strategies were applied in both regions after some insecticide resistance was first detected. The first objective to be achieved in the farms with high fruit damages was to reduce such damage within acceptable levels. Hence, plant protection strategies were developed that provided for integration between biological and chemical products. As regards the IGRs, diflubenzuron was substituted by flufenoxuron, which showed it could maintain a good degree of efficacy in populations resistant to this class of product. The use of flufenoxuron on pear orchards, however, is not advisable because of its secondary effects on psilla populations.

Chlorpyriphos, because of its demonstrated lack of cross-resistance with the azinphos-resistant strain, was critical in managing resistance in both regions. The use of chlorpyriphos has to be limited due to its high toxicity for humans and its negative side effects on beneficial organisms. The reduction of organo-phosphates use has for the moment been made possible by the use of the granulosis virus that demonstrates an efficacy equal to or superior to chemical products. Hence, it is now being widely employed in Emilia-Romagna. Moreover, in this region CM-GV and the application of mating disruption are the main strategies suggested for orchards with low CM population levels for the resistance management.

In Trentino, on the other hand, as the levels of the CM population are generally quite low, the wide application of mating disruption allowed pesticide resistance to be successfully managed. Furthermore, according to the results of the field trials carried out in these two fruit-growing areas, it has been shown that new pesticides, such as spinosad and thiacloprid, could be introduced into the IRM programs. Nevertheless, attention should be paid to their possible negative side-effects against the beneficial organisms controlling the pear-psilla populations.

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Fungicide Resistance

Resistance to QoI Fungicides in *Podosphaera xanthii* Associated with Reduced Control of Cucurbit Powdery Mildew in Research Fields in the Eastern United States

INTRODUCTION Application of fungicides continues to be the principal practice for managing powdery mildew, the most common disease of cucurbit crops throughout the world. Powdery mildew, which is caused by *Podosphaera xanthii*, needs to be controlled on both leaf surfaces to avoid premature death of leaves. It is especially important to control powdery mildew on the underside of leaves where conditions are more favorable for disease development than on upper surfaces. Using systemic or translaminar fungicides is the best approach. Unfortunately, most of these fungicides are at risk for resistance development because they have single-site modes of action. The cucurbit powdery mildew fungus has demonstrated a high potential for developing resistance. Each chemical class active for powdery mildew that is at risk for resistance somewhere in the world following repeated use has developed resistance. Presence of resistant strains has been associated with control failure. Thus managing fungicide resistance is an important aspect of effectively managing powdery mildew (McGrath 2001).

The fungicide program that has been recommended recently in the United States is a strobilurin fungicide (azoxystrobin formulated as Quadris® or trifloxystrobin formulated as Flint®) applied in alternation with the DMI fungicide myclobutanil (formulated as Nova® or Rally®) tank-mixed with a protectant fungicide. This program uses two strategies for managing resistance:

1. alternation among systemic fungicides in at least two chemical classes, and
2. inclusion of protectant fungicides which are not at risk for resistance development because they have multi-site modes of action.

Strobilurins are in fungicide group 11, the quinone outside inhibitor (QoI) activity group. Myclobutanil is a triazole fungicide in the DMI activity group, which is fungicide group 3. Quadris and Nova have been available for commercial use for powdery mildew on cucurbits in the US beginning in 1998 when they received Section 18 registration in some states. US federal (Section 3) registration was granted for Quadris in March 1999 and Nova in May 2000. Flint was registered in September 1999.

Resistance is a major concern with QoI fungicides used for cucurbit powdery mildew due to past history with this pathogen developing resistance. Therefore it is important to monitor fungicide efficacy and, if poor performance occurs, to determine if it is due to resistance. These were the goals of this study. Efficacy was monitored in NY. Pathogen isolates were also assayed for resistance from other areas where poor control was reported.

MATERIALS and METHODS Quadris applied in alternation with Nova tank-mixed with chlorothalonil (formulated as Bravo Ultrex®) was included in fungicide efficacy experiments conducted with pumpkin from 1998 to 2002 in Riverhead, NY. Quadris was used alone on a weekly schedule for another treatment in 2002 because other fungicides used with Quadris in a program designed for managing resistance, as recommended for production fields, might provide enough control of powdery mildew to mask the presence of strobilurin resistant strains, especially if they were at a low frequency. Fungicides were applied weekly with a tractor-mounted boom sprayer. Applications were initiated after the IPM threshold of one leaf with symptoms of 50 old leaves examined was reached in all (or almost all) plots (McGrath, 1996b). A randomized complete block design with four replications was used. Upper and lower surfaces of 5 to 50 leaves, depending on incidence, in each plot were examined weekly for powdery mildew. Average severity for the entire canopy was calculated from the individual leaf assessments. Area under disease progress curve (AUDPC) values were calculated as a summation measurement of powdery mildew severity over the treatment period. Cultural practices used and other fungicide treatments tested are described in previous reports (McGrath 2000, 2002b; McGrath and Shishkoff 1999, 2001, 2003). One other treatment was Flint applied alone weekly in 1998 to obtain manufacturer-requested efficacy data.

An additional opportunity to evaluate Quadris applied alone or with Nova plus Bravo was provided

through an experiment conducted with muskmelon in 1999 to evaluate resistance management strategies for DMI fungicides. A preliminary report has been published (McGrath and Shishkoff 2000).

Fungicide sensitivity was determined for isolates obtained in 2000 from a fungicide efficacy experiment conducted in research fields in Freeville, NY, where control obtained with pyraclostrobin formulated as BAS 500 was not as effective as expected based on previous results (Zitter et al 2001). Isolates were also collected from the efficacy experiment conducted in Riverhead, NY, for comparison. A leaf-disk bioassay with 0, 0,2, 2, 20, and 40µg/ml pyraclostrobin was used to determine strobilurin sensitivity (McGrath et al 1996). Assays were repeated at least once for each isolate. Fungicide sensitivity was determined for isolates obtained from commercial fields in CA and fungicide efficacy experiments conducted in research fields in AZ, CA, GA and NC, as well as NY, where control obtained with strobilurin fungicides was not effective in 2002, contrasting with previous years. Leaves were collected on 22 July, 8 Oct, and 17 Oct after the last of four, five, and six applications of a strobilurin fungicide (Flint or Quadris) made in experiments conducted by J. David Moore in Chula, GA, M. T. McGrath in Riverhead, NY, and Gerald J. Holmes in Clayton, NC, respectively. Leaves were also collected from non-treated (control) plants and plants that had been treated weekly with triadimefon formulated as Bayleton® in GA. Isolates were obtained from the leaves. Strobilurin sensitivity was determined using 0, 0.5, 5, 50, and 100µg/ml trifloxystrobin. Sensitivity to triazole fungicides was also determined using 5, 50, and 100µg/ml triadimefon.

RESULTS and DISCUSSION

Strobilurins used alone on a weekly schedule (use pattern not labeled) did not effectively control cucurbit powdery mildew in 2002 in several fungicide efficacy experiments conducted in research fields. Isolates of *Podosphaera xanthii* were collected for testing from fields in CA, GA, NC, and NY. Powdery mildew had been controlled well by Flint or Quadris applied alone and Quadris applied in alternation with Nova and Bravo in fungicide efficacy experiments conducted yearly from 1998 to 2001 in NY (Tables 1 and 2). Flint used alone effectively controlled cucurbit powdery mildew in efficacy experiments conducted in other states before 2002. Degree of control in 2001 was 100% in GA (Langston et al 2002), 80% in MI (Hausbeck et al 2002), 91% in VA (Alexander et al 2002), and 90% in DE (Everts et al, 2002). Reduced control in 2002 was evident for the treatment with Quadris alternated with Nova and Bravo as well as when Quadris was used alone (Table 1). Reduced efficacy was also observed in KY, NJ, IL, MI, and VA in 2002 (W. Nesmith, S. A. Johnston, M. Babadoost, M Hausbeck, and C. Waldenmaier, personal communications).

Isolates of *Podosphaera xanthii* resistant to strobilurin fungicides were obtained from the GA, NC, and NY research fields. Four of nine NY isolates, 19 of 21 GA isolates, and 13 of 15 NC isolates from plants treated weekly with Flint or Quadris were able to grow well on leaf disks treated with 100 µg/ml trifloxystrobin in the bioassay. Strobilurin resistance was also detected in the research fields in VA (Olaya, personal communication). Strobilurin sensitivity appeared to be qualitative as reported in other areas of the world (Ishii et. al. 2001). The maximum concentration tolerated by most of the 73 isolates from GA, NC, and NY in 2002 (89%) was either 0.5 or 100 µg/ml trifloxystrobin. Azoxystrobin baseline sensitivity distribution had been investigated in North America. In one study with *P. xanthii* isolates collected in 1998 and 1999 from several locations in North America, the geometric mean of the baseline was 0.258 µg/ml and the individual values ranged from 0.107 to 0.465 µg/ml (Olaya et. al 2000). In another study, 0.5-1 µg/ml was the maximum concentration tolerated by 60% of 72 isolates collected from 1990 to 1996 in six states; 6% were able to grow, but only slightly, on leaf disks treated with 5 µg/ml (Shishkoff and McGrath,

Table 1. Percent control of powdery mildew on pumpkin leaves obtained with Quadris or Flint applied alone or in alternation with Nova and Bravo Ultrex or Microthiol Disperss on a 7-day schedule in Riverhead, NY, 1998-2002.

