Resistant Pest Management

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Insecticide Resistance Action Committee (IRAC),
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EDITORIAL

A Letter from the Editor

Bacillus thuringiensis (*B.t.*) resistance has recently received considerable interest both nationally and internationally. At the recent USDA *B.t.* Workshop, held in Washington, D.C. last month, many issues were presented, analyzed and discussed. The unique difference of this Workshop was USDA's effort to involve agricultural producers and field decision makers from both conventional and organic agriculture along with academics, industry personnel, USDA administrators and USDA researchers.

The Workshop focused on the potential for resistance in both sprayed and transgenic *B.t.*, and explored some of the ecological, operational and policy options for managing *B.t.* resistance. A proceedings will be forthcoming. If you are interested, contact Dr. Bill McGaughey at USDA/ARS, 1515 College Avenue, Manhattan, Kansas 66502, e-mail: mcgaugh@crunch.usgmrl.ksu.edu, phone: (913) 776-2705, FAX: (913) 537-5584.

At another Workshop, Central American policy makers, researchers and extension personnel met in Zamorano, Honduras for an educational and policy exploration meeting on *B.t.* use and potential resistance. This group will develop a policy and educational effort to sustain *B.t.* usefulness in the region. Again, a proceedings will be forthcoming. If you are interested please contact Dr. Allen Hruska at Zamorano, Escuela Agricola Panamericana, Apartado Postal 93, Teucigalpa, Honduras, Phone: (504) 76-6140/50 Extension 2351, e-mail: allan%eapdpv%sdnhon@sdnhon.undp.org, FAX: (504) 76-6242.

A third workshop will be convened jointly by ISNAR and CamBio Tech. on *B.t.* deployment and other *B.t.* issues in October, 1996. This workshop will involve policy makers from Caribbean and South American countries, and address biotechnology issues including *B.t.* resistance. Again, a proceeding will be forthcoming. If you are interested please contact Joel Cohen, ISNAR, P.O. Box 93375, 2509 AJ The Hague, The Netherlands, Phone: (3170) 349-6100, FAX: (3170) 381-9677.

One might ask, "Why all the interest in *B.t.* when it occupies less than 2% of the total global insecticide sales?" Although the answer is complex, it has at least three elements:

- Unprecedented interest on the part of the environmental community and organic producers,
- The recent registration and deployment of transgenic plants in many countries, and
- Laboratory and field resistance to *B.t* in ten to twelve insect pest species.

The Newsletter will continue to update subscribers on these developments, and publish summaries of these Workshop Proceedings as they become available.

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NEWS AND REVIEW

The Role of Industry in Weed Resistance Management: The Herbicide Resistance Action Committee

Summary:

The Herbicide Resistance Action Committee (HRAC) is a body comprising of representatives of the major agrochemical companies. By fostering cooperation between industry members and with the scientific community, HRAC believes that resistance can be managed by developing effective, practical and economic strategies which allow farmers to prevent, or at least minimize the impact of a potentially serious problems. The mission of HRAC is to develop a partnership in resistance management between industry, scientists, advisors and the farmer, to provide support of research in key areas and to provide a communication channel through which information on resistance management can be shared.

Introduction:

Weed resistance to herbicides concerns many sectors of the agricultural community including farmers, advisors, researchers and the agrochemical industry. The fear exists that in an extreme case of resistance, farmers might lose a valuable chemical tool essential for the effective control of yield-reducing weeds.

Resistance is often seen as a problem caused by a particular product. This is an over-simplification and a misconception. Resistance results from mismanagement of the ecosystem, often where an overemphasis is placed on the use of a limited range of herbicides in limited crop rotations with little or no non-chemical weed control.

Experience has clearly shown that resistance problems are manageable through the integrated use of available weed control technologies. This approach utilizes both cultural methods as well as a diversity of herbicides. Such an approach reduces selection pressure in weeds and prevents further development of resistance to any single herbicide.

Those of us working in weed control have a major advantage over our fungicide and insecticide colleagues when it comes to managing resistance. The spread of weed resistance is rarely as explosive as with fungi and insects. Thus, we have time to develop management strategies that prevent the further spread of the problem so the farmer can continue to control his weed problems.

The management strategies devised must be effective, reliable, practical, economical and these strategies must be communicated to the farmer. To help achieve these goals, the Herbicide Resistance Action Committee (HRAC) fosters cooperation between industry, government researchers, advisors and farmers.

Aims of HRAC:

Table 1. HRAC Aims
To foster a responsible attitude to herbicide use.
To support the establishment of country or regional working groups.
To promote a better understanding of the causes and results of herbicide resistance.
To support work which defines the technical basis of resistance management strategies.
To communicate these strategies and encourage their implementation as practical guidelines.
To help define barriers that prevent farmers from adopting measures to manage resistance and then to find appropriate solutions.
To seek active collaboration with public and private researchers, particularly to identify problems, to devise and to implement management strategies.
To facilitate communication between industry representatives.

The Herbicide Resistance Action Committee (HRAC) was founded in 1989 by the agrochemical industry as part of the GIFAP organization (Groupement International des Associations Nationales de Fabricants de Produits Agrochimiques). The aims of HRAC (see Table 1) have the general purpose of supporting the cooperative approach to resistance management. We wish to ensure that the farmer benefits from a full range of weed control tools and that agriculture does not suffer the loss of key herbicides through resistance.

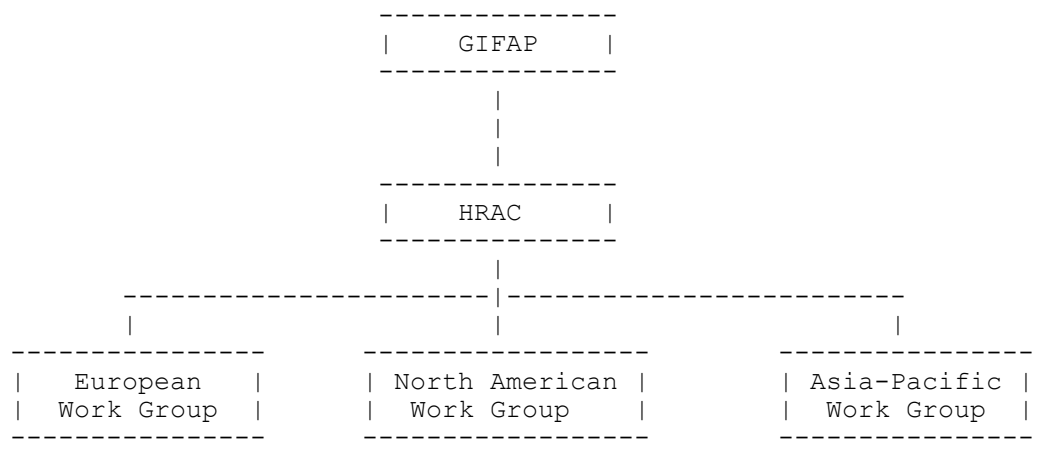
HRAC Membership and Structure:

The Herbicide Resistance Action Committee (HRAC) comprises of representatives from 13 major agrochemical companies (see Table 2).

Table 2. HRAC Membership	
AgrEvo	DuPont
BASF	Monsanto
Bayer	Rhône-Poulenc
Ciba	Sandoz
Cyanamid	Rohm & Haas
Dow Elanco	Tomen
Zeneca	

HRAC has recently re-organized its structure to focus better on the practical implementation of resistance management. This has meant a switch from working groups based on modes-of-action to a regional concept (Figure 1).

Figure 1. HRAC Structure



This organization reflects the HRAC belief that increased efforts are needed to ensure transfer of research results to the end-user. This should include the establishment of integrated use recommendations and their implementation through documentation, publications, training and education.

Practical solutions to resistance problems are dependent on local agronomic practices, weed populations and product registrations. Therefore an intensive local orientation is essential.

The European Work Group supports the establishment and work of country-based teams and currently cooperates with work groups in France, Germany, Spain, Britain, the Netherlands and Belgium. These country specific groups are autonomous groups, generally comprising of both public and industry members. They are very varied in their composition and activities, but all have the same aim of promoting resistance management according to their local needs and are keen to share information and experiences across Europe.

In North America, there were previously three working groups based on mode-of-action (ALS, ACCase and triazine), but these have now been combined into a single team. This work group consists of industry and public members and gives the opportunity to give a coordinated resistance management message across North America.

There is no team structure for the Asia-Pacific work group at present owing to the wide geographic spread and the diversity of problems. The coordination of HRAC actions is taken care of by an appointed HRAC member. However communication and support are essential since there is a great opportunity to learn from one another. For example, the integrated approach in Australia, inspired by the enthusiasm of Steve Powles from the Waite Institute in Adelaide, has combined the expertise of academic members, extension specialists, industry scientists and - most important - farmers, to manage the spread of resistance in Annual Ryegrass. The experience gained here is certainly of great value elsewhere; for example, in India where efforts are just beginning to manage weed resistance in *Phalaris.*

HRAC-funded Projects:

Until now HRAC has concentrated its funding in the areas of research and documentation/communication with the aim of understanding the basic principles of herbicide resistance and its prevention or management.

In research, HRAC has supported a wide range of projects including molecular biology, resistance monitoring and population genetics. Some examples are:

- S. Moss, Rothamsted - baseline monitoring of wild oats in the UK.
- M. Devine, Saskatchewan & I.N. Morrison, Manitoba - monitoring and forecasting of resistance in wild oats and green foxtail in Canada
- C. Mallory Smith, Idaho - survey and gene flow in Kochia and Russian Thistle in the USA.

- C. Eberlein, Idaho - genetics and molecular biology of ALS-resistance.
- R. Malik, Haryana - management of urea-resistant *Phalaris* in India.

In the area of documentation HRAC has funded monographs on:

- Graminicide resistance (A. Mortimer, Liverpool)
- Cross-resistance and multiple resistance (S. Powles and C. Preston, Adelaide)
- Triazine resistance (R. Ritter, Maryland, unpublished)

HRAC has also produced guidelines on how to minimize resistance risk and how to respond to cases of suspected and confirmed resistance.

Weed Management Practices:

Management techniques advocated by HRAC are detailed in the booklet "How to Minimize Resistance Risks and How to Respond to Cases of Suspected and Confirmed Resistance" (available from HRAC directly). These guidelines outline an approach which involves the consideration of the cropping system as a whole and suggest techniques which involve the use of cultural practices together with the careful use of herbicides to minimize selection pressure. Recommended practices are as follows:

Mixtures or sequences of herbicides with differing modes of action are important especially to prevent or overcome resistance based on target site. Although to be effective, the herbicides used in mixtures or sequences must have similar efficacy against the target weed.

Crop rotations may allow different herbicides or cultivation techniques to be used and may also provide different competitive environments to shift the weed flora. Set-aside programs also allow new opportunities to manage populations of resistant weeds.

Cultivation practices may be adjusted if this fits to general agronomic needs. Measures such as stale seedbeds, ploughing, stubble burning, grazing of weeds or mechanical methods can be very effective in reducing weed populations. Economic control levels should be the aim, not higher cosmetic levels that increase selection pressure without providing a financial return to the farmer. Generally, the best approach to resistance management is Integrated Weed Management using all available control methods in an economic and sustainable manner.

Future Directions for HRAC:

The guidelines provide a good starting point, but HRAC believes that specific strategies need to be developed at a local level and it is for this reason that HRAC will continue to support country work groups in the design and implementation of practical resistance management programs.

Other issues, however, need to be coordinated at a global level. Such an issue is the classification of herbicides according to their mode of action grouping. HRAC has recently proposed a herbicide mode of action grouping (Jutsum & Graham 1995) which builds on the system developed in Australia (Avcare 1995). This classification will allow the uniform establishment of a labeling system which identifies the mode(s) of action included in a product. It is proposed that an alphabetic letter(s) be added to all product labels to allow easy planning of herbicide rotations based on mode of action. HRAC will make every effort to have this classification standardized world-wide and will involve country groups such as the Weed Science Society of America (WSSA) to help implement the system where appropriate.

A further area that merits the support of HRAC is the establishment of a resistance database. WSSA has initiated such a project and HRAC is keen to see this established as definitive reference work.

Our approach over the next few years will be to concentrate on supporting the development and communication of programs which will provide the end-user with specific resistance management strategies. This will include:

- Establishment of clear labeling statements
- Support of science-based registration procedures
- Communication of resistance issues through congresses, monographs, newsletters, internet
- Support of research aimed at defining and verifying resistance management programs
- Support of the practical implementation of these programs
- Resistance testing methodology. This approach also has the consequence that we will reduce our support of basic research *e.g.* on mechanisms of resistance, unless the work has a direct practical relevance.

Conclusions:

HRAC is convinced that weed resistance to herbicides can be managed. We must make a major concerted effort now to ensure that farmers still have available the whole arsenal of weapons for the control of their weed problems. The combined expertise of public researchers, industrial scientists, and growers integrated into practical weed management programs can achieve this effort. HRAC will foster partnership between industry, researchers, advisors, dealers and farmers to ensure resistance is properly managed so growers can continue to win their battle against weeds.

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Susceptibility of Lygus Bug Populations to Acephate (Orthene®), Bifenthrin (Capture®) and Other Insecticides in Arizona

Executive Summary:

Adult lygus bugs, *Lygus hesperus* (Knight), were collected from alfalfa fields in 11 different cotton-producing areas of Arizona. A standardized, glass vial method estimated susceptibility of the collected populations to the organophosphate insecticide, acephate (Orthene®), and the pyrethroid, bifenthrin (Capture®). Overall, lygus throughout the state were significantly less susceptible to acephate and bifenthrin in 1995 than in 1994. Lygus resistance to acephate continues to be widespread and intense, but not uniform in Arizona. In 1995, all populations possessed individuals that survived exposure to vial treatments of 10,000 μg/ml acephate. Lygus bugs from Safford and Maricopa represented the most and least susceptible populations to both acephate and bifenthrin, respectively. These two populations were tested for susceptibility to the following nine insecticides: aldicarb (Temik®), dimethoate (Gowan Dimethoate E267®), endosulfan (Gowan Endosulfan 3EC®), imidacloprid (Admire 2F®), malathion (Gowan Malathion 8®) methamidophos (Monitor 4®), methomyl (Lannate LV®), oxamyl (Vydate 3.77L®), and oxydemeton-methyl (Metasystox-R SC®). The Maricopa population was significantly less susceptible to six of these insecticides. Our findings support the hypothesis that the intensive use of pyrethroid and organophosphate insecticides for whitefly control in cotton has selected for resistance in lygus. This result portends increased problems with lygus control in the future, points to the need for developing new

tools for controlling lygus bugs in Arizona cotton, and underscores the urgent need to find alternatives to the current heavy reliance on insecticides for managing whiteflies in cotton.

Introduction:

Lygus bugs are serious pests of cotton in the desert Southwest (Wene & Sheets 1994). Lygus populations in Arizona consist of four different species. *Lygus hesperus* is the most common in the cotton-growing areas. Cotton fields can be invaded between May and September. Great numbers of lygus migrate from nearby crops, such as alfalfa and safflower (Seveacherian & Stern 1974, Mueller & Stern 1974). Lygus feeding causes shedding of immature squares and damage to bolls, thus reducing cotton yields (Mauney & Henneberry 1978, 1984).

In Arizona cotton, the severity of invasions by lygus varies widely from year to year. Pest managers must be vigilant in monitoring for lygus and must routinely make decisions as to whether insecticide treatments are economically warranted for this pest. Unnecessary applications of insecticides are not only costly, but increase the possibility of secondary pest outbreaks (Leigh *et al*. 1970). Conversely, inadequate scouting or delaying required treatments for lygus results in severe yield losses.

Lygus resistance to insecticides has long been a concern of cotton pest managers. In California, Leigh *et al*. (1976) reported increased lygus resistance to organophosphate and carbamate insecticides at locations with greatest insecticide use. We continue to use many of these same organophosphates and carbamates to control lygus in Arizona. More recently, Knabke & Staetz (1991) reported substantial reductions in susceptibility within specific Arizona lygus populations to pyrethroids relative to populations from the Imperial Valley of California.