Year	Fungicide	Powdery mildew control on leaves (%)	
		Upper surface	Lower surface
1998	Flint	86.51%	78.37%
1998	Bravo + Nova alt Quadris	93.36%	57.84%
1999	Bravo + Nova alt Quadris	99.94%	84.18%
2000	Quadris alt Bravo + Nova	99.97%	98.42%
2001	Quadris alt Bravo + Nova	94.70%	71.70%
2002	Quadris	40.88%	48.97%
2002	Quadris alt Bravo + Nova	73.25%	44.35%
2002	Quadris alt Microthiol Disperss + Nova	86.88%	75.81%

Table 2. Percent control of powdery mildew on muskmelon leaves obtained with Quadris applied alone or in alternation with Nova and Bravo Ultrex in NY, 1999.

Year	Fungicide	Powdery mildew control on leaves (%)	
		Upper surface	Lower surface
1999	Quadris	98.20%	98.86%
1999	Bravo + Nova alt Quadris	99.81%	93.51%
1999	Quadris alt Bravo + Nova	99.98%	98.52%

unpublished). This indicates that poor control with strobilurins under field conditions was associated with reduced sensitivity in vitro. The resistant isolates were able to tolerate at least 100-fold higher concentration of strobilurins than isolates with baseline sensitivity.

Two strobilurin sensitive isolates and three resistant isolates collected in 2002 responded similarly when tested in another laboratory using kresoxim-methyl and pyraclostrobin (H. Ypema, personal communication). These findings and experiences in other areas of the world with strobilurin-resistant *P. xanthii* indicate that cross-resistance probably extends among multiple strobilurins (Ishii et. al. 2001).

All 14 isolates obtained from non-treated plants in the GA experiment were sensitive to trifloxystrobin (maximum concentration tolerated was 0 mg/ml for 14% of the isolates, 0.5 µg/ml for 79%, and 5 µg/ml for 7%). Thus applying Flint alone shifted the pathogen population substantially. Large changes in the frequency of resistance in *P. xanthii* populations during a growing season have been detected with triadimefon and benomyl (McGrath 1996a).

Results on resistance in the western US are inconclusive as only 1 isolate could be tested from AZ and only 5 from CA because spores obtained from these leaves grew poorly in culture. However, the AZ isolate tolerated 50µg/ml trifloxystrobin. Also, one CA isolate grew on disks treated with 50 and 100 µg/ml trifloxystrobin in one assay, but growth was reduced compared to lower concentrations. It did not survive to be re-tested. The maximum concentration tolerated by the other 4 CA isolates was 0.5µg/ml trifloxystrobin. Efficacy of strobilurin fungicides changed substantially from 2001 to 2002 in the CA research field (T. Turini 2002, 2003). Control of powdery mildew achieved on lower leaf surfaces with the strobilurin fungicides Cabrio (pyraclostrobin), Quadris, and Flint used alone was 65% - 94% in 2001 whereas severity did not differ significantly from non-treated plants in 2002. In contrast, the DMI fungicides Rally and Procure (triflumizole) used alone provided 82% - 100% control in 2001 and 82% - 98% control in 2002. Strobilurin fungicides used alone in AZ provided 46% - 77% control of powdery mildew on lower leaf surfaces in 2002 while triflumizole provided 91% control (Matheron et.al. 2003).

Strobilurin resistance did not appear to be a factor in pyraclostrobin efficacy being lower in 2000 (76%)(Zitter et al 2001) than in 1999 (92%)(Drennan et al 2000) in Freeville, NY. The highest concentration tolerated by *P. xanthii* isolates obtained from the Freeville experiment in 2000 was only 2µg/ml pyraclostrobin. This concentration was tolerated by 80% of Freeville isolates and 83% of isolates tested from Riverhead, NY, in 2000.

The strobilurin-resistant isolates also exhibited reduced sensitivity to DMI fungicides. All isolates but one tolerated 100µg/ml triadimefon. The one isolate unable to grow on leaf disks treated with 100 µg/ml was able to tolerate 50µg/ml triadimefon. Resistance to DMI fungicides is quantitative. Isolates able to tolerate 100µg/ml triadimefon are resistant to triadimefon but sensitive to myclobutanil because in fungicide efficacy experiments Bayleton was ineffective while Nova was effective where these isolates occurred (McGrath et. al. 1996). Applying the DMI fungicide Bayleton weekly in the experiment conducted in GA also shifted the pathogen population to a high frequency (71%) of isolates that were resistant to strobilurin fungicides and insensitive to DMI fungicides. Using strobilurin fungicides was shown in 1999 to be effective for managing DMI resistance (McGrath and Shishkoff 2000). In 2002, however, it appears that most individuals in the powdery mildew fungal population that were insensitive to one of these chemical classes were also insensitive to the other, consequently applying either a strobilurin or a DMI fungicide shifted the population towards insensitivity to both. None of the 73 isolates tested were strobilurin-resistant and DMI-sensitive; 7% were strobilurin-sensitive and DMI-insensitive. Only 2 isolates from NY, 1 from Flint-treated plants in GA, 4 from Bayleton-treated plants in GA, and 2 from NC were sensitive to both chemical classes.

Although isolates were not tested from commercial production fields, it is prudent for growers to consider improving their resistance management program. Using strobilurin fungicides alone, as was done in the research fields, exerts more selection pressure for strobilurin resistance than using them with DMIs and contact fungicides in a resistance management program; however, the size of the population exposed to this high selection pressure in research fields is extremely small compared to that in commercial fields. Strobilurin resistance likely occurs in commercial fields, but was more easily detected in research fields where plants treated with effective fungicides and non-treated plants provided comparisons. Strobilurin resistance appears to be widespread in the US. It was confirmed in GA, VA, NC, and NY. It is likely in AZ and CA. And efficacy of strobilurins was reduced in some mid-western states. The cucurbit powdery mildew fungus produces spores wind-dispersed over large areas. Inoculum for powdery mildew developing on cucurbit crops is thought to be wind-dispersed northwards through the eastern and mid-western US each year. Occurrence of resistance in commercial fields will reduce the utility of strobilurins, including those not yet registered, and eliminate an important tool for managing DMI resistance. Strobilurins and DMIs are the only systemic fungicides registered for cucurbit powdery mildew in the US. Current recommendations for managing fungicide resistance include using a diversity of fungicides within an

integrated disease management program that includes non-chemical practices, such as use of resistant cultivars (McGrath, 2001). Nova should be used at the manufacturer's highest label rate (full rate) and shortest application interval. One suggested change to improve resistance management is to apply a contact fungicide with strobilurins as well as DMIs. Sulfur (Microthiol Disperss®) and mineral oil (JMS Stylet-oil®) are recommended for resistance management because they are more effective than Bravo and other contact fungicides for powdery mildew on the lower leaf surface (McGrath 2002a). Quadris applied in alternation with Nova and Microthiol Disperss was more effective than Quadris alternated with Nova and Bravo in 2002 (Table 1). Sulfur is very inexpensive, but can be phytotoxic to melon (McGrath 2002a).

This is the first report of resistance in *Podosphaera xanthii* to this group of fungicides in the US. Resistance has already developed in *Didymella bryoniae*, which causes gummy stem blight, in the US (Stevenson et. al. 2002).

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Baseline Sensitivity of Cucurbit Powdery Mildew (*Podosphaera xanthii*) to the Fungicide Azoxystrobin in the United States

INTRODUCTION Azoxystrobin is the first synthetic fungicidal compound derived from naturally occurring strobilurins (Ypema and Gold 1999). It is in fungicide group 11, the quinone outside inhibitor (QoI) activity group. The purpose of this study was to examine baseline sensitivity of *Podosphaera xanthii* to azoxystrobin in the United States.

MATERIALS and METHODS Isolates of *Podosphaera xanthii* were obtained from six states in 1996 (Table 1). A reference isolate collected in 1990 was also included.

A leaf-disk bioassay was used to determine sensitivity to azoxystrobin (McGrath et al 1996). Two-week old squash seedlings ('Seneca Prolific') were sprayed with active ingredient (azoxystrobin 96%) dissolved in methanol: acetone: water (1:1:2 v:v:v) at 0, 0.25, 0.5, 1.0, 2.5, and 5 µg/ml. Test solutions were

sprayed onto plants using a DeVilbiss bottle attached to a compressed air source (20 psi). Treated plants were allowed to dry, then disks were cut from the cotyledons using a #9 cork borer (9 mm diameter). Five disks of each treatment were placed on water agar in divided petri plates (three treatments per plate). Disks were inoculated by transferring 5-10 conidia to the center of each disk. In each trial, duplicate sets of treated disks were inoculated with each mildew isolate, then plates were incubated for approximately 2 weeks at 24 C, at which time the control treatment showed good growth, with sporulating mildew covering an average of 40-60% of leaf disk area. For each fungicide concentration, growth of the mildew was considered to have occurred if sporulation was observed on 3 out of 5 disks (or 2 out of 4, if a disk died during incubation). The percent leaf disk area colonized by sporulating mildew was recorded for each disk and averaged for each treatment.

Table 1. Source of the groups of *Podosphaera xanthii* isolates used to determine baseline sensitivity to azoxystrobin and the range of maximum concentrations tolerated by the isolates in these groups.