While resistance can often be a the cause for control failures, many other factors may result in inadequate performance of pesticides, especially from highly mobile pests like lygus. In particular, a high rate of lygus immigration from refuges to cotton provides the appearance of insecticide failure. Only precision bioassays in the laboratory can determine whether resistance is a factor for reduced pesticide performance at any location.

In 1993, severe lygus infestations were experienced throughout the cotton crop in Arizona. At that time, some growers in Maricopa, Pinal and Pima counties reported inadequate performance of lygus treatments. In response we began investigations on the susceptibility of Arizona lygus populations to the pyrethroid insecticide, bifenthrin (Capture®) and the organophosphate, acephate (Orthene®) in 1994 and 1995. In 1995, we broadened our study to include evaluations of other insecticides used for lygus control. We selected two populations from our statewide resistance monitoring (least and most susceptible to bifenthrin and acephate), and tested the susceptibility of these populations to nine other insecticides. This study identifies resistant populations of lygus and evaluates insecticides that may control these resistant cotton pests in Arizona.

Materials and Methods:

Collection of Lygus

With sweep nets, approximately 400-600 adult Lygus bugs were collected from each field location. Bugs were emptied from the sweep nets into lunch-size bags with alfalfa cuttings. These bags were then placed in ice chests and transported to the laboratory in Tucson. Most lygus were tested the same day of collection. If necessary, they were held for 24-48 hours at 15-20°C. Lygus populations were sampled from Buckeye (3 locations), Casa Grande (3 locations), Cochise Country (2 locations), Gila Bend (3 locations), Gilbert (3 locations), Maricopa (3 locations), Marana (3 locations), Paloma (3 locations), Parker (3 locations), Safford (3 locations) and Yuma (3 locations), Arizona. In all but one case, collections were from alfalfa fields adjacent to cotton. The Maricopa 2 sample was collected from cotton.

Bioassay Method

We bioassayed with the glass vial technique described by Knabke & Staetz (1991). Modifications to this technique included drying treated vials on a commercial hot dog warmer, covering vials with dialysis membrane instead of vial screw caps, and eliminating carbon dioxide for anesthetizing bugs to facilitate handling.

Standard 20 ml screw-cap scintillation vials were treated with solutions of insecticide or acetone. A volume of 0.5 ml solution was placed in each vial. Vials were placed immediately on the hot dog warmer at room temperature and rotated slowly until the solvent evaporated. This provided thorough insecticide coverage on the inner vial surface.

For the statewide monitoring, acephate concentrations (w/v) used were 0 (control), 1,000 and 10,000 μg/ml. Acephate solutions were prepared and used within 24 hours. Bifenthrin stock solutions were 0, 10 and 100 μg/ml. Bifenthrin solutions were stored at 3°C for up to 4 weeks. Bifenthrin dilutions were prepared each day that vials were treated. Bifenthrin-tested vials were stored at 3°C for up to two weeks.

Susceptibility of Maricopa and Safford lygus bugs were contrasted with the following formulated insecticides: aldicarb (Temik®), dimethoate (Gowan Dimethoate E267®), endosulfan (Gowan Endosulfan 3EC®), imidacloprid (Admire 2F®), malathion (Gowan Malathion 8®) methamidophos (Monitor 4®), methomyl (Lannate LV®), oxamyl (Vydate 3.77L®), and oxydemeton-methyl (Metasystox-R SC®). Each insecticide was evaluated with 5-6 concentrations (w/v) ranging from 0.1 to 10,000 μg/ml. A total of 8-10 replications were conducted for each concentration test. Solutions were prepared in acetone and insecticide treated vials were rotated for 10 to 60 minutes. Treated vials were used in bioassays within 24 hours of preparation. One exception was imidacloprid solutions that were prepared in distilled water. Vials treated with imidacloprid/water solutions were rotated 24 hours to dry. Imidacloprid treated vials were stored up to two weeks in darkness before their use in bioassays.

Field-collected lygus were collected and held in one-quart plastic containers with hinged snap lids. Five adult lygus were aspirated into each bioassay vial and vials were sealed with 2.5 x 2.5 cm squares of dialysis membrane secured with a #8 rubber band. Prepared bioassay vials were then held at 27°C in an incubator. After 3 hours, mortality was recorded. Individuals unable to propel themselves the length of their body were scored as dead. Mortality values reported herein represent only the individuals scored as dead. Significant differences in mortality between populations was determined by ANOVA of mean mortality values, transformed with $\arcsin\sqrt{x}$. Moribund individuals (alive, but not able to walk) were scored, but did not affect this study.

Results and Discussion:

Statewide Surveys of Susceptibility to Bifenthrin and Acephate

The susceptibility of lygus populations throughout Arizona is illustrated in Fig. 1a-b (bifenthrin) and Fig. 2a-b (acephate). Control mortality was consistently below 10%. In 1995, lygus bug susceptibility to bifenthrin varied widely within the state. Populations most susceptible to bifenthrin originated from Cochise County, Safford and Yuma. The populations least susceptible to bifenthrin represented the major low desert cotton-growing areas of Buckeye, Paloma and Parker. Most populations had individuals that survived exposure to 100 μg/ml bifenthrin (Fig. 1b). However, this treatment yielded no survivors from the Cochise County and Safford populations.

Lygus resistance to acephate was widespread, but not uniform throughout the cotton-producing areas of Arizona. Populations most susceptible to acephate originated from Cochise County and Safford. Populations least susceptible to acephate were found in Gila Bend, Marana and Parker. Most populations had individuals that survived exposure to 10,000 μg/ml acephate (Fig. 2b). However, this treatment resulted in no survivors in the Cochise County #2 population. The extreme variation observed in lygus susceptibility within and between the major cotton-growing areas illustrates that generalizations regarding population susceptibility did not apply statewide. Insecticides that work very well in Safford, Yuma and Cochise County are likely to be less effective against populations in Parker, Marana and Gila Bend. Area-specific resistance monitoring is necessary to detect such differences.

1994-1995 Comparison of Statewide Surveys of Susceptibility

Figures 3a-b compare lygus susceptibility to bifenthrin and acetate at several locations in 1994 and 1995. Lygus populations were significantly less susceptible to bifenthrin in 1995 than in 1994 (Fig. 3a). The populations most susceptible to bifenthrin in 1994 were from Yuma, Safford, Parker and Marana. For bifenthrin, the only relatively susceptible populations observed in 1995 were from Safford. The least susceptible populations in 1994 were from Casa

Grande. Survivorship to 100 μg/ml bifenthrin ranged from 6-38%. In 1995, the least susceptible populations came from the Parker area. These lygus exhibited 82-100% survivorship to 100 μg/ml bifenthrin.

Temporal changes were observed in lygus susceptibility to acephate statewide (Fig. 3b). Lygus were significantly less susceptible to acephate in 1995 than in 1994. In 1995, the Safford collections were the most susceptible to acephate with survivorship to 10,000 μg/ml ranging from 52-100%. The two Marana populations exhibited an extreme reduction in susceptibility to acephate from 1994 (no survivors of 10,000 μg/ml acephate treatments) to 1995 (86-100% survivorship).

These substantial changes in susceptibility to bifenthrin and acephate support the hypothesis that resistance problems in Arizona lygus are increasing due to the intensive insecticide use against whiteflies in cotton. This underscores the importance of the multi-agency efforts underway in Arizona to reduce insecticide use and to develop alternative non-chemical controls for managing whiteflies.

Contrasts of Maricopa and Safford Populations

Figures 4a-b illustrate significant regional differences in lygus susceptibility to a broad range of insecticides. The Maricopa populations, tested because of its low susceptibility to acephate and bifenthrin, exhibited substantially reduced susceptibility (Figs. 5a-i). The Safford population was selected due to its comparatively high susceptibility to acephate and bifenthrin.

Our results indicted lygus resistance to aldicarb, dimethoate, imidacloprid, malathion, methomyl and oxamyl (Figs. 5a-i) in Arizona. These conclusions should be verified by evaluations of additional populations. Nonetheless, the large differences observed in susceptibility to recommended insecticides points to the essential role of resistance monitoring to help growers avoid less effective products for lygus control.

Conclusions:

We found that lygus bugs in Arizona cotton are becoming increasingly resistant to insecticides. It is likely that insecticides applied to suppress the severe whitefly infestations have selected for lygus resistance development. Resistance in lygus bugs is the indirect result of whitefly management. Therefore, chemical control and resistance management programs for both pests need to be developed jointly and harmonized. This is most important with the registration of new growth regulators for whitefly control and the 1996 Arizona whitefly resistance management strategy.

Finally, although it is expensive to monitor susceptibility to insecticides on a farm-by-farm basis, much information can be provided to growers by routine surveys of resistance focused on populations difficult to control. The University of Arizona's Extension Arthropod Resistance Management Laboratory will continue to work with growers, PCA's and chemical producers in Arizona in pursuit of these objectives.

Acknowledgments:

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RESISTANCE AROUND THE GLOBE

The Evolution of Insecticide Resistance in *Aphis gossypii* Glover (Hemiptera: Aphidiae) in Cameroon

The importance of *Aphis gossypii* Glover as a cotton pest is increasing throughout the cotton-producing regions of the world (Leclant & Deguine 1994). In central Africa, *A. gossypii* is the second most important economic cotton pest, following *Helicoverpa armigera* (Hübner). *A. gossypii* damage affects the yield of cotton seed as well as the fiber quality. 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.

Originally, new insecticide application methods used by farmers (ULV, VLV) considerably reduced pesticide effectiveness. However since 1993, there has been a general evolution of resistance in *A. gossypii* to most insecticides, particularly organophosphates. Frequently in farmers' fields, there is no significant decrease in aphid populations the day after treatment with monocrotophos. Often, the aphid populations in monocrotophos-treated fields exceed even those in

non-treated fields. Even under well-controlled research station conditions, weekly treatments of monocrotophos do not always control aphid populations. Similarly, biweekly applications of dimethoate (4 g a.i./1) at more than 100 1/ha in dry season irrigated fields are not consistently effective in reducing aphids populations. These findings have also been confirmed in bioassay trials comparing various aphicides.

Preliminary laboratory determinations for LC_{50} for several aphicides were carried out at CIRAD-CA in Montpellier, (France) with aphid clones collected in Maroua (Cameroon) (Amiot 1993). Results revealed that the resistance level in aphicides in these clones was identical to a known aphicide-resistant strain from Sudan (Gubran *et al.* 1992). It is important that these resistance studies continue. In Cameroon, we developed a simple method for rearing *A. gossypii* and evaluating the LC_{50} values. These methodologies make it possible to study the evolution of pesticide resistance in *A. gossypii*. This was done with other cotton pests, most notably *H. armigera*.

On-going studies will develop a simple field screening technique complementary to the laboratory evaluation technique. This field technique will expose *A. gossypii* to leaves soaked in aphicide solutions and estimate mortality. Once defined, this technique will allow rapid comparisons between clones of *A. gossypii* and allow us to study aphicide-resistance over space and time.

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Monitoring Susceptibility to Selected Insecticides in *Spodoptera frugiperda* and *Spodoptera latifascia* Populations: Two Main Cotton Pests in Northern Argentina

Introduction:

Fall armyworm, *Spodoptera frugiperda*, is generally a secondary, late season pest in the cotton cropping area in North Eastern Argentina. However in Northern Argentina, excessive economic damage to cotton by armyworm occurred in a large cotton growing area of 20,250 ha or approximately 2% of the whole cotton cropping area in the country. Recently, in the framework of a cotton IPM research project developing in Salta-Argentina (LIAG-CONICET Project - Basic research on cotton insect pests from Northern Argentina), a second *Spodoptera* species has been identified in that region. *S. latifascia* has a similar life cycle and habits as *S. frugiperda*. Flight periods may occur in alternate peaks or simultaneously with *S. frugiperda* populations and have not been distinguished from the former species during field monitoring trials.

Spodoptera sp. chew into leaves, squares and bolls producing injury similar to that caused by bollworms. Feeding is concentrated on terminals early in the season. This damage provokes excessive branching, may delay fruiting and induce boll rot. Late in the season, damage to small bolls can reduce yield drastically. In addition to cotton, the armyworm feeds on maize and probably on other favorable hosts from the native flora.

For several reasons, it is advisable to establish baseline toxicological information for both armyworm species. First, there are no baseline susceptibility data for *Spodoptera* populations in Argentina. Second, populations of *Spodoptera* from Northern Argentina have not been subjected to high selection pressure with insecticides. Third, the cotton cropping area in Northern Argentina rose from a few hectares in 1987 to more than 20,250 ha in 1995. Therefore, insecticide pressure on these insect pest populations will increase dramatically over future cropping seasons and eventually pest resistance will arise.

The purpose of this study was to determine the susceptibility of *S. frugiperda* and *S. latifascia* to the insecticides used to control both species in Northern Argentina, and establish the baseline for future assays with discriminating dose.

Materials and Methods:

For each species, 50 third instars were collected in a cotton field in Talavera, Salta-Argentina and reared separately under laboratory conditions. All cultures were reared in incubators maintained at 28 ± 1° C, 75% RH and 16:8 (L:D) photoperiod. Emerging adults mated and oviposited in the laboratory. The 3rd instar F1 generation were chosen for the bioassays because in the future, this instar can be collected in the field easily and tested with minimal effort (Ernst & Ditterich 1991). Furthermore, *S. frugiperda* and *S. latifascia* can be easily differentiated at this instar.

To derive concentration-mortality lines, five widely used cotton insecticides were chosen: cyfluthrin (Baythroid, BAYER AG), beta-cypermethrin (Atrion, Chem. SINTYAL), deltamethrin (Decis, AGREVO), metamidophos (Tanaron, BAYER AG) and azinphos-methyl (Azinphos-Methyl, BAYER AG). All insecticides were supplied as technical grade materials. The technical insecticides were dissolved in analytical grade acetone (Merck) to make stock solutions of 10 mg/ml. Before treatment, serial dilutions of each insecticide-stock solution were prepared with acetone and applied to Watman No. 1 filter paper discs. For the controls, paper discs were treated with acetone. Treated filter paper discs were left to dry for three hours before the bioassay was started. Newly molted 3rd instar *S. frugiperda* (100mg S=10mg) and *S. latifascia* (120mg S=8mg) were bioassayed.

The dose-response curve was determined for each species by placing 30 larvae in petri dishes on the filter papers impregnated with different concentrations of each insecticide. After the larvae were exposed to the insecticides for approximately 15 minutes, 500 mg artificial diet was added to each petri dish. Three replicates were treated to provide a sample size of 90 larvae per dose (Robertson & Preisler 1992).
Initially, tests with a wide range of concentrations to determined the range needed to obtain 0-100% mortality. After the proper range was obtained, six different concentrations of each insecticide were prepared within this range.

Mortality was recorded at 24, 48 and 72 hours after exposure. Larva that could not crawl in a coordinated manner were considered dead. Twenty-four hour dose-mortality data were analyzed by a probit analysis (Russell *et al.* 1977). When necessary, control mortality was corrected with Abbot's formula.

Results and Discussion:

Bioassay results on the F1 larvae from field-collected strains of *Spodoptera frugiperda* and *S. latifascia* are summarized in Tables 1 and 2.

Table 1. Response of 3rd instar *Spodoptera frugiperda* from Northern Argentina to selected insecticides

Insecticide	LC_{50}	95% FL	S	R
Cyfluthrin	4.50µg/cm2	3.24 - 5.76	2.5	0.9523
β-cypermethrin	2.40µg/cm2	1.77 - 3.09	1.9	0.9985
Deltamethrin	0.60µg/cm2	0.45 - 0.78	1.9	0.8948
Metamidofos	192.00µg/cm2	80.6 - 316.8	1.2	0.9886
Azinfos Methyl	147.00µg/cm2	95.5 - 264.6	2.4	0.7793

LC_{50} = Lethal concentration, 95% FL = Fiducidal Limit
S = Slope, R = Correlation

Table 2. Response of 3rd instar *Spodoptera latifascia* from

Northern Argentina to selected insecticides				
Insecticide	LC_{50}	95% FL	S	R
Cyfluthrin	1.55µg/cm2	1.16 - 1.98	3.9	0.9891
β-cypermethrin	1.20µg/cm2	0.85 - 1.51	4.7	0.9807
Deltamethrin	1.00µg/cm2	0.58 - 1.35	2.7	0.9835
Metamidofos	430.00µg/cm2	236.5 - 688.0	4.3	0.9976
Azinfos Methyl	99.40µg/cm2	63.6 - 181.9	1.7	0.7586

LC_{50} = Lethal concentration, 95% FL = Fiducidal Limit
S = Slope, R = Correlation

Based on the LC_{50} values and slopes from Table 1, we calculated the discriminating doses for each insecticide for both *Spodoptera* species (Roush & Miller 1986). Comparison between larval mortality at these discriminating doses is only an indication of resistance. There is a need for more detailed confirmatory tests to gain a more complete assessment of the resistance.

Methods for resistance detection in single insects based on immunity or biochemical assays and packed in commercial kits are much cheaper and simpler than conventional bioassays (Brodgon 1989). Nevertheless, conventional resistance detection based on dose-mortality tests is the most satisfactory means for monitoring resistance in areas in the southern hemisphere where it is common find a number of subspecies or biotypes of cosmopolitan pests species. Based on the sparseness of information on pest taxonomy and the complete lack of information about susceptibility of the major crop pests to insecticides, commercial kits for resistance detection need to be fitted for local circumstances.

Meanwhile, conventional dosage-mortality tests fulfill the basic requirements for the assessment of pest susceptibility to insecticides in Argentina. Data on larval susceptibility to insecticides from the F1 field populations of these two species serve as a proper baseline for resistance detection. In the future, resistance monitoring programs with a technique like the vial test (Kanga *et al.* 1993) fitted to local conditions will be useful for field monitoring for pesticide resistance in cotton pests in Argentina.

Successful pest management of *Spodoptera* populations depends on accurate monitoring for resistance to insecticides. Thus, high priority should be given to establishing reliable baseline levels of insecticide susceptibility to insecticides. Data obtained through the dosage-mortality relationship provides the information necessary for discriminating dose bioassays. Developing the ability to accurately and quickly identify resistance will keep farmers from using some insecticides after their effectiveness declines.

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Susceptibility of Tarnished Plant Bugs in Louisiana to Selected Insecticides

Abstract:

Tarnished plant bug (TPB) was collected from various hosts and locations throughout Louisiana to monitor for tolerance to carbamate, organophosphate, and pyrethroid insecticides. Insects were tested with the residual film vial bioassay. TPB collected April through July of 1994 and 1995 usually had significantly lower LC_{50} values (4-37 fold) to cypermethrin than collections in August. LC_{50}s for acephate ranged from 0.93 to 6.49 μg/vial, a 7-fold difference. The lowest LC_{50} for oxamyl was 0.92 μg/vial, while the highest value was 4.84 μg/vial, a 5-fold difference.

Introduction:

The tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois), is an important pest of cotton in the Mid-South United States. This insect has gained attention over the past few years due to the declining efficacy of insecticides registered for its control. Resistance of this species to several insecticide classes (carbamates, organochlorines, organophosphate, and pyrethroids) has been documented over the past two decades. Resistance to the organophosphates methyl parathion (Cleveland & Furr 1979) and dimethoate (Snodgrass & Scott 1988) was confirmed in TPB populations from Mississippi. Recently, another field collection of TPB from Mississippi exhibited resistance to the pyrethroids permethrin and bifenthrin (Snodgrass 1994). TPB from this same region of Mississippi also exhibited resistance to the organophosphates dicrotophos and methyl parathion (Snodgrass & Elzen 1995). In Arkansas, Hollingsworth *et al*. (1995 submitted) reported TPB resistant to the organophosphate dimethoate, the organochlorine endosulfan, the pyrethroid lambda-cyhalothrin, and the carbamate oxamyl.

Materials and Methods:

TPB was collected from various hosts and locations throughout Northeast Louisiana from April through September during both 1994 and 1995 (Table 1). Each collection from North Louisiana was from cotton-producing parishes with relatively high insecticide use. In 1995, a collection was made in South Louisiana (near Baton Rouge in East Baton Rouge Parish), where cotton acreage and insecticide use was relatively low. Collections were made with a standard 38 cm diameter sweep net. After collection, TPB were placed in a 46 x 92 cm wire mesh cage, fed green beans (*Phaseolus* spp.) and held overnight.

Table 1. Location, date, host, and sample size (n tested) of tarnished plant bugs collected for use in vial bioassays.

Location(Parish)	Date	Host	n
Bossier	June 1994	Cotton	48[1]
	June 1994	Alfalfa	174[1]
	June 1994	Alfalfa	150[2]
Caddo	May 1994	Alfalfa	207[1]
	June 1995	Alfalfa	210[1]
	June 1995	Horse weed	198[1]
	June 1995	Alfalfa	180[2]
	June 1995	Alfalfa	144[3]

	June 1995	Horse Weed	171[3]
Concordia	April 1994	Crimson Clover	237[1]
	June 1994	Cotton	120[1]
	July 1994	Cotton	54[1]
E. Baton Rouge	April 1995	Fleabane/Red Clover	251[1]
	April 1995	Fleabane/Red Clover	234[2]
Franklin	May 1994	Cutleaf Primrose	57[1]
	May 1994	Mustard	44[1]
	May 1994	Alfalfa	180[1]
	May 1994	Sericea lespedeza	93[1]
	June 1994	Alfalfa	330[2]
	June 1994	Sericea lespedeza	75[2]
	June 1994	Tickseed	49[2]
	June 1994	Mustard	179[2]
	June 1994	Tickseed	234[3]
	June 1994	Tickseed	150[3]
	June 1994	Mustard	165[3]
	April 1995	Crimson Clover	225[1]
	April 1995	Crimson Clover	210[2]
	May 1995	Cutleaf Primrose	164[1]
	May 1995	Cutleaf Primrose	180[2]
	May 1995	Cutleaf Primrose	216[3]
	June 1995	Tickseed	320[1]
	June 1995	Tickseed	216[2]
	June 1995	Alfalfa	216[3]
	July 1995	Mustard	208[1]
	July 1995	Mustard	142[2]
	July 1995	Cotton	195[3]
	August 1995	Cotton	216[1]
	August 1995	Cotton	216[2]
	August 1995	Cotton	180[3]
	Sept. 1995	Cotton	150[1]
Morehouse	June 1994	Cotton	45[1]
Ouachita	May 1995	Cutleaf Primrose	198[1]
	May 1995	Cutleaf Primrose	180[2]
	May 1995	Cutleaf Primrose	180[3]
Richland	June 1994	Black-eyed Susan	90[2]
	June 1994	Black-eyed Susan	135[3]
Tensas	May 1994	Cutleaf Primrose	57[1]
	May 1994	Mustard	39[1]
	May 1994	Cutleaf Primrose	229[1]
	June 1994	Cotton	77[1]
	June 1994	Mustard	75[2]
	June 1994	Mustard	354[3]
	July 1994	Alfalfa	325[1]
	July 1994	Mustard	120[2]
	July 1994	Cotton	150[2]
	July 1994	Mustard	268[3]
	August 1994	Cotton	112[1]
	August 1994	Cotton	99[1]
	August 1994	Cotton	75[3]
	August 1994	Cotton	150[3]
	August 1994	Cotton	126[2]
	August 1994	Cotton	120[2]
	May 1995	Pansy Dog Shade	150[1]
	May 1995	Pansy Dog Shade	135[2]
	May 1995	Pansy Dog Shade	180[3]
	August 1995	Cotton	174[1]
	August 1995	Cotton	174[2]
	Sept. 1995	Cotton	165[2]

[1]Tested with cypermethrin treated vials.
[2]Tested with acephate treated vials.

[3]Tested with oxamyl treated vials.

TPB adults were tested for their responses to acephate, cypermethrin, and oxamyl with the vial bioassay (Snodgrass 1994). Technical-grade samples of acephate (Valent USA, Walnut Creek, CA), cypermethrin (FMC Corporation, Middleport, NY), and oxamyl (Du Pont E.I de Nemours, Wilmington, DE) were obtained from the manufacturers and serial concentrations prepared in acetone. Vials were treated individually with each insecticide as described by Plapp *et al*. (1987) and stored in the freezer until bioassays were conducted. Five to six concentrations were tested against each population. Acetone treated vials served as controls and Abbott's formula (1925) was used to correct all mortality calculations for control mortality. Bioassays (three TPB/vial) were done at room temperature (70°F) and observations for mortality made 24 hours post-treatment. LC_{50} values, 95% confidence intervals, and slopes of ld-p lines were determined by probit analysis (Robertson & Preisler 1992). LC_{50} values were considered significantly different if 95% confidence limits did not overlap.

Results:

During 1994, all TPB collections, except the May collection from cutleaf primrose in Tensas Parish, tested with cypermethrin from April through July had significantly lower LC_{50}s (6-37 fold) compared to LC_{50}s for August collections (Table 2). The Caddo Parish population collected from alfalfa in May had a significantly lower LC_{50} compared to the Tensas Parish collections from cutleaf primrose and mustard in May. For collections made in the same month, there were no other significant differences in LC_{50}s among collections (Table 2). Slopes of ld-p lines ranged from 0.76 to 3.24. In 1995, TPB tested from April through June with cypermethrin had significantly lower LC_{50}s (4-22 fold) compared to LC_{50}s for August collections from cotton (Table 3). The LC_{50} for the Franklin Parish collection in July was significantly lower than the LC_{50} for collection in the August, but not from the LC_{50} for the Tensas Parish collection made in August. The East Baton Rouge and Franklin Parish collections in April had significantly lower LC_{50}s than the Franklin Parish collection made in September. The Franklin Parish collection from tickseed in June and the Caddo Parish collection from alfalfa in June also had significantly lower LC_{50}s than the Franklin Parish collection in September. The East Baton Rouge Parish collection had a significantly lower LC_{50} compared to the Franklin Parish collection from May. There were no other significant differences in LC_{50}s among collections from April through July (Table 3). Slopes of ld-p lines ranged from 0.99 to 2.74.

Table 2. Tarnished plant bug response to cypermethrin in Louisiana strains collected in 1994. Mortality assessed 24 hours after exposure.

Location (Parish)	April LC_{50} Slope ± SE (95% CL)	May LC_{50} Slope ± SE (95% CL)	June LC_{50} Slope ± SE (95% CL)	July LC_{50} Slope ± SE (95% CL)	August LC_{50} Slope ± SE (95% CL)
Concordia	1.71 0.76 ± 0.12[2] (0.65-4.67)	----[1]	0.58 1.19 ± 0.29[7] (0.17-1.00)	1.14 2.33 ± 0.54[7] (0.69-1.82)	----[1]
Franklin	0.91 1.51 ± 0.16[2] (0.65-1.19)	0.83 1.32 ± 0.32[3] (0.31-1.63)	----[1]	----[1]	----[1]
		2.45 1.62 ± 0.55[4] (0.59-6.26)			
		1.17 2.09 ± 0.25[5] (0.89-1.52)			
		1.42 2.06 ± 0.52[6] (0.62-2.40)			
Tensas	----[1]	2.99 0.83 ± 0.29[3] (1.07-19.43)	1.69 1.11 ± 0.28[7] (0.82-3.47)	2.49 1.23 ± 0.20[5] (1.49-3.81)	15.89 1.58 ± 0.49[7] (8.79-112.20)
		2.75 1.40 ± 0.41[4] (1.25-8.36)			21.22 1.54 ± 0.55[7] (10.73-518.6)
		0.80 1.24 ± 0.20[3] (0.28-1.50)			
Morehouse	----[1]	----[1]	0.65 0.94 ± 0.34[7] (0.08-1.82)	----[1]	----[1]
Caddo	----[1]	0.73 2.12 ± 0.25[5] (0.56-0.92)	--[1]	----[1]	----[1]
Bossier	----[1]	----[1]	0.93 3.24 ± 0.98[7] (0.61-1.37)	----[1]	----[1]
			0.65 1.91 ± 0.67[5] (0.02-1.49)		

[1] Collections not made.
[2] Collected from crimson clover, *Trifolium pratense*.
[3] Collected from cutleaf primrose, *Oenothera laciniata* Hill.
[4] Collected from mustard greens, *Brassica juncea*.
[5] Collected from alfalfa, *Medicago sativa* L.
[6] Collected from sericea lespedeza, *Lespedeza cuneata* [Dum.-Cours] G. Don.
[7] Collected from cotton, *Gossypium hirsutum* L.

Table 3. Tarnished plant bug response to cypermethrin in Louisiana strains collected in 1995. Mortality assessed 24 hours after exposure.

Location (Parish)	April LC_{50} Slope ± SE (95% CL)	May LC_{50} Slope ± SE (95% CL)	June LC_{50} Slope ± SE (95% CL)	July LC_{50} Slope ± SE (95% CL)	August LC_{50} Slope ± SE (95% CL)	September LC_{50} Slope ± SE (95% CL)
E. Baton Rouge	0.85 1.78 ± 0.18[2] (0.65-1.09)	----[1]	----[1]	----[1]	----[1]	----[1]
Franklin	0.68 1.27 ± 0.17[3] (0.23-1.34)	1.97 1.85 ± 0.27[4] (1.23-3.89)	1.09 1.58 ± 0.22[6] (0.65-1.59)	2.64 1.75 ± 0.50[9] (0.85-4.26)	12.69 1.47 ± 0.21[10] (6.38-59.97)	3.47 1.13 ± 0.21[10] (1.64-7.23)
Tensas	----[1]	1.10 1.03 ± 0.21[5] (0.35-3.40)	----[1]	----[1]	8.34 0.99 ± 0.18[10] (4.20-29.20)	----[1]
Ouachita	----[1]	1.21 1.78 ± 0.21[4] (0.69-2.05)	----[1]	----[1]	----[1]	----[1]
Caddo	----[1]	----[1]	0.57 1.70 ± 0.23[7] (0.40-0.76)	----[1]	----[1]	
			1.34 2.74 ± 0.56[8] (0.73-1.89)			

[1] Collections not made.
[2] Collected from daisy fleabane, *Erigeron philadelphicus* L. and red clover, *Trifolium pratense*.
[3] Collected from crimson clover, *Trifolium incarnatum* L.
[4] Collected from cutleaf primrose, *Oenothera laciniata* Hill.
[5] Collected from pansy dog shade, *Limnosciadium pinnatum*.
[6] Collected from tickseed, *Coreopsis tinctoria* Nuttall.
[7] Collected from alfalfa, *Medicago sativa* L.
[8] Collected from horse weed, *Erigeron canadensis* L.
[9] Collected from mustard greens, *Brassica juncea*.
[10] Collected from cotton, *Gossypium hirsutum* L.

The acephate LC_{50} values for the collections made in 1994 ranged from 1.10 (Bossier Parish in June) to 6.49 (Richland Parish in June), a 5.9-fold variation. The Richland Parish collection in June had a significantly higher LC_{50} compared to the Bossier Parish collection, Tensas Parish collection, and Franklin Parish collection from tickseed. Collections from Bossier Parish in June, Tensas Parish in June, and the Franklin Parish collection from tickseed in June were significantly lower (3- to 4 -fold) than the Franklin Parish collection from cotton in September. The Tensas Parish collection from cotton in July had a significantly higher LC_{50} compared to the collection from mustard during the same month. During June and July, there were no significant differences in LC_{50}s among populations collected from mustard in Franklin and Tensas Parishes. The Tensas Parish collection from cotton in September had a significantly lower LC_{50} compared to other collections made from cotton during July, August, and September. Slope values ranged from 1.51 to 3.22 during 1994. All collections made from April through June 1995 had significantly lower LC_{50}s (1.7- to 4.6-fold) than the Franklin Parish collection from cotton in August (Table 5). These same collections, except those from East Baton Rouge Parish in April, Franklin Parish in May and Ouachita Parish in May, had significantly lower LC_{50}s than the Tensas collection from cotton in August. Slopes of ld-p lines in 1995 ranged from 1.78 to 2.61.