Isolate Group	# Isolates Tested	Date Teceived	Collection Location	Host Crop	Max. Concentration Tolerated
4at*	1	9/90	Riverhead, NY	Pumpkin	1 µg/ml
Race1	3	10/7/1996	San Juan Batista, CA	Squash	1 - 2.5 µg/ml
Race2	9	10/7/1996	San Juan Batista, CA	Squash	0.5 - 2.5 µg/ml
CA1	6	9/19/1996	Woodland, CA	Pumpkin	1 - 2.5 µg/ml
CA2	7	9/19/1996	Yuba City, CA	Melon, honeydew	1 - 2.5 µg/ml
CA3	6	9/19/1996	Knights Landing, CA	Squash, banana	1 - 5 µg/ml
CA4	7	9/20/1996	Stockton, CA	Pumpkin	1 - 5 µg/ml
OK (1-4)	4	10/4/1996	Tulsa, OK	Pumpkin	1 – 2.5 µg/ml
OK (5-8)	4	10/4/1996	Payne Co., OK	Pumpkin	1 µg/ml
NY	10	10/25/1996	Riverhead, NY	Pumpkin	1 - 5 µg/ml
NJ	11	9/18/1996	Cornish Pt., NJ	Cucumber	0.5 – 2.5 µg/ml
96-75	1	8/20/1996	Sudlersville, MD	Cucumber	1 µg/ml
96-93	1	8/20/1996	Stockton, CA	Pumpkin	1 µg/ml
96-95	1	8/20/1996	Stockton, CA	Watermelon	0.5 µg/ml
96-114	1	8/20/1996	Spring Creek, TN?	Squash, crookneck	1 µg/ml

* Isolate shown previously to be resistant to triadimefon and benomyl.

RESULTS and DISCUSSION Seventy-two powdery mildew isolates from six states showed little variation in sensitivity to azoxystrobin. All were able to grow at 0.25 µg/ml, 60% could grow at 0.5-1 µg/ml but no higher, 35% grew slightly (0.4-11% disk area colonized) on leaf disks treated at 2.5 µg/ml, and 6% (4 isolates) grew slightly (0.2-2.8%) at 5 µg/ml. Some of the variation in fungus growth may have been due to variation in the actual concentration of test solutions used to spray test plants; the fungicidal active ingredient was not readily soluble in water at 5 µg/ml and took some time to dissolve in an acetone:methanol:water (1:1:2) solution. It was necessary to dissolve the fungicide in acetone:methanol (1:1) and then add water. There was no evident correlation between sensitivity and geographic location or sensitivity and race. An isolate with resistance to triadimefon and benomyl (isolate 4at) was unable to grow on disks treated with >1 µg/ml azoxystrobin, indicating that resistance to triazoles and benzimidazoles was independent of resistance to azoxystrobin.

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Managing Phenylamide Resistance in Potato Late Blight in Northern Ireland

INTRODUCTION Formulations containing phenylamides + mancozeb were approved for the control of potato late blight in the UK in 1978 and rapidly became widely used. In summer 1980, in the Republic of Ireland metalaxyl alone failed to control the disease and isolates of *Phytophthora infestans* from the foliage were found to be phenylamide-resistant (Dowley and O'Sullivan, 1981). In Northern Ireland, phenylamide-resistant isolates of *P. infestans* were obtained from blighted tubers from the 1980 crop and annual surveys of the incidence of phenylamide resistance were initiated starting in 1981 (Cooke, 1981). In the early 1980s, in Northern Ireland the percentage of isolates containing phenylamide-resistant strains was generally 10-20% (except in 1984), but in the late 1980s there was a dramatic increase to c. 90% in 1987-89. This was attributed to the selection pressure resulting from widespread and season-long use of formulations containing phenylamides + mancozeb (mainly metalaxyl + half-rate mancozeb) and a succession of very wet summers which favoured late blight. A similar build-up of phenylamide-resistant strains

occurred in the Republic of Ireland, Great Britain, and the Netherlands. In 1988, the metalaxyl + mancozeb formulation was changed to give a full-rate of mancozeb. In the early 1990s, an anti-resistance strategy based on the one developed in the Republic of Ireland (Dowley et al., 1995) was adopted in Northern Ireland with growers advised to use no more than three applications of phenylamides at the beginning of the spray programme only.

MATERIALS and METHODS Samples of infected potato foliage were obtained (mainly from seed crops) by members of the Department of Agriculture and Rural Development (DARD) Potato Inspection Service (Cooke et al., 2000). Isolates were derived by bulking together the sporangia obtained from all foliage samples within a single crop and maintained on detached glasshouse-grown potato leaflets then tested, using the floating leaf disc technique (Cooke, 1986), on 100 and 2 mg metalaxyl litre-1. Isolates were designated resistant if they sporulated on 100 mg metalaxyl litre-1-treated discs and sensitive if they sporulated on untreated discs, but not on any metalaxyl-treated disc. Isolates that grew on discs floating on 2 mg, but not on 100 mg metalaxyl litre-1, were designated intermediate. At the end of each season, inspectors provided estimates of fungicide usage for all seed potato crops in their areas.

RESULTS and DISCUSSION In the mid-1980s, a high proportion of Northern Ireland potato crops were sprayed with phenylamide fungicides for most of the spray programme. However, during most of the 1990s and up to 2002, Potato Inspectors' estimated that c. 40% of seed potato crops were phenylamide-treated (Fig. 1) and the majority of growers followed DARD advice and used no more than three phenylamide applications per season (Table 1). The proportion of isolates containing phenylamide-resistant strains declined in the early 1990s and appeared to have stabilised around 50% (Fig. 2) up to 1999. However, in 2000 and particularly in 2001, there was a marked increase in the incidence of phenylamide resistance. The reason for this was not clear, since fungicide usage had not changed. The weather in the summer months of 1998-2000 was unusually wet

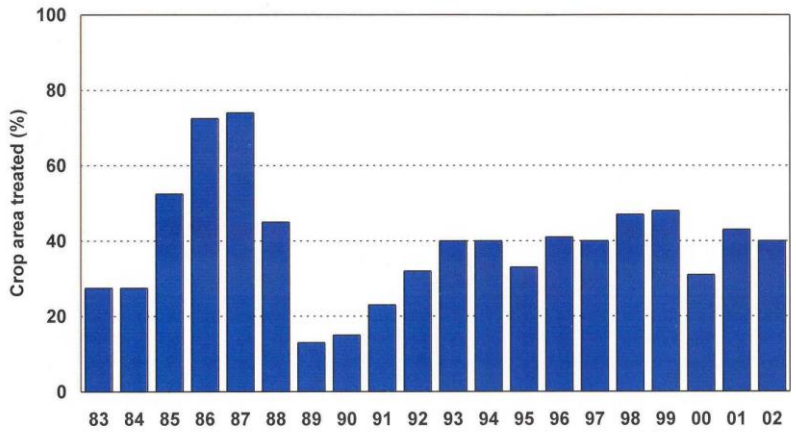

Figure 1. The proportion of Northern Ireland seed potato crops treated with phenylamides, 1983-

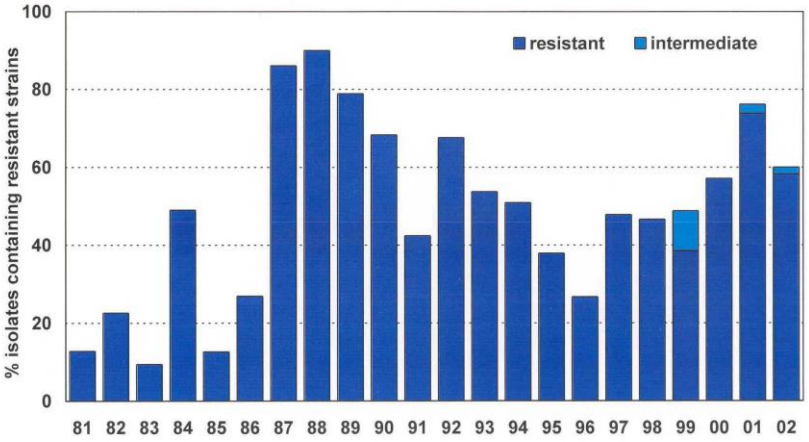

Figure 2. The proportion of Northern Ireland *Phytophthora infestans* isolates containing phenylamide-resistant strains, 1981-2002

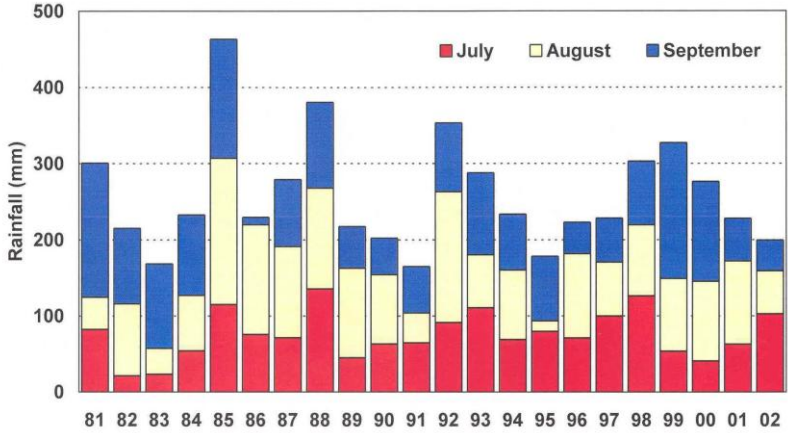

Figure 3. Northern Ireland rainfall, July-September 1981-2002

Table 1. Number of applications used by growers applying phenylamides, 1993-2001*

No. of phenylamide applications	Of growers using phenylamides (%) using								
	1993	1994	1995	1996	1997	1998	1999	2000	2001
one	25	33	33	17	22	17	14	13	17
two	27	29	18	36	44	42	46	50	58
three	23	26	25	24	15	22	28	25	19
four	18	9	17	9	8	10	9	10	5
five or more	7	3	7	14	11	9	3	2	1

* data for 2002 not yet available

and it is possible that this may have increased the number of generations of *P. infestans* within each season and favoured the buildup of resistant strains (Fig. 3). In 2002, DARD and Syngenta agreed revised recommendations for phenylamide usage: growers were advised to use no more than two applications per season and to switch to an alternative product type no later than 15 July. Subsequently, the proportion of isolates containing resistant strains declined from 76% in 2001 to 60% in 2002. These grower recommendations will be continued in 2003 and the incidence of resistant strains again monitored. It is concluded that in a region such as Northern Ireland, where fit phenylamide-sensitive and -resistant strains of *P. infestans* co-exist, resistance may be managed by a strategy of limited use of phenylamides early in the season only. During the winter period when the pathogen survives in infected tubers, more resistant than sensitive strains tend to be lost by tuber rotting and this helps to stabilise the situation (Walker and Cooke, 1990). However, in regions where aggressive phenylamide-resistant strains have been introduced by migration rather than *in situ* selection, as occurred recently in Taiwan (Deahl et al, 2002), such resistance management may not be possible.