Table 4. Tarnished plant bug response to acephate in Louisiana strains collected in 1994. Mortality assessed 24 hours after exposure.

Location (Parish)	June LC_{50} Slope ± SE (95% CL)	July LC_{50} Slope ± SE (95% CL)	August LC_{50} Slope ± SE (95% CL)	September LC_{50} Slope ± SE (95% CL)
Bossier	1.10 1.79 ± 0.29[2] (0.33-2.15)	----[1]	----[1]	----[1]
Franklin	4.29 2.54 ± 0.42[2] (2.93-5.58)	2.85 2.74 ± 0.33[5] (1.80-4.37)	----[1]	4.60 1.83 ± 0.29[7] (3.42-6.68)
	2.31 1.65 ± 0.34[3] (1.32-3.74)			
	1.16 2.44 ± 0.86[4]			

	(0.30-1.83)			
	1.80 2.11 ± 0.31[5] (0.54-3.64)			
Tensas	1.49 2.20 ± 0.43[5] (0.93-2.30)	1.99 2.59 ± 0.56[5] (1.50-2.64)	6.17 1.51 ± 0.26[7] (4.20-9.83)	1.46 3.22 ± 0.43[7] (1.19-1.77)
		5.69 2.73 ± 0.38[7] (4.55-7.15)	3.65 1.99 ± 0.30[7] (2.03-6.70)	
Richland	6.49 2.52 ± 0.69[6] (2.75-10.6)	----[1]	----[1]	----[1]

[1] Collections not made.
[2] Collected from alfalfa, *Medicago sativa* L.
[3] Collected from sercia lespedeza, *Lespedeza cuneata* [Dum.-Cours] G. Don.
[4] Collected from tickseed, *Coreopsis tinctoria* Nuttall.
[5] Collected from mustard greens, *Brassica juncea*.
[6] Collected from Black-eyed susan, *Rudbeckia hirta*.
[7] Collected from cotton, *Gossypium hirsutum* L.

Table 5. Tarnished plant bug response to acephate in Louisiana strains collected in 1995. Mortality assessed 24 hours after exposure.

Location (Parish)	April LC_{50} Slope ± SE (95% CL)	May LC_{50} Slope ± SE (95% CL)	June LC_{50} Slope ± SE (95% CL)	July LC_{50} Slope ± SE (95% CL)	Augus LC_{50} Slope ± SE (95% CL)
E. Baton Rouge	1.70 1.82 ± 0.22[2] (0.87-2.85)	----[1]	----[1]	----[1]	----[1]
Franklin	1.44 1.99 ± 0.25[3] (1.10-1.85)	2.53 2.28 ± 0.28[4] (1.96-3.22)	0.93 1.78 ± 0.25[6] (0.63-1.24)	6.18 2.36 ± 0.70[8] (2.21-9.01)	4.24 2.09 ± 0.24[9] (3.36-5.39)
Tensas	----[1]	1.59 2.61 ± 0.37[5] (1.23-2.03)	----[1]	----[1]	2.76 2.58 ± 0.31[9] (2.18-3.50)
Ouachita	----[1]	2.23 2.46 ± 0.31[4] (1.53-3.18)	----[1]	----[1]	----[1]
Caddo	----[1]	----[1]	0.99 2.48 ± 0.72[7] (0.16-1.82)	----[1]	----[1]

[1] Collections not made.
[2] Collected from daisy fleabane, *Erigeron philadelphicus* L. and crimson clover, *Trifolium pratense*.
[3] Collected from crimson clover.
[4] Collected from cutleaf primrose, *Oenothera laciniata* Hill.
[5] Collected from pansy dog shade, *Limnosciadium pinnatum*.
[6] Collected from tickseed, *Coreopsis tinctoria* Nuttall.
[7] Collected from alfalfa, *Medicago sativa* L.
[8] Collected from mustard greens, *Brassica juncea*.
[9] Collected from cotton, *Gossypium hirsutum* L.

There were no significant differences in oxamyl LC_{50}s among populations tested in 1994 except that the LC_{50}s for the Tensas and Richland Parish collections in June were significantly lower than one of the Tensas collections from cotton in August (Table 6). A Tensas Parish collection from cotton in August had the highest LC_{50} (3.46) whereas the Tensas Parish collection from mustard in June had the lowest LC_{50} (1.32). Slope values ranged from 1.62 to 2.51. During 1995, all May and June collections tested except for the Franklin Parish in June had significantly lower LC_{50}s compared to the Franklin Parish collection from cotton in August (Table 7). The LC_{50}s of all May collections and the Caddo collection from alfalfa in June also were significantly lower from the LC_{50} of the Franklin Parish collection in July. Slope values ranged from 1.38 to 2.76.

Table 6. Tarnished plant bug response to oxamyl in Louisiana strains collected in 1994. Mortality assessed 24 hours after exposure.

Location (Parish)	June LC_{50} Slope ± SE (95% CL)	July LC_{50} Slope ± SE (95% CL)	August LC_{50} Slope ± SE (95% CL)
Franklin	1.64 2.06 ± 0.24[2] (0.96-2.51)	----[1]	----[1]
	1.99 2.51 ± 0.49[2] (0.54-3.61)		
	1.59 1.81 ± 0.26[3] (0.81-2.72)		
Tensas	1.32 1.70 ± 0.20[3]	2.14 1.94 ± 0.34[3]	3.46 1.97 ± 0.41[5]

	(0.88-1.80)	(0.74-3.65)	(2.17-5.16)
			1.61 1.62 ± 0.25[5] (0.73-2.80)
Richland	1.39 1.64 ± 0.28[4] (0.92-1.94)	----[1]	----[1]

[1] Collections not made.
[2] Collected from tickseed, *Coreopsis tinctoria* Nuttall.
[3] Collected from mustard greens, *Brassica juncea*.
[4] Collected from Black-eyed susan, *Rudbeckia hirta*.
[5] Collected from cotton, *Gossypium hirsutum* L.

Table 7. Tarnished plant bug response to oxamyl in Louisiana strains collected in 1995. Mortality assessed 24 hours after exposure.

Location (Parish)	May LC_{50} Slope ± SE (95% CL)	June LC_{50} Slope ± SE (95% CL)	July LC_{50} Slope ± SE (95% CL)	August LC_{50} Slope ± SE (95% CL)
Franklin	1.54 2.07 ± 0.25[2] (1.03-2.18)	2.58 2.15 ± 0.40[4] (1.32-3.82)	3.03 1.88 ± 0.21[7] (2.31-4.02)	4.84 2.49 ± 0.31[8] (3.71-6.15)
Tensas	1.78 2.42 ± 0.31[3] (1.38-2.26)	----[1]	----[1]	----[1]
Ouachita	0.96 1.38 ± 0.23[2] (0.55-1.41)	----[1]	----[1]	----[1]
Caddo	----[1]	0.92 2.76 ± 0.42[5] (0.41-1.53)	----[1]	----[1]
		1.78 2.27 ± 0.32[6] (1.15-2.80)		

[1] Collections not made.
[2] Collected from cutleaf primrose, *Oenothera laciniata* Hill.
[3] Collected from pansy dog shade, *Limnosciadium pinnatum*.
[4] Collected from tickseed, *Coreopsis tinctoria* Nuttall.
[5] Collected from alfalfa, *Medicago sativa* L.
[6] Collected from horse weed, *Erigeron canadensis* L.
[7] Collected from mustard greens, *Brassica juncea*.
[8] Collected from cotton, *Gossypium hirsutum* L.

Discussion:

Resistance to cypermethrin in Louisiana populations of TPB was documented in 1994 and 1995. The range in LC_{50} values over both years was 0.57-21.22 µg/vial (37-fold). The highest values were 21.22 µg/vial in 1994 and 12.69 in 1995. These values were not as high as others previously reported, but they were within the range of values for TPB populations reported as resistant to pyrethroids in other states.

LC_{50}s values followed a trend associated with seasonal use of pyrethroids. The lowest values were recorded during April, May and June. During this period of the cotton growing season, pyrethroid use was low. The highest values were generally observed in August when pyrethroid use was more common. For the single collection made in September 1995, the LC_{50} was not significantly different from that of several collections made during May, June and July. Reversion of pyrethroid resistance may occur during September because pyrethroid use generally declined in late August and September.

Low levels of resistance to acephate were recorded in both 1994 and 1995. There was a 7-fold difference (0.93-6.49 µg/vial) in values over the two years. The highest LC_{50} for 1994 (6.49) was recorded in June, while the highest value in 1995 (6.18) was recorded in July. The LC_{50}s for acephate of Louisiana collections made in the early season were significantly lower than the LC_{50} (12.60 µg/vial) (95% CL = 11.06-14.51) for the Stoneville lab colony (Snodgrass 1994). The LC_{50} (8.90 µg/vial) (95% CL = 7.65-10.22) for a Mississippi collection from cotton (Snodgrass and Elzen 1995) was significantly higher than LC_{50}s of all Louisiana collections from cotton except for the Tensas Parish collection from August, 1994. During May and June, acephate was an insecticide commonly used to control TPB. Other organophosphates such as azinphosmethyl, dicrotophos, dimethoate, methamidophos, and methyl parathion were used to control early season insect pests of cotton. Later in the season, organophosphates such as profenofos and sulprofos are

used for control of pyrethroid-resistant tobacco budworms (*Heliothis virescens* (F.)). The continuous use of insecticides in this class throughout the cotton growing season probably contributed to the overall tolerance of TPB to acephate.

There were low levels of resistance to oxamyl recorded in 1994 and 1995 based on the vial bioassay. The LC_{50}s ranged from 0.92-4.84 µg/vial (5-fold variation) over both years. The highest LC_{50}s for oxamyl were recorded in August of both years. Although oxamyl was used to control insect pests frequently during May and June in cotton, this use was probably not enough to cause TPB tolerance to carbamates. However, other carbamates such as aldicarb, methomyl, and thiodicarb were used to control cotton insect pests. Carbamates have a similar mode of action as organophosphates and cross-resistance may occur. LC_{50} values for oxamyl from Arkansas collections (7.2-26.0 µg/vial) were higher than those observed for Louisiana collections (Hollingsworth *et al*. 1995 submitted).

There was variation in the TPB susceptibility from Louisiana to all three classes of insecticides, but the changes in LC_{50}s generally corresponded with the seasonal use pattern of that insecticide class. Although the data in this study was limited, the hosts from which the TPB were collected did not influence insecticide susceptibility. Based on comparisons with previously published data, TPB in Louisiana have varying levels of susceptibility to pyrethroids, organophosphates, and carbamates that could result in control failures during late July and August.

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An Overview of Insecticide Resistance in *Plutella xylostella* L. in India

The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Yponomeutidae), has an extraordinary propensity to develop resistance to every synthetic insecticide used to control it in crucifers. India has not escaped the devastation of this pest. Efforts to control this pest solely through conventional insecticides led to resistance development to most insecticides available in India. The escalating cost of managing this pest on commercially grown crucifers reveals the inadequacy of existing management efforts. This article provides an overview of resistance development in DBM to various insecticides in India.

In India, Fletcher (1914) first recorded DBM on crucifer vegetable crops. The first record of pesticide resistance in DBM was in 1966 when DDT and parathion failed to control DBM around Ludhiana, Punjab (Verma & Sandhu 1968). DBM resistance to several organochlorine and organophosphate insecticides in the neighboring state, Haryana, was reported by Verma *et al.* (1972). Deshmukh & Saramma (1973) confirmed the resistance problem in this moth and observed that populations of DBM collected from Jullandhar were less susceptible to ethyl parathion than those found in Ludhiana district of Punjab. Three years later, Chawla & Kalra (1976) observed that resistance had extended to multiple insecticides including fenitrothion and malathion in major vegetable growing areas of Punjab. All these observations suggest that by 1976, DBM had developed cross resistance throughout India. Resistance to diazinon in DBM from Punjab was observed in 1978-79 (Anonymous 1986) compared to baseline data prepared by Kalra & Chawla (1977).

Pesticide resistance was regularly monitored at Punjab Agricultural University, Ludhiana, during the late 1970s and mid 1980s. DBM was susceptible to quinalphos during 1980; but was resistant by 1984, three years after the pesticides' introduction in Punjab. Nevertheless, introduction of synthetic pyrethroids (SPs) in the Indian market during 1980 assured control of DBM in crucifers. However by 1982, DBM showed tolerance to the pyrethroids frequently used because of their initial promise. Since truly susceptible populations of the insect were not available and the discriminating dose for resistance monitoring was not used for DBM in India, the resistance ratios of different populations to these pyrethroids were calculated by LC_{99} values based field rates recommended for Maharastra state (Awate *et al.* 1982, Gandhale *et al.* 1986).

In the absence of alternative control measures, the application of SPs in the field continues. Resistance to cypermethrin, fenvalerate, deltamethrin and also to quinalphos was found in DBM populations collected from areas where growers relied most heavily on pyrethroids (Saxena *et al.* 1989). Saxena *et al.* (1989) observations revealed that DBM developed resistance levels of 144-fold against cypermethrin at Panipat (Haryana), 178-fold against fenvalerate at Ranchi (Bihar), 191-fold and 115-fold against deltamethrin at Delhi and Bangalore (Karnataka), respectively, and 628-fold against quinalphos at Jaunpur (Uttar Pradesh). All these observations indicate that DBM developed multiple resistance in India during the 1980s. Mehrothra (1991, 1993) reported widespread unacceptable control of this pest with current control strategies and expressed concern about the rising importance of this pest at national level.

Recently, DBM resistance to cypermethrin, fenvalerate and deltamethrin was encountered in Punjab (Chawla & Joia 1991). For the past ten years, persistent DBM resistance to quinalphos from various regions of Punjab was calculated after determining the base-line toxicity (Joia & Chawla 1992). Joia *et al.* (1994) recorded DBM resistance to quinalphos at 170-fold. A new insecticide, cartap hydrochloride, was successful in controlling multiresistant populations of DBM.

These insecticide failures to control DBM were observed from North and Northwest part of India. Few studies assess pesticide resistance in Central and Southern regions, including the Varanasi region, an important cruciferous vegetable production center for whole Northeastern part of the country. In 1994, we evaluated the resistance levels in DBM populations collected from two different locations around Varanasi region against five commonly used insecticides. We observed 25-fold resistance to cypermethrin and fenvalerate and 5-fold resistance to endosulfan and quinalphos (Raju & Singh 1995). In the second year since its introduction, Cartap hydrochloride showed high LC_{50} values indicating nearly 8-fold resistance in the field populations. This shows the true threat of DBM multiresistance in India.

Resistance monitoring is an indispensable prerequisite in designing any integrated pest management program. Insecticide resistance is a complex and relative phenomenon. Nevertheless, the withdrawal of selection pressure for those insecticides that exaggerate the differences between susceptible and resistant pest population is one of the basic objectives in insecticide resistance management (IRM). The observed decline in efficacy of various insecticides against DBM indicates that insecticide resistance in DBM has become a limiting factor in the commercial cultivation of cabbage and cauliflower in India. Immediate action is needed to develop a resistance management strategy for this pest. Both the acylureas and *Bacillus thuringiensis* (*Bt*)-based insecticide formulations are now available in India to use against resistant

DBM. However, the higher cost and multiple reports of DBM resistance to acylureas and *Bt*s in other countries can not be overlooked. The success of these insecticides against DBM in India is very much in question. Concerted efforts are needed to devise an effective IRM strategy that is within the economic reach of marginal/small scale farmers in India.

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Laboratory and Field Evaluations of Virginia Peanut Cultivars for Resistance to Southern Corn Rootworm

The southern corn rootworm (SCR) (*Diabrotica undecimpunctata howardi* Barber) is the primary soil insect pest to peanut (*Arachis hypogaea* L.) in Virginia and North Carolina. The newer cultivars, planted on the majority of acreage, have not been extensively screened for rootworm resistance. The objective of this two-year study was to evaluate five new Virginia cultivars and 18 breeded lines for resistance to southern corn rootworm in the laboratory and in the field. NC 7 and NC 9 were used as susceptible checks. NC 6 was used as a known resistant check. Rootworm mortality and feeding were measured from bioassays in the lab. Pod damage data was obtained from field studies.

At the Tidewater AREC, eleven laboratory bioassays were conducted with peanut seedlings or field tissue (peg and immature pod) against rootworm development from the neonate to the adult stage. Rootworm mortality and feeding damage determined which cultivars showed the most resistance (Table 1). The cultivars AgraTech VC-1, NC 7 and VA 93B were very susceptible to southern corn rootworm and should not be planted in problem fields without an insecticide treatment. The cultivars NC 9 and NC-V11 were more resistant than the former, but not as resistant as NC 6. In these studies, VA-C 92R caused lower rootworm mortality than NC 6, but not significantly less in any test. This may indicate some level of resistance that needs to be confirmed in field studies.

Table 1. Peanut cultivar resistance to rootworm by scoring each cultivar compared with NC 6. If score is ≥ 0, the cultivar is as resistant as NC 6; or, if score is < 0, the cultivar is less resistant than NC 6.

Cultivar	SCR Mortality*	SCR Feeding **	Score
NC 6	0	0	0
VA-C 92R	0	1	1
NC-V11	-1	0	-1
VA 861101	-3	1	-2
NC 9	-2	0	-2
NC 7	-3	0	-3
AgraTech VC-1	-3	0	-3
VA 93B	-5	1	-4

* Cultivar receives a -1 if significantly less total mortality than NC 6 in each of 6 bioassays (max. -6).
** Cultivar receives a +1 if significantly less feeding or No. of punctures than NC 6 in each of 5 bioassays (max. +5).

The field studies were conducted at the Tidewater AREC and at a grower's field in Greensville county. NC 6, NC 7, AgraTech VC-1 and VA 861101 were planted in fields with a history of SCR infestation. Percent pod damage was determined at the end of the season by assessing rootworm damage on 100 randomly sampled pods from five plants in each of four replicants for each cultivar. NC 6 sustained significantly less pod damage than NC 7 from natural SCR infestations in both years and at both sites (Table 2). Results from this study indicate that NC 6 is still the only cultivar that demonstrates resistance to rootworm in the field and should be maintained in the breeding program as a future parent to other peanut lines.

Table 2. Mean percentages of total pod damage by southern corn rootworm on three peanut cultivars and one advanced breeding line under natural SCR infestations, Tidewater AREC and Greensville County, VA (1994-1995).

	Total pod damage (%)

	1994	1995	
Entry	Tidewater AREC	Tidewater AREC	Greensville
NC 6	5.0 a*	5.5 a	6.0 a
VA 861101	15.3 ab	9.0 ab	10.2 a
AgraTech VC-1	15.6 ab	11.0 ab	13.2 a
NC 7	27.0 b	18.0 b	24.5 b

* Means within columns followed by the same letter are not significantly different (df = 12, N = 16, P = 0.05) as determined by MSD

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Characters of Fenvalerate Resistance in *Helicoverpa armigera* (Hubner) from China

The cotton bollworm, *Helicoverpa armigera* (H.), is one of the most damaging cotton pests in China. Since early 1980s, pyrethroids have been used widely to control this insect. The cotton bollworm in cotton-growing areas of northern China developed resistance to pyrethoids, such as fenvalerate and deltamethrin, by the end of 1980s. Pyrethroid failure to control cotton bollworm presents a critical problem to cotton growers due to the lack of new and effective insecticides. Therefore, it is very urgent to develop resistance management strategies to preserve pyrethroids, the most effective insecticides in use against cotton bollworm.

Fenvalerate resistance in cotton bollworm in China is more serious than other pyrethroid resistance problem so we must know the characters of fenvalerate resistance and its cross resistance to other pyrethroids. This information will better assist us to design an effective resistance management strategy. Thus, the aim of present study is to investigate some important characters of fenvalerate resistance including the cross-resistance spectrum, resistance mechanism, resistance inheritance mode and fitness of the resistance strain.

Laboratory selection and cross-resistance:

A field strain (YG) of cotton bollworm, *Helicoverpa armigera* (Hubner), was collected from Yanggu County, Shandong Province in 1990. After nine selections with fenvalerate over fifteen generations, the selected colony (YG-R) exhibited about 2,471-fold resistance to this insecticide compared with susceptible strain collected from Dongtai County, Jiangsu Province in 1983. A fenvalerate susceptible strain (YS-S) was developed by selection of the clones from single-pair matings in a colony (YS) collected from Yanshi County, Henan Province in 1991. The LD_{50} value of YS-S strain was 0.01132 μg/larva and close to that value from the Dongtai susceptible strain (0.0098 μg/larva).

Dose-mortality regressions were estimated for ten insecticides applied to third instar YG strain (non-selected) and to YG-R strain (fenvalerate-selected), respectively. A spectrum of cross-resistance was detected to fenpropathrin (LD_{50} YG-R strain/LD_{50} YG strain = 25.9-fold), deltamethrin (5.9-fold), and cypermethrin (2.7-fold). No cross-resistance was detected to cyhalothrin (0.6-fold), permethrin (0.9-fold), methomyl (0.8-fold), methamidophos (1.8-fold) and monocrotophos (1.5-fold). The YG-R strain may possess negative cross-resistance to methylparathion (LD_{50} YG-R strain/LD_{50} YG strain=0.3-fold). Thus, the fenvalerate resistant strain shows considerable cross-resistance to pyrethroid insecticides and to non-pyrethroid insecticides.

Mechanism of fenvalerate resistance in *H. armigera*:

Fenvalerate plus the synergist piperonyl butoxide (PBO) significantly enhanced the toxicity of fenvalerate in the third instars from both the laboratory-selected resistant strains (YG-R, Rd) and the field-collected resistant strains (YG9221,

YG9241 and YG9321) of *H. armigera*. Synergism ratios ranged from 19.5 to 169.5-fold at the LD_{50} level. The fenvalerate toxicity was not synergised by *O,O,O*-triphenyl phosphate (TPP) or *S,S,S*-tributyl phosphorotrithioate (DEF). The aldrin epoxidase activities of mixed function oxidase (MFO) in the 3rd instar from Rd strain was 1.36 times higher than the YS-S strain and there was no difference in the esterase activities on alpha-naphthylacetate among the resistant strains (YG-R, Rd) and susceptible strain (YS-S). This indicates that fenvalerate resistance was principally due to the metabolic mechanism of MFO and esterases were not important in causing fenvalerate resistance in this population of *H. armigera*. Since cytochrome P450, the key component of MFO system, has stereo-specifity to substrates, it was possible that cross-resistance did not extend to all pyrethroids. We are examining the relationship between cytochrome P450 and pyrethroid resistance in cotton bollworm.

Penetration of ^{14}C-fenvalerate into the 3rd instars from the Rd strain and the YS-S strain was measured at specific times after topical application. At 8 hours, 51.7% of ^{14}C-fenvalerate penetrated into the insect body in YS-S strain but only 31.4% in Rd strain. This revealed that the rate of penetration of ^{14}C-fenvalerate was higher in the susceptible strain than in the resistant strain and decreased cuticular penetration was a factor responsible for fenvalerate resistance.

In the selection process of YG-R strain, we found that YG-R strain possessed cross resistance to DDT. This suggests that nerve insensitivity may be another mechanism of fenvalerate resistance in *H. armigera*.

Inheritance pattern of fenvalerate resistance in *H. armigera*:

The inheritance mode of resistance to fenvalerate was evaluated from log dosage- probit mortality curves constructed from the third instar response to fenvalerate treatment. The insects used for the study were taken from laboratory selected susceptible and resistant strains. The resistant strain exhibited over 2,000-fold resistance to fenvalerate compared with the susceptible one. The genetic analysis indicated that fenvalerate resistance in *H. armigera* was controlled by two or more autosomal genes and the major gene(s) involved was incompletely dominant. The MFO gene primarily responsible for resistance was found to be incompletely dominant.

Relative fitness of fenvalerate resistant and susceptible strains of *H. armigera*:

The fitness of resistant *H. armigera* was evaluated based on developmental and reproductive characteristics. Life tables of YG-R strain (fenvalerate-resistant) and YS-S strain (susceptible) were constructed to determine relative fitness by the net reproductive rate (R_0). The results indicated that YG-R strain possessed reproductive disadvantages such as reduced percentage of females mated, lower mean number of eggs per female and decreased percentage of eggs hatched when compared with YS-S strain. No developmental defects in the YG-R strain were observed. A fitness value of 0.69-0.88 for the YG-R strain relative to the susceptible YS-S strain was calculated.

Stability of pyrethroid resistance in *H. armigera*:

A regression of pyrethroid resistance in *H. armigera* colonies following several generations of non-selection was observed. Although laboratory-selected YG-R resistant strain had extremely high level resistance to fenvalerate (3,166-fold), that resistance was non-stable and decreased to 61.4-fold after 14 generations Studies with several field-collected resistant strains were conducted. These studies showed that resistance to the three pyrethroids (fenvalerate, deltamethrin and cyhalothrin) was not stable. Initially resistance declined rapidly, then stabilized at 2.0- to 9.0-fold. Complete susceptibility to the three pyrethroids, however, may be difficult to recover.

Resistance monitoring of *H. armigera* in Yanggu County, Shandong Province from 1990 to 1995:

Resistance monitoring of *H .armigera* in Yanggu County, Shandong Province was carried out from 1990 to 1995 by topical application with the 3rd instars. Eight conventional insecticides were tested on the second and fourth generations captured in cotton each year. The results indicate that fenvalerate resistance ranged between 40.5- to 542.8-fold; resistance to deltamethrin, cyhalothrin, fenpropathrin, esfenvalerate, cyfluthrin and methomyl ranged between 10- to 50-fold; resistance to monocrotophos and phoxim was less than 5-fold.

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Preventing the Development of Insecticide Resistance in *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) Based on Laboratory Studies

Introduction:

Cotton entomologists must manage pests with insecticides while preventing resistance selection. At the present time, cotton protection in francophone Africa consists of 4 to 6 calendar-based applications of a pyrethroid for bollworm control, and an organophosphate (OP) for control against mites and leaf-eating caterpillars or biting-sucking insects. Field and laboratory results demonstrated that concurrent use of selected pyrethroids and OPs could increase the effectiveness of both applications against bollworms (Vaissayre 1985). We evaluated the effect of such a recommendation on resistance development, especially for *Helicoverpa armigera* (Hubner), a key pest in Africa. A lab study was conducted in CIRAD-CA's facilities in Montpellier (France) on an African field and a laboratory strain. This strain was obtained through the introduction of resistant individuals in a susceptible field strain.

Materials and Methods:

Strains

Three *H. armigera* strains were used in CIRAD laboratories in Montpellier: a susceptible field strain (Bk), collected in Côte d'Ivoire, a resistant field strain (Th), coming from Thailand and a laboratory strain (BKTH), initiated by mixing adults of BK (90%) and TH (10%) strains.

Applications

Topical applications (Arnold microapplicator) of the active ingredient diluted in 1 microliter of acetone were applied to individuals following the recommendations of the Entomological Society of America (Anon. 1970). The active ingredients, deltamethrin and triazophos, were supplied by Roussell Uclaf (AgrEvo).

LD_{50} value

Insect mortality was observed 48 hours after insecticide application. The LD_{50} values were calculated with statistical software developed by CIRAD's computing unit (Joly & Giner 1993) and based on Finney's Probit Analysis method (Finney 1971). When insecticide mixtures were applied, LD_{50}s were calculated for the pyrethroid only.

Selection pressure

For each strain, selection pressure was applied to the 4^{th} instar each generation. The selection treatments were:

- Strain D1 (Bk): LD_{80} deltamethrin
- Strain A1 (Bk): alternation LD_{80} deltamethrin and LD_{80} triazophos
- Strain M1 (Bk): LD_{80} mixture deltamethrin-traizophos (1/10)
- Strain T2 (BKTH): no pressure
- Strain D2 (BKTH): LD_{50} deltamethrin
- Strain A2 (BKTH): alternation LD_{50} deltamethrin and LD_{50} triazophos
- Strain M2 (BKTH): LD_{50} mixture deltamethrin-traizophos (1/10)

Results :

Tables 1-3 present baseline data on *H. armigera* susceptibility to deltamethrin and triazophos prior to laboratory selection.

Table 1. Mortality of BK strain 48 hours after topical treatment with different insecticides.

Active Ingredient	LD_{50}	95% FL	Slope ± S.E.	χ^2
Deltamethrin	0.11	0.07 - 0.16	1.88 ± 0.42	n.s.
Deltametrin + triazophos (1/30)	0.09	0.06 - 0.13	2.14 ± 0.50	n.s.
triazophos	100.7	71.9 - 141	2.78 ± 0.66	n.s.

Table 2. Mortality of TH strain 48 hours after topical treatment with different insecticides.

Active Ingredient	LD_{50}	95% FL	Slope ± S.E.	χ^2
deltamethrin	10.41	6.55 - 16.6	1.15 ± 0.17	n.s.
triazophos	118.4	99.3 - 141	3.11 ± 0.51	n.s.

Table 3. Mortality of BKTH strain 48 hours after topical treatment with different insecticides.

Active Ingredient	LD_{50}	95% FL	Slope ± S.E.	χ^2
Deltamethrin	0.19	0.13 - 0.28	1.52 ± 0.21	n.s.
Deltametrin + triazophos (1/30)	0.16	0.11 - 0.23	1.48 ± 0.21	n.s.
triazophos	116.6	97.9 - 139	3.12 ± 0.51	n.s.

Results obtained after 12 generations under selection pressure on an African (Bk) strain did not show a significant increase in pyrethroid resistance (Figure 1). Nevertheless, significant differences in the LD_{50} values appeared between strains selected under alternations verses mixtures of pyrethroids and OPs. This resistance disappeared as soon as the pressure ceased (Figure 2). This suggests that insecticide mixtures were better than alternations in reducing resistance development.

These experiments on the laboratory (BKTH) strain show how easy and rapidly we can select for resistance in *H. armigera* (Figure 3). In the laboratory, we obtained a statistically significant difference between alternations and mixtures of insecticides after eight generations of selection. The results confirm that insecticide mixtures may decrease resistance selection in a population compared to constant pressure with a single insecticide (Figure 4).

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Figure 1. Evolution of the sensitivity to deltamethrin (LD_{50} and 95% FL) to the D1 strain selected with a single continuous deltamethrin treatment regiment.

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Figure 2. Evolution of the sensitivity to deltamethrin (LD_{50} and 95% FL) to the A1 strain selected with an alternating deltamethrin-triazophos treatment regimen and to the M1 strain selected with deltamethrin-triazophos (1:10) mixture treatment regiment.

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Figure 3. Evolution of the sensitivity to deltamethrin (LD_{50} and 95%FL) to the D2 strain selected with a single continuous deltamethrin regiment to the T2 strain (no pressure).

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Figure 4. Evolution of the sensitivity to deltamethrin (LD_{50} and 95%FL) to the A2 strain selected with an alternating deltamethrin-triazophos treatment regimen and to the M2 strain selected with deltamethrin-triazophos (1:10) mixture treatment regiment.

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Evaluating Resistance to CryIA(b) in European Corn Borer (Lepidoptera: Pyralidae) with Artificial Diet

Transgenic corn hybrids expressing the *Bacillus thuringiensis* protein, CryIA(b), will be introduced to the commercial market in the 1996 growing season for European corn borer (ECB) control. There is concern that the deployment of such hybrids will result in pest resistance to the CryIA(b) protein. This will not only render these hybrids ineffective, but will also reduce the efficacy of *B.t.*-based bio-insecticides.