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Research in Resistance Management

Activity Spectrum of Spinosad and Indoxacarb: Rationale for an Innovative Pyrethroid Resistance Management Strategy in West Africa

ABSTRACT To face pyrethroid resistance in the cotton bollworm *Helicoverpa armigera* (Hübner), endosulfan (700 g/ha) has been used in a resistance management strategy for four years in Côte d'Ivoire, West Africa. Actually, its recommendation is being questioned with regard to its acute toxicity and environmental issues. Earlier prospects revealed that insecticides such as spinosad (48 g/ha) and indoxacarb (25 g/ha) proved as effective as endosulfan in controlling *H. armigera*. In contrast to endosulfan, the activity spectrum of these non-pyrethroids insecticides appears to be restricted to a few bollworm and leaf pests. The present study pointed out the strength and weakness of these new insecticides with respect to major insect pests and beneficial species. On the basis of their activity spectrum and in the light of cotton crop phenology and main pest seasonal occurence, a differential scheme was designed. Indoxacarb is more appropriate to the fruiting stage (101-115 DAE (Day After Emergence)) as it appeared very effective against the cotton stainer *Dysdercus voelkeri* (Schmidt) while showing lower performance against *Earias* spp and the mite *Polyphagotarsonemus latus* (Bank). In contrast, spinosad is to be used preferably at the vegetative stage (45-66 DAE) as it proved safer to coccinellids, more effective against *Earias* spp while its lower effectiveness against *D. voelkeri* suggests avoiding its positioning at a late stage of cotton. Various benefits related to these new insecticides strongly advise their use as alternatives to pyrethroids. Still, to be more attractive, their activity needs to be reinforced by other insecticides in such a way to control the whole arthropod pest complex.

KEY WORDS: Cotton, *Helicoverpa armigera*, pyrethroid resistance management strategy, Spinosad, Indoxacarb, Côte d'Ivoire.

INTRODUCTION

The development of resistance in H. armigera:

Known as very effective in controlling *Helicoverpa armigera* (Hübner) and most cotton bollworm pests, pyrethroids have been widely used for more than twenty years in Côte d'Ivoire. Recently, laboratory data obtained on *H. armigera* strains within 1996-1998 pointed out significant increase in the LD50

for both deltamethrin (Figure 1) and cypermethrin (Vassal et al., 1997; Vaissayre et al., 1998; Martin et al., 2000). Field data recorded for eight consecutive years (Figure 2) revealed that the pest infestation profiles changed deeply from 1991 to 1998 (Ochou et al., 1998). Moreover, cases of ineffectiveness of the pest control programme against *H. armigera* have been reported during exceptional pest outbreaks in Côte d'Ivoire. With this regard, the routine calendar-based programme applying six fortnightly sprays of pyrethroid-organophosphate insecticide mixtures over the whole cotton season has been questionned as the pyrethroid resistance in *H. armigera* was confirmed (Ochou & Martin, 2000). Similar cases of resistance were reported in *H. armigera* in most West African countries (Benin, Burkina Faso, Guinea, Mali, Senegal, and Togo) (Anonymous, 1999).

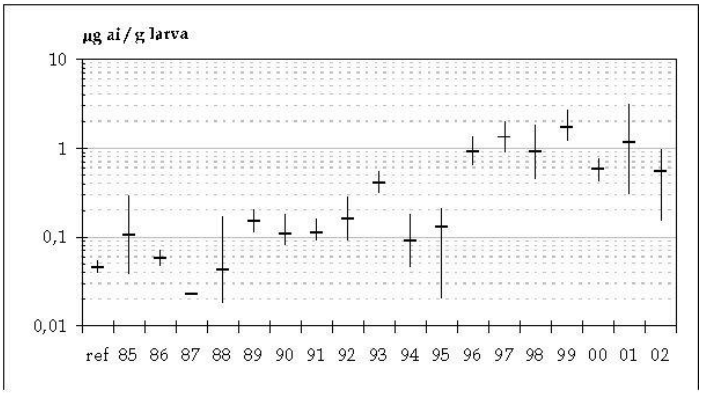

Figure 1: LD_{50} survey of deltamethrin from 1985 to 1998 with topical application tests on *Helicoverpa armigera* Bouaké strain

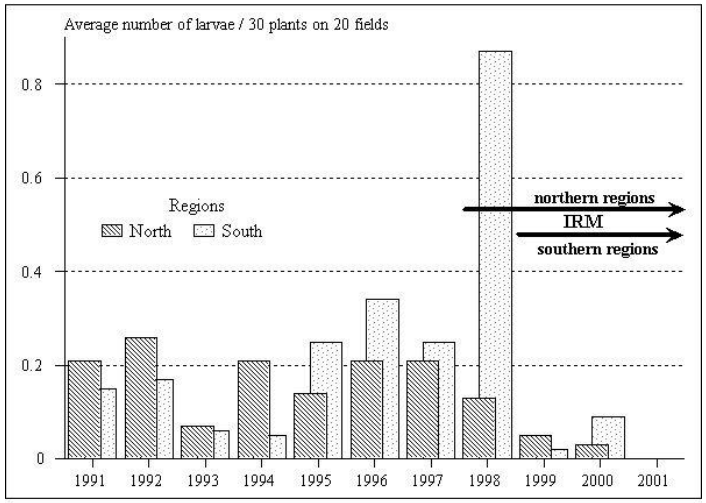

Figure 2: Annual variations of average field infestation levels of *H. armigera* in cotton areas of Côte d'Ivoire before and after IRM

Development of the IRM strategy against H. armigera:

To face pyrethroid resistance in the cotton bollworm, *H. armigera*, an Insect Resistance Management (IRM) programme, inspired from the "Australian" strategy (Sawicki and Denholm, 1987), was designed in Côte d'Ivoire. In practice, the strategy has led to the determination of a pyrethroid-free season nationwide by using non-pyrethroid insecticides (endosulfan 700-750g/ha and profenofos 750 g/ha) in a kind of "window" programme in order to lessen pyrethroid selection pressure. The pyrethroid-free season is established according to cotton growing zones (August 10 and August 20 respectively for northern and southern regions). The main picture which has come out from the nationwide adoption of the pyrethroid resistance management programme by cotton farmers is the important decrease in the field populations of *H. armigera* (Figure 2) since 1998 (Ochou & Martin, 2002).

Endosulfan has been widely used in the current pyrethroid resistance management programme over the last four years in Côte d'Ivoire, and so far, no resistance to endosulfan has been detected (Martin et al., 2002). However, its recommendation is being actually questioned with regard to its toxicity, environmental issues, and farmer practices. To tackle this problem, investigations are being undertaken to adapt a relatively low dose of endosulfan (525 g/ha) to the actual field infestation of *H. armigera* (Ochou & Martin, 2000) and to assess microencapsulated formulations of endosulfan, assumed safer than the EC formulations. At the same time, further investigations focused on new insecticides such as spinosad and indoxacarb as potential alternatives to endosulfan. Spinosad is a naturally produced mixture of the actinomycete *Saccaropolyspora spinosa*. Its mode of action is described as an activation of the nicotine acetylcholine receptor, but at a different site from nicotine or imidachloprid. It is activated by contact and ingestion, causing paralysis (Pesticide Manual, 12th edition, v2). Indoxacarb is an oxadiazine product where the active component blocks sodium channels in nerve cells. It is activated by contact and ingestion, and affected insects cease feeding, with poor co-ordination, paralysis, and ultimately death (Pesticide Manual, 12th edition, v2). Due to their novel mode of action, both insecticides appear ideal for resistance management programmes. However, to be rationally used, there is a need for a precise activity spectrum of these new insecticides that proved as effective as endosulfan in controlling *H. armigera* (Ochou & Martin, 2002).

The present study is to assess the activity spectrum of spinosad and indoxacarb with regard to beneficials and major components of the cotton pest complex in Côte d'Ivoire. The need to reinforce their activity by other insecticides will be also assessed. On the basis of the strength and weakness of these new insecticides and with respect to cotton crop phenology and seasonal occurence of main pests, appropriate recommendations will be stated to justify their integration into the pyrethroïd resistance management programmes.