The two primary objectives of this study are to : 1) determine the potential of ECB to develop resistance to the CryIA(b) *B.t.* toxin, and 2) develop a CryIA(b) resistant colony of ECB to aid in the discovery and development of novel ECB resistant genes with a different mode of action.

Materials and Methods:

In June 1994, we initiated two separate laboratory colonies of European corn on wheat germ based diets incorporated with purified CryIA(b) protein. The solubilized form of the protein simulated the form expressed in transgenic corn. One colony was continually reared on 0.03 ppm *B.t.* protein and the other on 0.015 ppm *B.t.* in the diet. The ECB were exposed to the toxin throughout their entire larval cycle to mimic the duration of exposure encountered in *B.t.* transgenic corn. The higher dose inflicted mortality, but allowed a sufficient number of ECB to complete development and sustain the colony.

Dose response bioassays in artificial diet were conducted for each generation with comparisons made between the two *B.t.*-stressed colonies and a standard, non-challenged ECB colony. *B.t.* concentrations of 0.0, 0.005, 0.015, 0.05, 0.15, 0.5, and 1.5 ppm were employed. Each bioassay contained of 20.0 mL treated diet dispensed into 16 cells in a tray. Each cell was infested with one ECB neonate. The bioassay trays were incubated in the dark for seven days at 28 C° and

70 - 80% RH. Surviving larvae were weighed (to the nearest 0.1 mg) and mortality counts taken. One bioassay was conducted each generation for the first six generations then three bioassays were performed per generation.

Adult fecundity tests were conducted every other generation beginning with seventh generation. Two pairs of freshly emerged moths/colony were put into cages. Ten replications (cages)/colony were performed. The cages were incubated at 28 and 21°C (12:12 hours), 70 - 80% RH and 8:16 hour photoperiod (light:dark). Eggs were collected daily and weighed throughout the adults lifespan.

Greenhouse studies were conducted with the ECB colonies on CryIA(b) transgenic corn. The test plants were a non-transformed Pioneer hybrid (PHI-416), a transgenic of the same hybrid (PHI-416K) with low *B.t.* expression (<1 μg protein/gram fresh weight whorl tissue), and a second transgenic of the hybrid (PHI-416P) expressing *B.t.* at a high level (>10 μg protein/gram fresh weight whorl tissue). The protein levels were determined by ELISA. For each hybrid, ten plants (reps) at the V5-6 vegetative stage were artificially infested with one of the three ECB colonies. Each plant was infested (with approximately 50-100 neonate ECB) three times at 2 - 4 day intervals. The insects were allowed to feed on the non-transformed control plants for approximately 3 weeks. The plants were then visually scored for damage on a 1 - 9 scale (a score of 1 = majority of leaves with long lesions and 9 = no visible leaf injury). This process was repeated for each colony. To date, we have tested generations 10, 12, and 13 in the greenhouse.

Analysis of variance were performed on all data. Mean separations were determined by Tukey's Studentized Range Test.

Results:

No significant differences in weight loss or mortality occured in the colonies at the lower concentrations of 0.005 and 0.015 ppm (Graph 1). Significant differences in larval weight reduction among the ECB colonies were detected in diet bioassays after two generations of selection pressure. The differences were detected at the *B.t.* concentrations of 0.05 and 0.15 ppm (Graphs 2 and 3). These observed differences remained constant between generation seven to thirteen. When exposed at the higher concentrations of 0.5 and 1.5 ppm, all colonies experience mean larval weight loss of >98%, although there were more survivors in the *B.t.* reared colonies (data not shown).

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Fecundity tests revealed high variability in mean egg production among replications and generations (Graph 4). When analyzed across the generations there are no significant differences in egg production possibly because of a small sample size.

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The greenhouse studies show significant differences between the non-transformed hybrid (control) and the two transgenic *B.t.* hybrids for first generation ECB damage. The mean damage of all ECB colonies on non transgenic hybrid was 2.6 (heavy damage) while the low and the high expressing *B.t.* transgenic hybrids had damage means of 8.6 and 8.7 respectively (Graph 5).

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Discussion:

Results show a significant change in the susceptibility to CryIA(b) protein for two colonies of ECB selected on low concentrations of the toxin. Thus, ECB was able to develop some tolerance to low levels of CryIA(b) in the diet. We were unable to initiate, let alone sustain, an ECB colony with a higher *B.t.* concentration in the diet closer to the actual levels expressed in our transgenic plants (1 - 10 μg protein/gram fresh weight whorl tissue). After 13 generations of selection pressure, no colony survived on transgenic *B.t.* corn hybrids in the greenhouse. These "resistant" ECB should not be considered as resistant to hybrid strains of *B.t.* transgenic corn. This indicates that corn borers in the wild may be

unable to develop resistance on CryIA(b) transgenic corn. However, the small genetic pool of our colonies and lack of knowledge correlating *B.t.* activity in artificial diet versus a corn plant makes this a speculative statement.

We continue to increase *B.t.* protein levels in the rearing media of corn. To date, we are able to rear corn borers on media treated with 0.075 ppm *B.t.* protein.

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Transformation and Expression of the Resistance Gene to Carbendazim into *Trichoderma harzianum*

Carbendazim is a benzamidazole fungicide that plays a very important role in plant disease control. Carbendazim has been applied in disease control for a long time and resistance is a very serious problem (Qian 1995). Our approach to resistance management is to develop an integrated plant disease management program that relies on both chemical and biological tactics. Typically, chemicals kill biological control agents, as well as disease agents, when they are applied in field. We are developing a strain of *T. harzianum* resistant to carbendazim that allow us to implement an integrated plant disease management program. Protocols to develop such a strain have been improved and applied to other fungi (Case *et al*. 1979, Yelton *et al.* 1984, Parsons *et al.* 1987, Turgeon *et al.* 1987, Huang *et al.* 1989, Goettel *et al.* 1990). The strain of *T. harzianum* resistant to carbendazim was developed with a plasmid containing the TUB2 gene from *Saccharomyces cerevisiae*, and transforming the TUB2 gene into *T. harzianum*. This strain of *T. harzianum* can grow on medium containing 10 μg/ml carbendazim. Carbendazim resistance was stable after 5 successive subcultures in non-selective medium. Further tests show that the transformant can grow on the medium containing 50 μg/ml carbendazim. This resistance level is more than 150 times the original *T. harzianum* strain (original strain did not survive on medium with 0.8 μg/ml). Our resistant strain survives carbendazim doses much higher than the dose recommended for disease control in the field. We conclude that it is safe to use this transformant in an integrated plant disease management program.

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Cotton Bollworm Resistance and its Development in Northern Cotton Region of China 1984 - 1995

Among cotton pests, the resistance development in the cotton bollworm (*Heliocoverpa armigera*) is the fastest. We present the resistance development situation in the northern cotton area of China for the last 12 years (1984 - 1995).

Four major pesticides, parathion, monocrotophos, deltamethrin and methomyl are applied to control cotton bollworm in northern China. Over 0.1 million tons of each pesticide has been applied and contributes to the rapid development of resistance in the cotton bollworm. There are nearly 60 other pesticides applied to cotton.

Our studies were done in the Liaocheng district, Shangdong province, China. This district has an extremely high incidence of resistant cotton bollworm.

Our data indicates:

- Resistance in cotton bollworm was present in 1984. In 1986, resistance distinctly increased then peaked and stabilized after 1990 (Figure 1).
- Over the past 12 years, resistance increased or decreased by less than 2-fold. However, we observed two surges in resistance during the 12 year period for all pesticides (Table 1). These years were called periods of "sudden resistance."
- The continual use of pesticides in the early years contributed to periods of sudden resistance. After two such periods, the resistance level remained very high.
- Figures 1-4 display cumulative (system) change in resistance as compared to the initial year, 1984, and an annual change in resistance as compared to the previous year. The latter can possibly be used to forecast the beginning of a resistance peak in a population.

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Table 1. Monitoring cotton bollworm resistance to four pesticides in China between 1984 to 1990.

Year	Deltamethrin			Parathion			Monocrotophos			Methomyl		
	LD_{50}	±	Year	LD_{50}	±	Year	LD_{50}	±	Year	LD_{50}	±	Year
1984	0.002274	1.0	1.0	0.2384	1.0	1.0	22.7513	1.0	1.0	2.5832	1.0	1.0
1985	0.005002	2.2	2.2	0.3306	1.4	1.4	100.5041	4.4	4.4	5.3597	2.0	2.0
1986	0.029618	13.0	6.0	2.7742	11.6	8.4	263.6474	11.6	2.6	40.6105	15.7	7.5
1987	0.094372	41.5	5.6	4.9444	20.7	1.8	277.1125	12.2	1.1	32.3926	12.5	0.8
1988	0.113792	50.0	1.2	6.1721	28.1	1.3	292.7662	12.8	1.1	25.9817	9.7	0.8
1989	0.283567	124.7	2.5	13.4305	56.3	2.0	529.3575	23.2	3.0	13.2541	5.1	0.5
1990	0.537346	236.3	1.9	65.4005	274.3	4.8	584.6033	25.7	1.1	80.3214	31.1	6.1
1991	0.667930	293.7	1.2	70.9679	297.7	1.1	413.8990	18.2	0.7	129.6355	50.2	1.6
1992	8.015167	3524.7	12.0	77.3832	324.6	1.1	352.5256	15.5	0.8	146.3587	56.6	1.1
1993	11.645911	5121.3	1.4	87.0160	365.0	1.1	409.6387	18.0	1.2	157.3254	60.9	1.1
1994	14.772557	6496.3	1.3	91.8793	385.4	1.1	356.0717	15.6	0.8	141.8415	54.9	0.9
1995	17.873466	78.59.9	1.2	98.6022	413.6	1.1	300.8438	13.2	0.8	119.1698	46.1	0.6

LD50: μg/g [In Liaocheng Cotton Area]

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Resistance Remediation in the German Cockroach

It is in the agroecosystem that insecticide resistance receives the most attention and where effective strategies for prevention and management have been devised. Here, there are economic opportunities and incentives for areawide and nationwide programs to limit insecticide use and manage the susceptibility in pest populations (Cox & Forrester 1992, Untung 1996). The result is insecticide resistance management (IRM) programs for several crop systems.

In recent years, IRM was extended to the urban ecosystem, specifically to *Blattella germanica* (L.). This cockroach has a long history of exposure to insecticides, and has developed resistance to nearly every class of insecticide used for its control. With the advent of pyrethroids, there was concern that this pest would quickly develop high-level (control failure) resistance to this entire class (Cochran 1990). Although this prediction has not come true, the potential for increased and widespread resistance in this species lead to IRM in cockroach control.

Methods to prevent resistance in this species include mixtures and alternations of pesticide classes in control programs (Cochran 1990). However, preventing resistance is difficult since most German cockroach populations have been exposed to pyrethroids, and moderate to high levels of resistance may be achieved with only limited exposure (Robinson & Zhai 1990, Zhai & Robinson 1991). In urban ecosystems, there are opportunities or economic incentives to limit insecticide use and prevent resistance. Nevertheless, there is a critical need for programs to restore susceptibility in *B. germanica* populations that are resistant to pyrethroids or other insecticides.

Reports on resistance in the German cockroach consist primarily of one-time profiles of several insecticides on laboratory populations, often with little information on the history of insecticide use (Cochran 1994). The value of long-term monitoring programs include linking insecticide use patterns and percentage reduction with resistance ratios (RR), and evaluating insecticide programs for the prevention and remediation of resistance. This paper documents the gradual decline in efficacy of a pyrethroid insecticide (cypermethrin) in a field population (Lincoln Terrace apartments, Roanoke, VA), and the gradual remediation of resistance in this population. The Urban Pest Control Research Center monitored the levels of resistance to chlorpyrifos and cypermethrin in this German cockroach population (RHA) for 10 years.
History of Insecticide Use

From 1970-1975, cockroach control at the Lincoln Terrace apartments was unscheduled and based on chlorpyrifos. From 1975 to mid-1985, chlorpyrifos was used on a scheduled basis (2 times per year). From 1985 to mid-1990, cypermethrin was used exclusively (2 times per year). From 1990-1993, the apartments were treated exclusively with chlorpyrifos, and no pyrethroids. From 1994-1996, they were treated about once every 3 months with either chlorpyrifos or cypermethrin.

Cypermethrin Field Efficacy and Resistance:

Cypermethrin was used experimentally in the apartments in 1981 and provided >90% reduction in the pest population. Following exclusive use (1985-1990), RHA susceptibility to cypermethrin (and other pyrethroids) gradually declined. In 1988, cypermethrin provided about 70% reduction; by 1989, there was a complete loss of flushing action and control failure. High-level resistance (RR=180 at LC_{50}) was confirmed in 1990 (Zhai & Robinson 1991). During this time (1981-1990), the efficacy of chlorpyrifos in the apartments fluctuated between 54% to 63% and the RR at KT_{50} was 1.7-1.8.

Remediation of Cypermethrin Resistance:

From 1990-1993, all pyrethroid use in the apartments stopped and control was relied on chlorpyrifos. During this 3-year period, the cypermethrin susceptibility in RHA returned so that by late 1993 the RR at LC_{50} was 3.0. The remediation of cypermethrin resistance in RHA generations was documented periodically (Table 1). Susceptibility was reconfirmed in 1993 by the 76% reduction provided by applying cypermethrin in apartments. From 1990-1993, the level of chlorpyrifos resistance increased to RR 2.2 at KT_{50}.

Table 1. Cypermethrin resistance (LD_{50}, µg per cockroach) and resistance ratios (RR) in Lincoln Terrace German cockroach population.

Year/ RHA Generation	LD_{50} (+ 95% FL)	Slope + SE	RR
1990/F0	11.69 (2.50)	2.45 + 0.32	180
1991/F2	6.82 (1.76)	3.15 + 0.65	123
1991/F5	2.62 (1.12)	2.28 + 0.41	66
1992/F9	1.56 (0.54)	2.61 + 0.35	41
1993/F13	0.10 (0.18)	1.91 + 0.34	3

1994/F17	0.77 (0.78)	2.22 + 1.03	20
1994/F20	1.25 (0.74)	2.79 + 0.66	33
1995/F21	0.78 (0.27)	4.59 + 1.12	21

Cypermethrin Resistance Management:

Control failure due to cypermethrin resistance in RHA occurred in about 13 generations with only limited selection pressure. It may be difficult to design a IRM program for cypermethrin based on rotation of less than three applications/year and still utilize the benefits of low odor, low rates and effectiveness. There is considerable client satisfaction and incentive for the applicator to use this and other pyrethroids, and little incentive to restrict usage to preserve susceptibility. The restoration of susceptibility in RHA was accomplished in about 13 generations. A moderate level of effectiveness was achieved (76% reduction), as well as the return of the flushing action that characterizes pyrethroids. However, periodic (every 3 months) use of cypermethrin resulted in a percentage reduction that ranged from 54 to 62%, and a RR at LC_{50} that ranged from 20 to 33.

When insecticide resistance was first observed, reversal of this phenomenon was discussed. At that time, there were few effective insecticides, but now a wide range of chemicals are available and discussion might seem unimportant. However, there is considerable incentive to reverse resistance. Some insecticides may be more effective and less expensive than alternative insecticides. Ideally, a modern pest control program can be designed to include several strategies and insecticides that contribute to overall population reduction.

The desirable characteristics of pyrethroids for household pest control will insure their continued use by professionals and homemakers. It is inevitable that control failures due to resistance will develop in some populations. To date, there has not been widespread resistance to these insecticides. Resistance remediation may be effective and practical for some German cockroach populations, especially in commercial establishments where insecticide use can be monitored and controlled.

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Controlling a Population of Norway Rats Resistant to Anticoagulant Rodenticides
(based on a paper published in Pesticide Science Vol. 45 Pages 247-256 (1995).