MATERIALS and METHODS The study was carried out for three consecutive years (1999-2001) at the cotton research station of CNRA based at Bouaké and at the experimental station of LCCI at Nambingué. At first, the biological activity of the two specific insecticides (spinosad 48g/ha (Laser 480 SC, Dow AgroSciences) and indoxacarb 25g/ha (Avaunt 150 SC, Du Pont)) was assessed in reference with endosulfan 750 g/ha (Phaser 375 EC, Aventis), and deltamethrin 12 g/ha (Decis 12 EC, Aventis) through a Complete Bloc Design with six replicates. Individual plots were of 10 rows x 15 m. Further field trials were undertaken in a similar design with the two insecticides in association with other insecticides. Tested mixtures included spinosad 48g/ha + profenofos 300g/ha, spinosad 48g/ha + acetamiprid 10g/ha, indoxacarb 25g/ha + profenofos 300g/ha, indoxacarb 25g/ha + acetamiprid 10g/ha, and cypermethrin 36g/ha + profenofos 300g/ha.

Insecticides sprays were performed with an adapted horizontal boom knapsack sprayer debiting 60 l/ha of product-water mixture. Plots were treated every 14 days from 45th to 115th DAE (day after emergence of cotton). Fields were scouted directly on plants once a week from 30th to 122nd DAE for sucking pests, leafworms, and exocarpic bollworms pests, and every two weeks on green bolls from 70th to 112th DAE for endocarpic bollworms. Target pests and beneficials were recorded as follows:

1. mite *Polyphagotarsonemus latus* infested plants p. 3 rows x 15m;
2. aphid *Aphis gossypii* infested plants p. 3 rows x 15m;
3. jassid *Jacobiella fascialis* infested plants p. 30 plants;
4. individual sucking pests (*Dysdercus voelkeri*, *Bemisia tabaci*), leafworms (*Spodoptera littoralis*, *Anomis flava*, *Syllepte derogata*), and exocarpic bollworms (*H. armigera*, *Earias* spp, *Diparopsis watersi*) p. 30 plants;
5. endocarpic bollworms (*Cryptophlebia leucotreta*, *Pectinophora gossypiella*) p. 100 green bolls; and
6. individual beneficials (ladybirds, spiders, etc.) p. 30 plants.

Three year average data for all bollworms and one-two year average data for sucking pests, leaf pests, and benefials were considered.

RESULTS

Effectiveness of spinosad and indoxacarb against cotton bollworms:

Data presented in Figures 3a-d show compared effectiveness of the pyrethroid deltamethrin and the non pyrethroïd insecticides on cotton exocarpic bollworm species (*H. armigera*, *Earias* spp., *D. watersi*) and endocarpic bollworm species (*C. leucotreta* and *P. gossypiella*).

Spinosad activicty on the exocarpic bollworm species was at least equivalent to endosulfan as a reference: *H. armigera* (3.1 vs 3.4 larvae p. 30 plants), *Earias* spp, and *D. watersi*. Overall activity of spinosad against the exocarpic bollworm species was higher than deltamethrin. Indoxacarb activity was at the level of deltamethrin for *H. armigera* (4.9 vs 5.1 larvae p. 30 plants), and to a certain extent less effective against *Earias* spp. In contrast, the activity of both insecticides (spinosad and indoxacarb) on endocarpic species remained low in relation to deltamethrin (6.4 and 7.1 vs 3.2 endocarpic larvae p. 100 bolls, respectively for spinosad, indoxacarb, and deltamethrin).

Effectiveness of spinosad and indoxacarb against sucking pests:

Data presented in Figures 4a-d reveal compared activity of the pyrethroïd deltamethrin and the non pyrethroïd insecticides on cotton sucking pests *J. fascialis*, *A. gossypii*, *D. voëlkeri*, and the mite *P. latus*.

The effect of spinosad was at least equivalent to deltamethrin on the jassid *J. fascialis* (1.2 vs 1 jassid attacked plants p. 30 plants) and on the mite *P. latus* (4 mite infested plants p. 3 rows). In contrast, spinosad appeared less effective than endosulfan against the aphid *A. gossypii* (56.8 vs 36.8 aphid infested plants p. 3 rows) and the cotton stainer *D. voëlkeri* (169 vs 140.8 Dysdercus p. 30 plants). Contrary to spinosad, the effect of indoxacarb was equivalent to deltamethrin on *D. voëlkeri* (110.3 vs 101.8 Dysdercus p.30 plants) and on the aphid *A. gossypii* (43.3 vs 48.8 aphid infested plants p. 3 rows) while showing less effectiveness compared to endosulfan against the mite *P. latus* (11.5 vs 2.4 mite infested plants p. 3 rows).

Effectiveness of spinosad and indoxacarb against cotton leafworms:

Data presented in Figures 5a-b show the compared effect of the pyrethroid deltamethrin and the non pyrethroïd insecticides on cotton leafworm *S. littoralis* and *A. flava*.

Spinosad and indoxacarb proved very effective against the leafworm *S. littoralis* (0.7 and 0.8 vs 1.5 larvae p. 30 plants, respectively for indoxacarb, spinosad, and deltamethrin). Their activity of on *A. flava* remained equivalent to deltamethrin and endosulfan (1.2 and 2.2 vs 1.8 larvae p. 30 plants, respectively for spinosad, indoxacarb, and endosulfan).

Activity of spinosad and indoxacarb on beneficials:

Figures 6a-b show data on the compared activity of the pyrethroïd deltamethrin and the non pyrethroïd insecticides on beneficial predators.

Spinosad and indoxacarb to a lesser extent proved safer on ladybirds, *Coccinela* spp., as compared to endosulfan (10.7 and 5.8 respectively for spinosad and

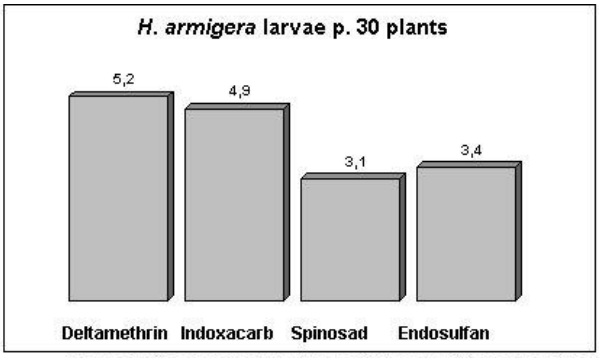

Figure 3a: Compared activity of insecticides on Helicoverpa armigera

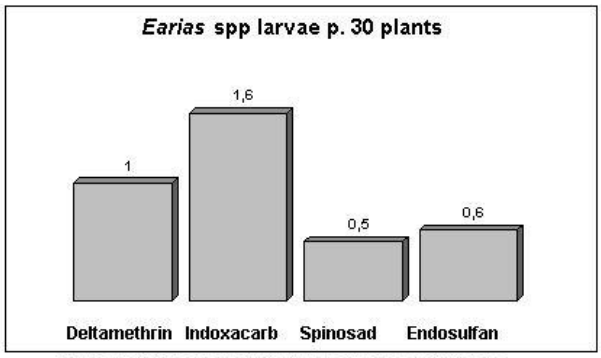

Figure 3b: Compared activity of insecticides on Earias spp

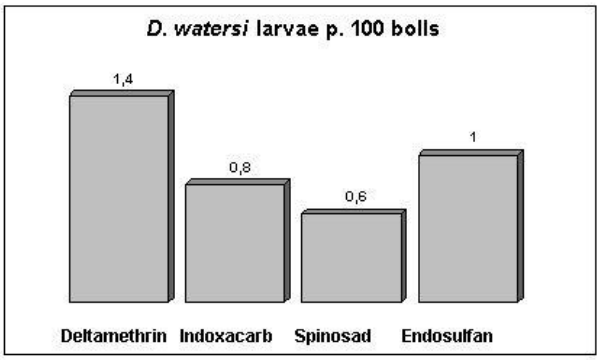

Figure 3c: Compared activity of insecticides on Diparopsis watersi

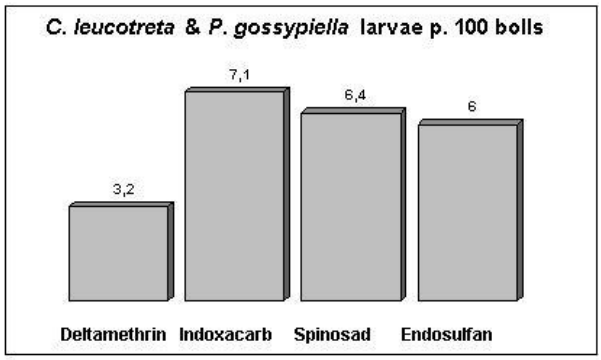

Figure 3d: Compared activity of insecticides on endocarpic bollworms

Figure 3 : Compared effectiveness of spinosad and indoxacarb against cotton bollworms in Côte d'Ivoire