The poor performance of anticoagulant rodenticides in controlling Norway rats (*Rattus norvegicus* Berk.) is frequently attributed to resistance when other factors such as re-invasion or poor operating techniques are the real causes of failures. However, resistance to warfarin is well documented. In response to resistance, pesticide manufacturers developed more potent toxicants, referred to as the 'second-generation' anticoagulants such as difenacoum and bromadiolone. Despite these new rodenticides, control problems reappeared in southern central England. Apparently, these rat populations developed resistance to the new materials. Nevertheless, the rat populations resistant to second-generation anticoagulants could be controlled with difenacoum and bromadiolone provided bait consumption was adequate. In the early 1980s, bromadiolone bait trials killed half the resistant rats in 14 days, but total eradication was not achieved (for comparison, the same baits achieved complete control in 11-25 days against susceptible populations). However in 1991, an unusually large rat infestation on a farm in the north proved very difficult to control. Over the past 4-5 years, the farmer reported variable control with anticoagulant rodenticides. In June 1991, local pest controllers attempted to reduce the population. By October 1991, no apparent reduction was achieved with the anticoagulant baits bromadiolone, brodifacoum and flocoumafen (the latter two used indoors only) and the non-anticoagulant rodenticides zinc phosphide and calciferol.

This article reports how the prevalence and degree of resistance within the population had a significantly detrimental effect on the effectiveness of second-generation anticoagulants. We also investigated one approach to managing populations with a high-level resistance to second-generation anticoagulants. We relied on the non-anticoagulant rodenticide, calciferol, despite the earlier failure of this compound.

Methods

The study site was located in the north-west corner of Berkshire county in south-central England. This farmer reared pigs, beef bulls, sheep and a few free-range chickens. Wheat and barley were stored in barns on the floor and in open-topped bins throughout autumn and winter. In response to earlier control failures, two more bromadiolone treatments with increased numbers of bait points were carried out. The baits contained a liquid bromadiolone concentrate added to either ground pig feed (with <3 mg/kg vitamin K_3) or pinhead oatmeal, corn oil and caster sugar. The final concentration of bromadiolone in the feed was 0.005%. The first treatment with the pig-feed bait began in November 1991 and ended in April 1992. We intended to maintain a surplus of bait, but consumption was much larger than expected. Over 5 months 585 kg bait was consumed by rats. Although the rat population was not monitored, discovery of rat carcasses followed a reduction in bait take in some areas. However, consumption quickly increased presumably through rat recolonization. The second treatment with the oatmeal bait began in June 1992 and lasted 23 days. This time, the rat population was monitored with two techniques -- a tracking plate technique and a chemical bait marker. The chemical bait marker was decachlorobiphenyl (DCBP) added to the bait at 0.01% so that the amount of bait eaten by survivors could be determined. During this treatment, 248 kg bait was eaten by rats and it was impossible to maintain a surplus of bait despite more bait points and more frequent inspections. The average bait consumption was 10.3-11.8 kg and there was no measurable reduction in the size of the rat population, despite rat mortality (Fig. 1). Rapid re-invasion was ruled out and an analysis of 63 survivors showed that 51% ate >100 g bait, including one 543 g male that ate an estimated 450 g bromadiolone bait.

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Figure 1. The outcome of the bromadiolone treatment carried out in June 1992. The dotted line is the bait take and the bars are population estimates.

There was little doubt that the treatments failed due to resistance to bromadiolone. To gain insight into the degree and prevalence of resistance within this population, rat samples were trapped and bioassayed. At the Central Science Laboratory, we developed non-lethal bioassays to measure the degree of resistance to difenacoum and bromadiolone in individual rats. These bioassays are based on the earlier warfarin blood clotting response (BCR) test, that observes changes in blood clotting activity after administrating a sub-lethal dose of rodenticide. Those animals with a percentage clotting activity (PCA) <10% normal on day 4 were classified as susceptible; whereas those whose PCA is >10% were considered resistant. First, the bioassay tested rats for difenacoum resistance. Three weeks later, all resistant animals were tested for bromadiolone resistance. All rats trapped in September 1991 before the first bromadiolone treatment and all rats trapped before the second treatment (June 1992) were resistant to both difenacoum and bromadiolone (Table 1). This implies that difenacoum would perform no better than bromadiolone.

Table 1. Results of blood clotting response tests to determine the degree of resistance to difenacoum and bromadiolone. The figures show the mean percentage clotting activity (PCA) on day 4 of the test. PCAs above 10% indicate varying degrees of resistance.

Active Ingredient	Date rats trapped	Sex	No. Resistant/ No. tested	PCA ± se
Difenacoum	Sept. 91	M	4/4	69.9 ± 23.0
		F	12/12	48.1 ± 5.0
	June 92	M	13/13	89.1 ± 9.5
		F	12/12	72.9 ± 8.1
	Dec. 93	M	9/9	69.5 ± 15.2
		F	10/10	53.3 ± 5.3
Bromadiolone	Sept. 91	M	4/4	137.9 ± 12.9
		F	12/12	140.2 ± 8.7
	June 92	M	10/10	108.0 ± 11.7
		F	9/9	106.4 ± 5.8
	Dec. 93	M	7/7	66.3 ± 13.8
		F	8/8	82.7 ± 9.9

Would more potent anticoagulants, such as brodifacoum or flocoumafen, perform any better? Trapped rats were given a 7-day no-choice feeding test on 0.0005% brodifacoum bait. Although this concentration is one tenth the normal concentration in commercial baits, test survivors were considered resistant to brodifacoum. None of the 37 males tested survived, but 15 of 57 females survived. Doses ingested by the survivors ranged from 1.55-3.22 mg/kg body weight. Based on the amount of bait eaten by 63 rats following the June 1992 treatment, 71% would have died if the poison had been brodifacoum. Thus, a brodifacoum (and probably flocoumafen) treatment would result in a significant reduction in the rat population, particularly if the treatment extended beyond 23 days. However if brodifacoum becomes the main control agent, the long-term effects of selection pressure on a population with a low degree of resistance must be considered.

History shows that the response to the rodent resistance problem is to formulate more potent anticoagulants. In the UK, brodifacoum and flocoumafen are restricted to indoor use only, thus they have a marginal effect since most rats lived outdoors. An alternative approach is to use toxicants with different modes of action. Zinc phosphide and calciferol can be used outdoors, but their rapid action may lead some rats to develop an aversion to them before a lethal dose is ingested. Therefore, it is necessary to lay an unpoisoned bait to encourage rats to feed from the bait points before the poison is added. The absence of prebaiting might explain the poor result with calciferol. In March 1993, eight months after the second bromadiolone treatment, the rat population on the farm had become more extensive. An unpoisoned prebait of pinhead oatmeal, corn oil, caster sugar and a dye was laid in surplus for 3 weeks. By the third week, consumption was relatively stable at 29.4-31.2 kg/day and the bait marker DCBP was added. For the poison bait, an oil-based concentrate of ergocalciferol was added plus another chemical bait marker -- hexachlorobiphenyl (HCBP) at 0.01%. The final calciferol concentration in the bait was 0.1%. The markers determined the extent of any aversion that might develop to the poison. The total amount of poison bait eaten over 3 weeks was 48.5 kg, but 74% was taken over the first 2 days. The rat population, monitored by tracking plates, was reduced by 69%, but still an estimated 320 rats were alive, representing a significant infestation. Quantities of prebait and poison bait eaten by survivors and non-survivors were determined from the residues of DCBP and HCBP in their tissues. It was apparent that if the amount of calciferol ingested was insufficient to kill, the animals developed a learned aversion to the poison bait and reverted to the alternative foods. Only one survivor of 45 ate no bait at all. Animals that fed well on the prebait invariably died.

The most practical method to remove large numbers of bait-shy, anticoagulant-resistant rats was trapping. Between 148-170 spring-loaded kill traps (Fenn MkIV) were set each weekday for 3 weeks. The final population estimate was 10 rats, an overall population reduction of 99%. Unfortunately, the farm habitat was not cleaned up sufficiently and the population recovered somewhat. In December 1993, another rat sample was trapped and tested for resistance to difenacoum, bromadiolone and brodifacoum. Prevalence of resistance to difenacoum and bromadiolone remained unchanged, but the degree had reduced significantly, although not enough that difenacoum or bromadiolone were effective (Table 1). No rats survived the brodifacoum feeding test. No anticoagulants were used on the farm between the end of June 1992 and December 1993. This suggests that a key factor in the long-term management of resistant populations is to relax the selection pressure imposed by anticoagulant use. Effective use of non-anticoagulants could

hasten the selection of anticoagulant-susceptible phenotypes without waiting for susceptible rats to outbreed less hearty resistant ones. Such a strategy depends on the the development of non-anticoagulant rodenticides alongside the more easy-to-use anticoagulants.

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Natural Variation in Response to Insecticides as a Factor in Bioassays

Entomologists rely on bioassays as the principal means to determine and compare the toxicity of insecticides to insect pests. Normally, a dose-mortality technique is employed that gives a standard measure, such as the 50% or 90% mortality point to compare between populations. However, one must recognize that each value obtained is an estimate of the true value for that population. If one were to put aside the original set of data and get additional independent responses, these responses would show a range of variability about a mean value. Indeed, from a statistical viewpoint, the mean of several sets of data would give a better estimate of the true mean than any individual estimate. Thus, natural variability in response to insecticides does occur and researchers must interpret that variability. I was interested in how much variability occurs if a large number of independent tests were conducted.

Robertson *et al.* (1995) examined this question in three insect species against several insecticides. In the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), they found a 12.8-fold variation in response at LC_{50} to *Bacillus thuringiensis* subsp. *tenebrionis* in the same strain of beetles over time. In a diamondback moth, *Plutella xylostella* (L.), strain, the corresponding variation was 3.7 against *B. thuringiensis* subsp. *kurstaki.* In the western spruce budworm, *Choristoneura occidentalis* Freeman, the variation was 12.7 against DDT and 4.3 against pyrethrins. The results illustrate that considerable natural variation exists in these populations.

I have tested insecticide resistance in the German cockroach, *Blattella germanica* (L.) for many years. I consistently tested a susceptible strain for comparison with the resistant strain and assessed day-to-day variation (Cochran 1989, 1995). In the process, I accumulated much data on a susceptible strain against a series of insecticides. I present this data to demonstrate the range of variation that exists in this strain's response to insecticides.

Materials and Methods:

Insects

The Virginia Polytechnic Institute & State University (VPI) susceptible strain was collected on campus in Blacksburg, Virginia in 1947, and maintained in a pesticide-free environment. It is reared on 'rodent chow' under conditions described by Cochran (1979).

Toxicity Bioassays

Insect exposure to the insecticides was conducted by a tarsal-contact method -- the jar test (Cochran 1989). In each bioassay, a known amount of insecticide, dissolved in acetone, was placed in a 0.5 liter jar (Cochran 1989, 1995). The jar was rotated in a fume hood until the acetone evaporated leaving a thin insecticide coat on the inside surface. Ten 5^{th} or 6^{th} instars were placed in each jar and mortality recorded over time until 90% of the insects were killed. Each test was replicated three times.

Data Analysis

At the end of each test, the data from the replicates were pooled and analyzed by probit analysis (SAS 1985). The data reported here are LT_{50} values (minutes needed for toxicant to kill 50% of insect populations). Normally, this bioassay establishes significant differences (= resistance) between LT_{50} values based on non-overlap of the 95% confidence limits.

Results and Discussion:

Table 1 shows the roach response to four organophosphate (OP) insecticides. The mean LT_{50} values were different for each OP. Relative to other insecticides, the mean LT_{50} was high since the insect must activate the insecticide to its toxic form. Variation in response ranged from 2.3 to 4.1.

Table 1. Response of the VPI-susceptible strain of German cockroaches to several organophosphate insecticides

Insecticide	LT_{50} Values (min.)		X-Range Span	n
	Mean ± SEM	Range		
Diazinon	61.1 ± 4.2	34.5-110.6	3.2	30
Chlorpyrifos	113.8 ± 5.0	75.4-174.1	2.4	30
Malathion	60.1 ± 4.7	26.5-109.7	4.1	25
Acephate	115.8 ± 5.5	82.4-192.3	2.3	20

Results with two carbamate insecticides are presented in Table 2. The LT_{50} values were shorter than with OPs, and the range of responses to both carbamates were small, nearly identical.

Table 2 Response of the VPI-susceptible strain of German cockroach to two carbamate insecticides

Insecticide	LT_{50} Values (min.)		X-Range Span	n
	Mean ± SEM	Range		
Propoxur	32.9 ± 1.4	23.5-62.1	2.6	38
Bendiocarb	28.4 ± 1.5	17.5-46.7	2.7	32

Pyrethroid insecticides were fast acting with relatively short LT_{50} values (Table 3). Of those pyrethroids tested, allethrin was the fastest acting while fenvalerate was the slowest. Variation in responses ranged from 2.4 to 3.7.

Table 3. Response of the VPI-susceptible strain of German cockroach to several pyrethroid insecticides

Insecticide	LT_{50} Values (min.)		X-Range Span	n
	Mean ± SEM	Range		
Pyrethrins	11.3 ± 0.4	5.6-15.6	2.8	42
Allethrin	7.8 ± 0.4	4.3-16.0	3.7	45
Permethrin	18.5 ± 0.8	11.1-36.5	3.3	43
Phenothrin	14.6 ± 0.8	10.4-31.7	3.0	39
Fenvalerate	37.6 ± 1.8	19.5-63.3	3.0	39
Esfenvalerate	23.5 ± 1.1	11.1-36.6	3.3	36
Cyfluthrin	23.9 ± 0.8	23.9-0.8	2.9	45
Cypermethrin	21.5 ± 1.1	13.8-33.7	2.4	28
Tralomethrin	29.5 ± 2.5	17.4-44.7	2.6	12

These results show that natural variation in response to insecticides occurs in this species. Natural variation in the susceptible strain can influence the resistance level indicated by the data when comparing resistance ratios. However, the range of responses was less than reported by Robertson *et al.* (1995) indicating that varaiation in response is generally quite low in the German cockroach with the jar test. The average variation range was 2.8 for OPs; 2.6 for carbamates and 3.0 for pyrethroids. I did not find any extremely high values.

Likewise, a range of natural variation in response may occur in resistant strains as well. The presumption is that day-to-day variations in response are similar in both resistant and susceptible strains. Natural variation in response to insecticides should not be a major variable in calculating resistance ratios in this species with this test. Nevertheless, variations in the response of an insect population to an insecticide occurs normally and should not be ignored. Natural variation is a factor that should be considered when calculating resistance ratios for any test species.

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Survey of Susceptibility to Imidacloprid (Admire®) in Colorado Potato Beetle (Coleoptera: Chrysomelidae)

Introduction:

The Colorado potato beetle (CPB) is the most serious pest on potatoes and tomatoes in Maryland. CPB has developed resistance to virtually every insecticide class. New insecticides introduced for CPB control will most likely encounter the same fate unless proactive resistance management plans are implemented.

Imidacloprid was registered for CPB control in potatoes in 1995. Imidacloprid belongs to a new class of insecticides and shows high activity against CPB. Due to its long-term systemic action, as well as the insect's resistance to other chemicals, imidacloprid is used extensively as a soil treatment. This approach will result in continuous exposure to the chemical and heavy selection pressure for CPB resistance. The objectives of this study were to: 1) monitor CPB susceptibility to imidacloprid and establish baseline levels of susceptibility to quantify future shifts in responses 2) determine whether imidacloprid has cross resistance to other insecticides and predict where imidacloprid resistance may occur, and 3) determine if CPB susceptibility to imidacloprid changed in any field populations after one season of exposure to this insecticide.

Methods:

CPB adults were collected by cooperators from potato fields across the US and Canada and reared in field cages. A feeding bioassay with a modified Forrester potato leaf-agar diet was conducted on each population. Larvae were

allowed to feed on the egg chorion and potato leaves for five hours before dosing. Each replicate test consisted of five imidacloprid concentrations plus a water control, and 50 larvae per concentration. At least three replicates were conducted per population. Mortality was assessed after 48 hours and larvae were considered alive if they could move one leg. Concentration-mortality responses were calculated with the POLO-PC probit regression program.