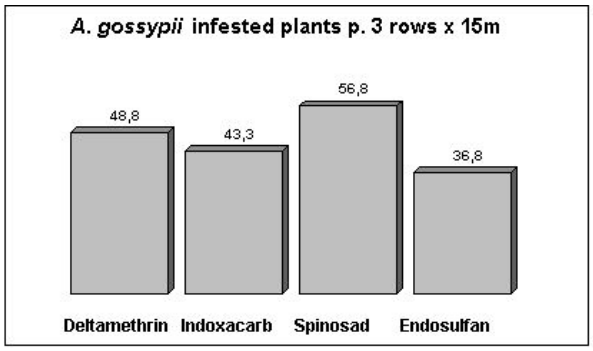

Figure 4a: Compared activity of insecticides on Aphis gossypii

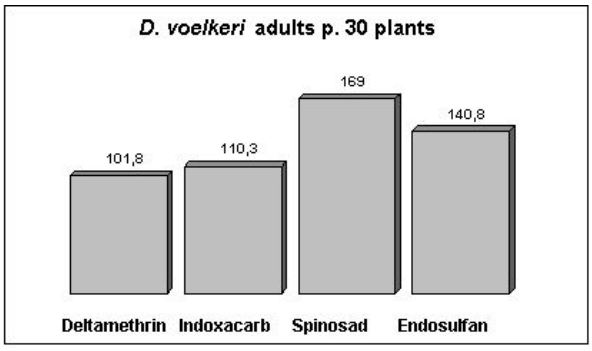

Figure 4b Compared activity of insecticides on Dysdercus voelkeri

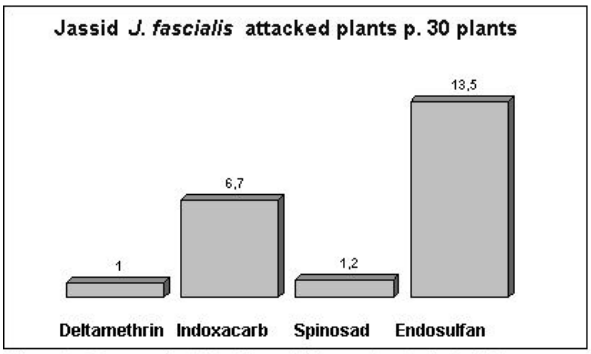

Figure 4c: Compared activity of insecticides on Jacobiella fascialis

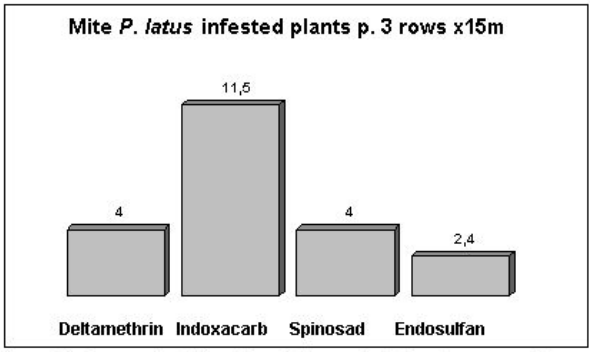

Figure 4d: Compared activity of insecticides on Polyphagotarsonemus latus

Figure 4 : Compared effectiveness of spinosad and indoxacarb against cotton sucking pests in Côte d'Ivoire

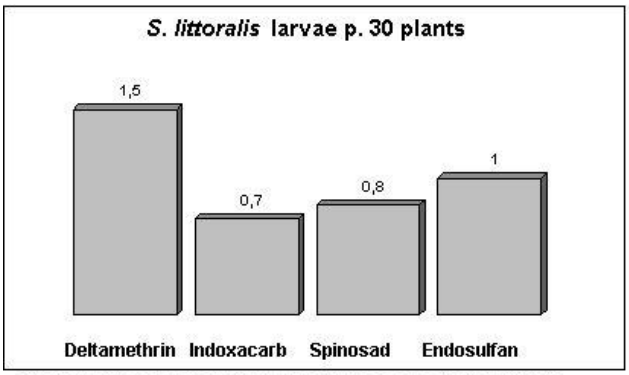

Figure 5a: Compared activity of insecticides on Spodoptera littoralis

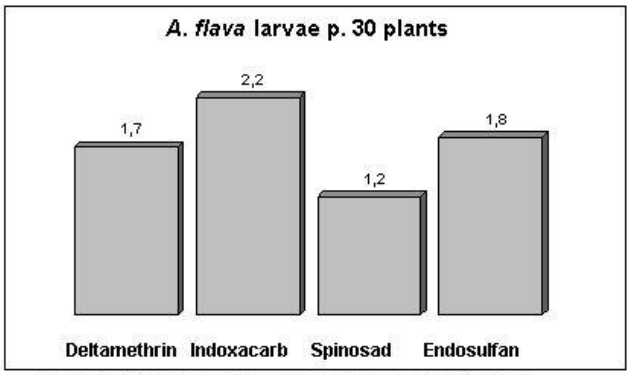

Figure 5b: Compared activity of insecticides on Anomis flava

Figure 5 : Compared effectiveness of spinosad and indoxacarb against cotton leafworm pests in Côte d'Ivoire

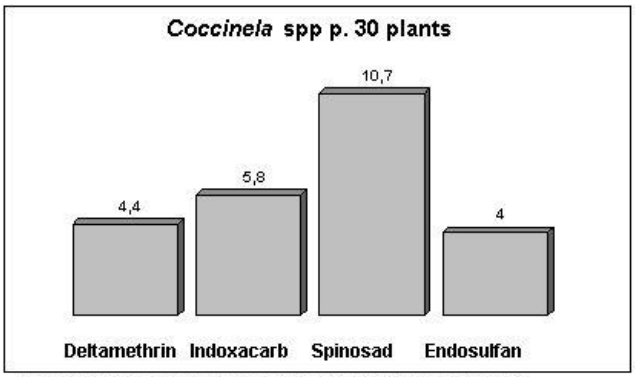

Figure 6a: Compared activity of insecticides on Coccinela spp

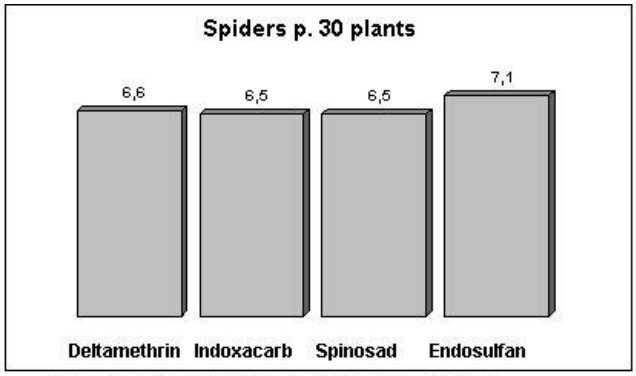

Figure 6b: Compared activity of insecticides on spiders

Figure 6 : Compared activity of spinosad and indoxacarb on beneficials in Côte d'Ivoire

indoxacarb vs 4 coccinellids p.30 plants). The effect of both insecticides on the spiders was equivalent to endosulfan (6.5 vs 7 spiders p.30 plants).

Effectiveness of spinosad and indoxacarb in mixture with other insecticides:

Data presented in Figures 7a-d showed compared activity of spinosad or indoxacarb based associations with profenofos and acetamiprid, and pyrethroïd based associations on cotton bollworms and some sucking pests.

The profenofos based association with spinosad or indoxacarb provided an activity level at least equivalent to cypermethrin-profenofos association against *H. armigera* (0.3 and 1 vs 1.1 larva p. 30 plants, respectively for indoxacarb-profenofos, spinosad-profenofos and cypermethrin-profenofos). The same tendency was observed against the mite P. latus (0.1 and 2.5 vs 2.9 mite infested plants p. 3 rows).

The acetamiprid-based association with spinosad was at least equivalent to the cypermethrin-acétamiprid association against *D. voëlkeri* (74.2 vs 90.7 Dysdercus p. 30 plants). This association was much more effective against *D. voelkeri* than the indoxacarb-acetamiprid association (109.3 Dysdercus p. 30 plants). Concerning the endocarpic bollworm species (*C. leucotreta* & *P. gossypiella*) the spinosad-acetamiprid association showed an activity level equivalent to the cypermethrin-acetamiprid (4 vs 2 larvae p. 100 bolls) while the activity remained very low for the indoxacarb-acetamiprid association (9.5 larvae p. 100 bolls).

DISCUSSION The present study pointed out the strengths and weaknesses of spinosad and indoxacarb with respect to major insect pests and beneficial species. The activity of spinosad and indoxacarb varied significantly according to insect pest species or beneficial species.

The spinosad activity spectrum comprised exocarpic bollworm species (*H. armigera*, *Earias* spp, and *D. watersi*) and cotton leafworms *S. littoralis* and *A. flava*. It appeared to have a certain activity against the endocarpic bollworm species (*C. leucotreta* & *P. gossypiella*), the jassid *J. fascialis*, and the mite *P. latus*. This activity noticed especially on sucking pests such as the jassid *J. fascialis* and the mite *P. latus* need to be confirmed in more field trials, for the manual pesticide states that spinosad is non toxic to sucking pests. Indeed, spinosad appeared very limited against the aphid *A. gossypii* and the cotton stainer *D. voëlkeri*. With regard to benefials, spinosad proved safer to *Coccinela* spp and spiders.

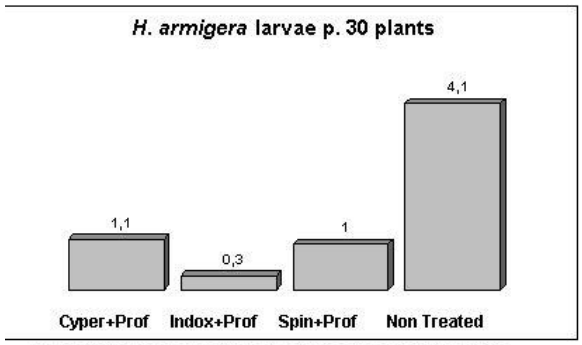

Figure 7a : Compared activity of profenofos based mixtures with spinosad and indoxacarb on Helicoverpa armigera

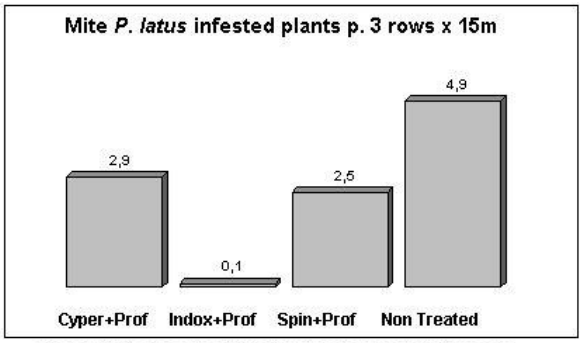

Figure 7b: Compared activity of profenofos based mixtures with spinosad and indoxacarb on Polyphagotarsonemus latus

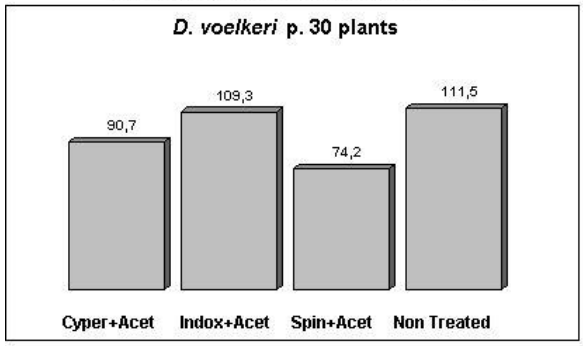

Figure 7c : Compared activity of acetamiprid based mixtures with spinosad and indoxacarb on Dysdercus voelkeri

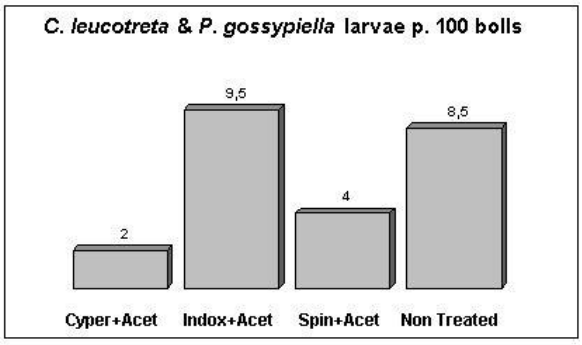

Figure 7d : Compared activity of acetamiprid based mixtures with spinosad and indoxacarb on endocarpic bollworms

Figure 7 : Compared activity of profenofos or acetamiprid based mixtures with spinosad and indoxacarb on cotton pests in Côte d'Ivoire

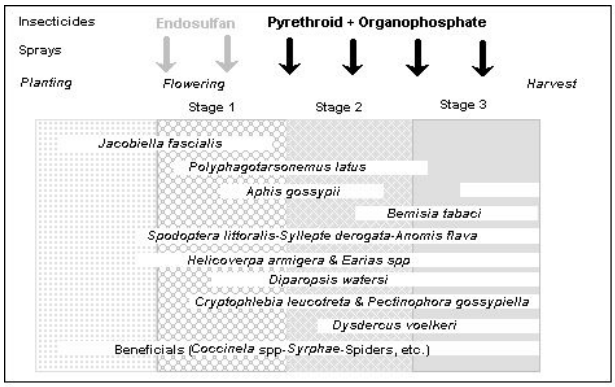

Figure 8a: Endosulfan based IRM programme

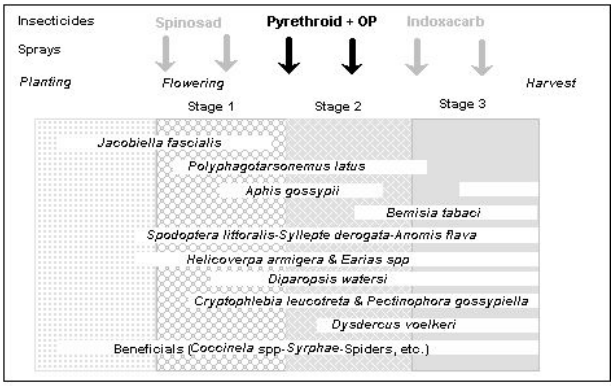

Figure 8b: Spinosad and Indoxacarb based IRM programme

Figure 8: Current and innovative pyrethroid management programmes

In contrast to spinosad, indoxacarb activity spectrum was restricted to a few bollworm species (*H. armigera*, *D. watersi*) and the cotton leafworm *S. littoralis*. In addition, it appeared to have a certain effectiveness against the jassid *J. fascialis*, the aphid *A. gossypii*, and the cotton stainer *D. voëlkeri*. Indoxacarb appeared somehow inactive on *Earias* spp., the mite *P. latus*, and the endocarpic bollworm species (*C. leucotreta* & *P. gossypiella*).

On the basis of their activity spectrum and in the light of cotton crop phenology and seasonal occurence of main pests, differential pyrethroïd resistant management plans could be designed (Figures 8a-b) considering the positioning of spinosad and indoxacarb either at the vegetative or fruiting stages of cotton.

Due to its high effectiveness on exocarpic bollworm species mainly *H. armigera* and *Earias* spp and its relative safety to major beneficials such as ladybird *Coccinela* spp, spinosad could be used preferably at cotton vegetative stage (45-66 d.a.e.). The relatively broad activity spectrum of spinosad makes it ideal for use at the vegetative stage of cotton, appearing as a true alternative to endosulfan. Its positioning at the late stage of cotton could be more suitable provided it would be used in association with other insecticides such as acetamiprid, effective against *D. voelkeri* and *A. gossypii*. Due to its activity

spectrum, which is relatively restricted in relation to spinosad, indoxacarb appears more appropriate to the cotton fruiting stage (101-115 d.a.e), as it proved effective against the cotton stainer *D. voelkeri* while showing lower performance against *Earias* spp and the mite *P. latus*. Association of indoxacarb with other insecticides such as profenofos could enhance its activity mainly on the mite *P. latus*. The use of indoxacarb is not advisable during the period that coincides with maximum flowering for it had a limited effect on endocarpic bollworm species (*C. leucotreta* and *P. gossypiella*) which occur in largest numbers at this stage; it is therefore necessary to maintain a pyrethroid based association in order to control endocarpic bollworm species.

Various benefits related to these new insecticides strongly advise their use as alternatives to pyrethroids. Still, to be more attractive, their activity needs to be reinforced by other insecticides in such a way as to control the whole arthropod pest complex. Conjoined laboratory activities are being achieved to help set more reliable strategies and improve the whole pest management strategy. Bioassays performed with several classes of insecticides, especially non pyrethroïd insecticides such as DDT, endosulfan, profenofos, indoxacarb, and spinosad did not show any cross-resistance with pyrethroids in *H. armigera* (Martin, unpublished data), knowing that pyrethroid resistance in *H. armigera* from West Africa was due to greater degradation of pyrethroids involving oxidases from the P450 family (Martin et al., 2002).

CONCLUSION The earlier use of endosulfan and profenofos as pyrethroid alternatives in *H. armigera* resistance management in Côte d'Ivoire helped substantially reduce field infestations of *H. armigera* for the last four years. No resistance was detected for endosulfan and profenofos in field populations indicating the success of these pyrethroid alternatives. However, endosulfan and profenofos resistance was shown in *H. armigera* from Pakistan (Ahmad et al., 1995) and Australia (Forrester et al., 1993; Gunning et al., 1993) indicating the risk to select resistant larvae in Côte d'Ivoire if those insecticides are to be used for years without alternatives. For the pyrethroid resistance management to be sustainable, there is a clear need to adopt alternative insecticides such as spinosad and indoxacarb in a rational non pyrethroid insecticide rotation plan. Spinosad and indoxacarb could be used in appropriate resistance management programmes either alone or reinforced in mixture by other insecticides or in mosaic with endosulfan and profenofos in such a way to avoid the selection of new cases of resistance.

ACKNOWLEDGEMENTS The authors acknowledge the research and development staff of cotton companies of Côte d'Ivoire (CIDT, IC, LCCI) and MM. Konan K. Jérôme, Kouadio René, and Kouadio Gérard of the cotton entomology technical research team of CNRA for their assistance in collecting field data. Thanks are due to chemical companies Dow AgroScience, Du Pont de Nemours, Aventis CropScience, and Syngenta for insecticide samples provided.

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Resistance Management News

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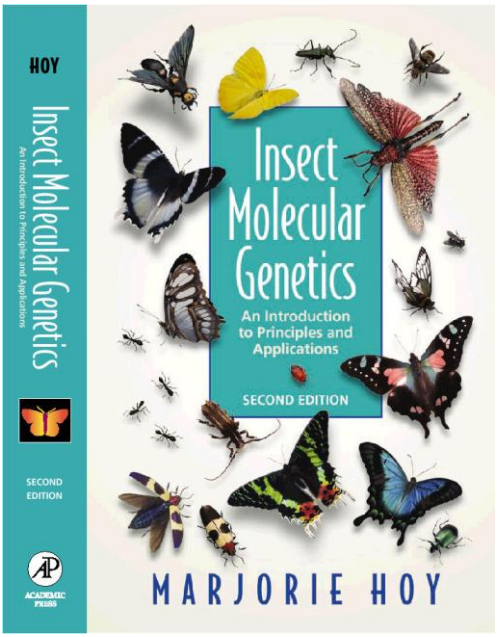

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Abstracts

Effect of Systemic Acquired Resistance on the Susceptibility of Insect Herbivores to Entomopathogens

Induced systemic resistance and systemic acquired resistance in plants involve major biochemical changes resulting in resistance to pathogens, reduced disease expression, and direct effects on herbivores. Tritrophic effects on the pathogens of herbivorous insects have not yet been described, however. If an insect feeding on induced plants is stressed in some manner it may be more susceptible to pathogens. We are studying three model systems to examine these tritrophic effects: the susceptibility of the orthopteran *Melanoplus sanguinipes*, and the lepidotperans *Ostrinia nubilalis* and *Agrotis ipsilon*, all feeding on Systemic Acquired Resistance (SAR)-induced corn plants, to *Beauveria bassiana* Strain GHA. SAR was induced by application of a commercial preparation of harpin. Immature insects were reared on induced and corn plants and

then bioassayed as young adults (*M. sanguinipes*) or 4th instar larvae (the two lepidopterans) with the fungus.

From the Annual Meeting of the Entomological Society of America, Nov. 17-21, 2002, Ft. Lauderdale FL.

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Kit for the Detection of *Echinochloa colona* and *Ischaemum rugosum* Susceptibility Status to the Herbicide Fenoxaprop-p-ethyl

A kit for the detection of the status of susceptibility of *Echinochloa colona* and *Ischaemum rugosum* to the herbicide fenoxaprop-p-ethyl is a new tool that allows verification of the loss of susceptibility from these two grass weeds to the herbicide in a reliable, quick, and simple way. The kit is the result of several years of research in two different laboratories working simultaneously: the Laboratory of Herbicides of "La Tupia" Bayer Crop Science Experimental Station located at Cauca Valley-Colombia and the Weed Science Laboratory of the Agronomy Faculty-National University of Colombia, Bogotá. Previous and experimental work included more than 60 experiments and kit validation under field conditions. Initially, different techniques were evaluated by dose-response bioassays considering seeds, meristems, seedlings (shoots) growing in nutrient solutions, and foliar treatment with a microsyringe, compared with the traditional methodology of herbicide treatment in a wide dose range to plants growing in pots. Two standards (purified biotypes) - one sensitive and one resistant - of each one of the two weed species were considered in all experiments. "Seedling (shoots) test" showed the best performance and is therefore the one on which this kit has been based. To develop the kit, the following were determined: flask size, plant state, time of activity of the herbicide solutions once prepared, optimal date of visual fitotoxicity testing, DG50, discriminatory dose (dose that marks the biggest difference between the sensitive standard and the resistant one), and optimal population sample size. DG50 was calculated by a log-logistic model. The kit manual instruction explains in a simple and practical way the procedure of gathering the plants in the field, of seedling assembly in the flasks, and of evaluating herbicide fitotoxicity. This kit will not be marketed. It will have restricted use for the technical staff of Bayer Crop Science in Colombia and in any other country.

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Santiago Montoya, Bernard Jacqmin & Norberto Hernández
Bayer Crop Science
Colombia

Relative Susceptibility in Open and Greenhouse Populations of Two-Spotted Spider Mite, *Tetranychus urticae* Koch, on Rose to Dicofol

Dicofol is a very commonly used acaricide for the management of spider mites. Development of resistance to miticides in spider mites (Tetranychidae) is often so rapid that effective spider mite management is difficult in many agricultural systems (Jeppson et al., 1975). There are extensive reports worldwide regarding resistance of the two-spotted spider mite, *Tetranychus urticae* Koch, to different groups of chemicals. However, there is no information available regarding relative susceptibility of *T. urticae* to dicofol from open and greenhouse populations from India. In response to an increasing number of treatment failures of dicofol against *T. urticae* on rose, as expressed by farmers in and around Bangalore, this study was taken up. Bioassays were carried out during April-May 2002 to assess the relative susceptibility of open and greenhouse populations of this mite on rose to dicofol 18.5 EC (Kelthane) at the Indian Institute of Horticultural Research (IIHR), Bangalore. Leaf residue method was used for the bioassays as described by FAO (1980). Each concentration was replicated three times. Mite mortality was observed 24 h after release of mites to the insecticide treated leaves of different concentrations. The LC50 values of dicofol to *T. urticae* were recorded as 0.0404 % for greenhouse populations and 0.0195 % for open field populations. Thus, greenhouse population of *T. urticae* had developed 2.1fold resistance to dicofol when compared to the open field. Relatively more tolerance of greenhouse populations of the mite to dicofol might be attributed to more number of generations of mites subjected to acaricidal sprays when compared to open cultivated roses. The development of cross resistance to different chemicals frequently used on this crop needs to be further studied.

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Symposia

CAST Pesticide Resistance Management Symposium Provides Cross-Disciplinary Dialogue

The Council for Agricultural Science and Technology (CAST) held a two-day symposium on April 10-11, 2003 in Indianapolis, Indiana, entitled "Management of Pest Resistance: Strategies Using Crop Management, Biotechnology, and Pesticides." CAST is a nonprofit organization composed of 38 scientific societies and many individual, student, company, nonprofit, and associate society members. CAST assembles, interprets, and communicates science-based information regionally, nationally, and internationally on food, fiber, agricultural, natural resource, and related societal and environmental issues to our stakeholders - legislators, regulators, policymakers, the media, the private sector, and the public.

This symposium provided a cross-disciplinary approach to management of pest resistance and brought together 120 professionals concerned with resistance management involving pathogens, insect pests, and weeds. There were representatives from the pesticide industry, seed companies, extension, academia, state and federal government (U.S. and Canada), pesticide education, consulting agencies, and grower organizations.

The overall goal of the symposium was to provide a collective framework in which more proactive resistance management could be developed in the future. The major objectives of the symposium were to:

1. identify the common issues related to pesticide resistance management across disciplines;
2. identify ways to remove barriers that prevent proactive resistance management;
3. provide opportunities for future discussions on pesticide resistance management;
4. identify future research activities in resistance management; and
5. provide this information to lawmakers and federal agencies, especially the United States Environmental Protection Agency (EPA) and the United States Department of Agriculture (USDA), academia, extension, industry, consulting agencies, and the public.

Forty-seven speakers from industry, academia, extension, consulting agencies, federal and state government, grower organizations, and public interest groups gave presentations at the Symposium. Most of these presentations will be available on-line at the CAST website (http://www.cast-science.org). The symposium agenda was developed by a steering committee consisting of representatives from USDA, EPA, industry (Resistance Action Committees), academia, public interest groups, and grower organizations. The agenda is available at the CAST web site. The Symposium was organized into the following eight sessions:

1. Scope of North American Pest Resistance Problems in 2003
2. Issues in Pest Resistance Management
3. Lessons Learned I: Balance between Industry, Academia, Users, and Regulators
4. Lessons Learned II: Have Models Helped?
5. Role of Stakeholders
6. Lessons Learned III: How Can We Work to Alleviate Barriers to Comprehensive Resistance Management Implementation? How Can We Work Together Better?
7. Pest Resistance Management Goals
8. Symposium Recommendations for Pest Resistance Management - Where to Now?

The symposium provided a fruitful opportunity for all stakeholders involved in insect, weed, and pathogen pest management to come together to discuss issues and lay the foundation for future collaborations to address pest resistance management. Several major interest areas were explored:

1. targeting research funding for pest resistance management with federal competitive grant programs;
2. improving pesticide education programs to address pest resistance management;
3. improving transparency of the EPA's regulation of pesticide resistance management;
4. evaluating of potential economic impacts of pest resistance management;
5. targeting consumer education programs on the use of reduced risk pesticides, resistance management, and the cost of producing quality food in the marketplace;
6. standardizing of definitions for resistance, methods of resistance documentation, etc.;
7. focusing on goals of resistance monitoring programs; and
8. changing national farm policy to better fund research and education for resistance management.

Stakeholders agreed that proactive resistance management is a desirable goal, but the path to reach this goal is unclear.

CAST is in the process of developing an on-line Proceedings of the Symposium that should be available on the CAST website in Fall 2003

(http://www.pestmanagement.info/rmworkshop/). This publication will be available at no cost to CAST members, the media, and government policymakers, and will be accessible to others for a fee. For further information contact, Sharlene Matten, 202-675-8333, ext. 16 or smatten@cast-science.org.

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Announcements and Submission Deadlines

Due to lack of subscriber interest and use we will no longer be offering our **Perspectives Forum** section in the newsletter. This site, intended as an online discussion forum to express your views on issues and/or problems that are of general reader interest, was not being utilized. Perhaps if there is interest, this feature will be reintroduced in the future.

Thank you to those who contributed to this issue - you have really made the newsletter a worthwhile reading experience! Our contributors truely increase the newsletter's success at sharing resistance information worldwide.

We encourage all of our readers to submit articles, abstracts, opinions, etc (see the newsletter online at http://whalonlab.msu.edu/rpmnews/general/rpm_submission.htm for submission information).

The Newsletter is a resource to many around the globe. It is also a wonderful and effective way to enhance the flow of ideas and stimulate communication among global colleagues. We appreciate your efforts to support the newsletter and we look forward to your continued contributions.

The next two **submission deadlines** are:

Monday, September 15th, 2003
Monday, March 15th, 2004

We hope you continue to consider the newsletter as a forum for displaying your ideas and research.

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