An exposure bioassay with discriminating concentrations of esfenvalerate and azinphosmethyl was also used to classify the resistance status of each population. Each insecticide, dissolved in acetone, was applied to filter paper. Twenty neonates, fed on egg chorion only, were placed on the filter paper and sealed in petri dishes. Mortality was observed after 24 hours and larvae were considered alive if they could move one leg.

Results:

Significant variation in susceptibility existed among populations. LC_{50} values ranged from 0.28 ppm to 4.4 ppm imidacloprid and exhibited a 16-fold difference between the least and most sensitive populations (Table 1, Figure 1). Response slopes ranged from 0.9 to 2.3 and were negatively correlated with LC_{50}s ($r = -0.67$, $P < 0.001$). Populations that were less sensitive to imidacloprid had a shallower and more heterogeneous response.

Of the 34 populations tested, 20 populations were highly resistant to esfenvalerate and azinphosmethyl, 10 were susceptible, and 4 were intermediate (Figure 2). There was no strong evidence of cross resistance with esfenvalerate ($r = -0.24$, $P < 0.17$) or azinphosmethyl ($r = -0.29$, $P < 0.09$). However, many populations with high pyrethroid and organophosphate resistance had higher LC_{50}s on average (Figures 3 and 4).

There was no evidence of any shift toward tolerance to imidacloprid after one season of selection pressure. Interestingly, five of the six populations assayed before and after field exposure exhibited a significant shift towards greater sensitivity to imidacloprid in the summer generation (Table 2). One explanation is that the source of late summer infestations came from susceptible adults immigrating from refugia outside the potato fields (*i.e.* nearby wild host plants and untreated volunteer potatoes). These late colonizers invaded fields when the systemic toxicity of imidacloprid was no longer effective; thus, the selection pressure exerted on these CPB may have been minimal. Another explanation is that we collected overwintered beetles on imidacloprid-treated plants and even though the exposure time was relatively short, we collected survivors already selected by imidacloprid.

Acknowledgments:

Thanks to the USDA/ARS (Potato Competitive Grant Program) and to Dr. Walter Mullins (Bayer Corporation) for funding; and to the many cooperators who provided collections of adult beetles.

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Figure 1. Mortality response (LC50 and 95%Cl) of the populations to imidacloprid incorporated diet bioassays.

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Figure 2. Location of CPB populations assayed for imidacloprid susceptibility. Populations are group according to their resistance to pyrethroids and organophosphates.

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Figure 3 Scatter plot of susceptibility to imidacloprid and esfenvalerate in the CPB populations

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Figure 4. Scatter plot of susceptibility to imidacloprid and azinphosmethyl in the CPB populations.

Table 1. Susceptibility of Colorado potato beetle populations to imidacloprid, esfenvalerate, and azinphosmethyl. Populations are listed in order of most to least susceptible to imidacloprid. 1995.

Grower/ Location	Full series diet bioassay[1] response to imidacloprid				% Mortality ± SEM based on discriminating concentration[2] of	
	No. of insects tested	LC_{50} ppm	95% CL	Slope ± SEM	Esfenvalerate100 Tg/ml	Azinphosmethyl 2500 Tg/ml
Coloma / Coloma, WI	1451	0.28	0.2 - 0.35	1.5 ± 0.11	95.0 ± 1.8	55.0 ± 5.8
Roper / Roper, NC	821	0.33	0.26-0.40	2.3 ± 0.17	97.9 ± 0.8	61.0 ± 3.3
Okray / Central, WI	898	0.36	0.26-0.47	1.8 ± 0.15	93.5 ± 2.6	81.9 ± 2.3
Black / Cape Charles, VA	1323	0.36	0.28-0.44	2.1 ± 0.14	24.6 ± 4.8	26.8 ± 2.6
McPherson / Mapleton, ME	1426	0.37	0.27-0.48	1.8 ± 0.12	30.0 ± 4.7	27.5 ± 5.3
Baker / Middleton, DE	751	0.41	0.32-0.51	1.7 ± 0.16	12.4 ± 4.6	4.8 ± 1.3
Circle Farm / Patterson, WA	851	0.42	0.31-0.55	1.8 ± 0.15	98.0 ± 1.4	99.7 ± 0.3
Jackewicz / Rising Sun, DE	879	0.42	0.29-0.57	1.7 ± 0.15	17.3 ± 3.0	14.6 ± 3.0
Kelly#1 / Horntown, VA	1415	0.43	0.31-0.55	1.4 ± 0.12	16.4 ± 3.6	7.3 ± 2.2
G. Holland / Pocomoke, MD	1500	0.43	0.33-0.54	1.7 ± 0.12	14.2 ± 3.3	15.3 ± 3.5
Steward / PEI Canada	822	0.45	0.40-0.51	2.2 ± 0.14	82.0 ± 2.3	78.0 ± 3.3
Burgold / Little Creek, DE	1000	0.50	0.28-0.76	1.7 ± 0.15	23.7 ± 3.3	18.0 ± 3.5
Pries / Felton, DE	1198	0.51	0.38-0.64	2.1 ± 0.17	13.0 ± 2.3	13.8 ± 2.4
Wicks / Leipsic, DE	1155	0.52	0.42-0.61	1.8 ± 0.14	7.0 ± 2.5	2.5 ± 0.8
PSU Farm / Rock Spring, PA	1131	0.52	0.42-0.63	1.9 ± 0.14	22.5 ± 2.5	20.5 ± 2.8
Flewelling / Easton, ME	1169	0.52	0.41-0.65	2.1 ± 0.13	51.3 ± 5.1	44.2 ± 6.1
UM Farm / Queenstown, MD	651	0.55	0.41-0.88	1.9 ± 0.37	26.6 ± 3.9	38.4 ± 4.4
UM Farm / Salisbury, MD	1175	0.57	0.37-0.82	1.7 ± 0.12	27.8 ± 3.9	16.1 ± 3.4
UW Farm / Hancock, WI	999	0.58	0.44-0.75	2.0 ± 0.13	96.0 ± 4.0	52.0 ± 12.5
USDA Farm / Beltsville, MD	1479	0.59	0.48-0.72	1.6 ± 0.10	85.7 ± 2.9	60.3 ± 5.4
Martens / Port Byron, NY	988	0.60	0.42-0.85	1.3 ± 0.11	45.3 ± 6.8	19.6 ± 5.3
Grellinger / Southern, NJ	1200	0.67	0.58-0.77	1.9 ± 0.14	15.0 ± 5.2	10.5 ± 2.5
Gerritsen / Bridgewater, ME	870	0.68	0.52-0.89	1.4 ± 0.13	43.1 ± 7.7	31.0 ± 6.3
UM Farm / Marlsboro, MD	1167	0.70	0.52-0.91	2.2 ± 0.16	18.5 ± 4.4	37.5 ± 6.9
Diercks / Central, WI	908	0.78	0.47-1.17	1.9 ± 0.18	90.0 ± 4.0	65.1 ± 5.2
Miller / Hancock, WI	898	1.00	0.78-1.33	1.5 ± 0.14	97.0 ± 1.1	95.0 ± 2.0
Kelly#2 / Horntown, VA	1420	1.06	0.78-1.44	1.9 ± 0.18	31.5 ± 4.1	16.8 ± 2.4
NJ Reference Colony	1202	1.12	0.91-1.42	1.9 ± 0.14	64.4 ± 4.4	8.8 ± 2.7
Hickman#2 / Horntown, VA	540	1.12	0.75-1.80	1.3 ± 0.19	15.9 ± 3.2	7.3 ± 1.7
Hickman#1 / Horntown, VA	1174	1.30	0.93-2.01	1.7 ± 0.17	15.3 ± 2.6	7.2 ± 2.0
Voorhies / Rush, NY	897	1.33	0.81-2.43	1.2 ± 0.17	35.5 ± 5.2	15.9 ± 3.1
D. Holland / Stockton, MD	873	1.64	0.85-4.89	1.0 ± 0.17	21.7 ± 5.4	12.7 ± 2.7
B. Holland / New Church, VA	1175	2.13	1.46-3.91	1.0 ± 0.11	22.7 ± 2.6	16.1 ± 3.3
Wells / Riverhead, NY	644	4.08	3.06-5.30	1.0 ± 0.16	28.8 ± 3.9	16.5 ± 2.8
Wulforst / Calverton, NY	1125	4.40	2.21-24.3	0.9 ± 0.15	14.4 ± 4.2	5.8 ± 1.6

[1] At least three replicate tests were performed on each CPB population. Each test consisted of the 0.5x series of five concentrations of imidacloprid and a water control. Fifty larvae per concentration were fed incorporated-treated diet (modified Forrester recipe) for 48 hours.
[2] Standard filter paper assay of 200 neonate larvae exposed to a discriminating concentration of each insecticide for 24 hr.

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Response of Hygienic Honey Bees to *Varroa jacobsoni* Mites

The parasitic mite, *Varroa jacobsoni*, is the most destructive pest of honey bees (*Apis mellifera*) in the U.S. and Europe. Since its introduction into the U.S. in 1987, this mite has reduced the quality and quantity of bee colonies available for honey production and pollination. Currently, the only approved treatment for the mite is the pesticide Apistan® (fluvalinate) applied in strips within the bee hive. The risks of contaminating honey with pesticides and selecting mites for resistance to the pesticide (*e.g.*, Lodesani *et al.* 1992, 1995) are formidable. Therefore, it is important to determine if

honey bees have any natural, heritable defense mechanisms against the mite that may be readily incorporated into breeding programs.

The mite lifecycle is as follows: A mated, female mite is phroretic ("hitchhikes") on a young adult bee. The mite leaves the adult bee to enter a cell with an immature bee. The mite lays eggs on the immature bee then she and her offspring feed on the bee's hemolymph. The offspring mate within the cell and mated female mites leave when the bee emerges as an adult. Parasitized bees have reduced life-spans and may be deformed. Severe infestations lead to both individual and colony mortality.

A balanced host-parasite relationship has evolved between *Varroa* and its natural host, *Apis cerana*, in Asia. This honey bee species has two behavioral defenses that maintain the numbers of mites within tolerable limits -- grooming and removal (reviewed in Buchler 1994). While grooming, adult bees remove mites from themselves or from nestmates, damaging mite legs and cuticle in the process. Removal behavior involves the ability of some bees to detect, uncap and remove infested worker pupae from the cells (hygienic). The removal of infested pupae interrupts the reproductive success of the mites.

Removal behavior is analogous to "hygienic" behavior in honey bees. Hygienic behavior is considered the primary mechanism of resistance to at least two diseases of larval and pupal honey bees -- American foulbrood caused by the bacterium, *Bacillus larvae* (Rothenbuhler 1964) and chalkbrood caused by the fungus, *Ascosphaera apis* (Gilliam *et al.* 1983). Hygienic bees have the ability to detect, uncap and remove diseased brood from the nest before the causative organisms reach the sporulating stage. Rothenbuhler (1964) postulated that hygienic behavior is controlled by two independently assorting, recessive genes. Rapid hygienic behavior occurs at a relatively low frequency in most honey bee populations thus far studied (Spivak & Gilliam 1993).

A two-way selection program for hygienic behavior was initiated at the University of Minnesota in 1992. Lines of hygienic and non-hygienic colonies were bred and tested for their ability to remove pupae infested with *Varroa* mites. The hygienic and non-hygienic lines used in the experiment were bred from "Starline" stock, derived from Italian *A. mellifera ligustica*. Hygienic behavior in the colonies was determined by a freeze-killed brood assay where the amount of time was recorded for bees to detect, uncap and remove a comb section containing freeze-killed pupae (frozen at -20 C° for 24 hours). Colonies that removed the freeze-killed brood within 48 hours were considered hygienic and colonies that took longer than one week to remove the dead brood were considered non-hygienic. To establish and maintain the lines, queen bees were raised from colonies that displayed the most rapid and least rapid removal rates. The daughter queens were inseminated with semen from drones from different hygienic or non-hygienic colonies.

A commercially available Jenter Box® was used to test whether the selected hygienic and non-hygienic colonies of bees would remove pupae artificially infested with *Varroa* mites (following methods of Boecking & Drescher 1991, 1992). The box contains approximately 300 plastic worker cells and fits into a standard brood frame. Ninety cells within the box have false bottoms fitted with removable plugs that allows access to individual larvae or pupae within the box through the base of the cell.

The inseminated queens in each experimental colony were confined within the box until they laid eggs in most of the cells (6-24 hours). Eight or nine days later, *Varroa* mites were introduced through the plugs in the cells containing fifth instars using a fine, camel-hair paint brush. In 1994, one *Varroa* mite per cell was introduced into 10 - 20 cells containing fifth instars. Another group of cells served as controls and the plugs removed and replaced with no mite introduction. The infested and control cells were marked on a transparent sheet of plastic and inspected 1, 2, 4, 7, and 10 days after infestation to determine if the bees had detected and removed the infested brood. In 1995, two mites per cell were introduced onto other larvae within the box as well as to larvae infested with one mite and to the controls.

In 1994, the experiments included four hygienic and three non-hygienic colonies; and in 1995, seven hygienic and four non-hygienic colonies. The ability of the hygienic colonies to detect, uncap and remove mite-infested pupae from the cells within the Jenter Box are given in Figures 1a and 1b (Spivak 1996). In 1994, the four hygienic colonies removed significantly more pupae infested with one mite per cell by day 10 (69.2% ± 16.4) than the three non-hygienic colonies (10.0% ± 10.0) and the controls for the hygienic and non-hygienic treatments (21.1% ± 19.9 and 10.4% ± 10.0, respectively).

The same assay in 1995 yielded different results. When one mite per cell was introduced, the seven hygienic colonies did not remove significantly more infested pupae (24.7% ± 20.1) than the non-hygienic colonies (11.3% ± 6.3). When two mites per cell were introduced no significant difference in removal behavior was detected in hygienic colonies (49.8% ± 30.5) compared to non-hygienic colonies (22.5% ± 3.5). However, in both colony types significantly more pupae were removed than were infested with two mites per cell than the controls (9.9% ± 7.5 and 3.1% ± 6.3 for hygienic and non-hygienic, respectively).

There was considerable variation in the amount of infested brood removed by the seven hygienic colonies in 1995. Four hygienic colonies removed $\leq$ 15% infested pupae when one mite per cell was introduced. The remaining three removed 45.5% ± 6.5 and 69.6% ± 26.7 pupae when one and two mites per cell were introduced, respectively.

Many factors seem to regulate the expression of hygienic behavior in honey bees and the removal of pupae infested with *Varroa*. Further tests will determine if the variation between years was due to genetic or environmental causes. Bees may have a threshold response to the cues elicited by the mite or infested brood. If the colony is highly infested, it may not be advantageous for the bees to remove all infested worker pupae. This could substantially reduce the adult population of the colony. This may explain why some hygienic colonies did not remove higher numbers of infested pupae in 1995. Despite the plastic nature of the hygienic response, this defense against *Varroa* that can reduce the mite load within honey bee colonies. Studies will determine if open mated hygienic colonies are non aggressive, easy to handle and good honey producers (M. Spivak unpublished data). If so, inclusion of hygienic behavior as a selection criterion in breeding programs to provide a natural defense against American foulbrood, chalkbrood, and *Varroa* may be highly desirable.

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Figure 1a.

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Figure 1b.

Figure 1a & 1b. The mean percent removal of mite-infested pupae from the cells of the Jenter Box by the hygienic and non-hygienic colonies in 1994 (a) and in 1995 (b) on days 1,2,4,7 and 10 after the mites were introduced. One mite or two mites per cell were introduced into 10-20 cells in each colony through the plug at the base of the cell. The controls were cells containing 5th instar larvae from which the plug was removed and replaced with no mite introduction. Results of split-plot 2-way ANOVA on arcsine transformed data:

- 1994: bee type F = 45.87, df = 1,5, *P* = 0.001; treatment F = 6.35, df = 1,5, *P*= 0.05; interaction F = 4.86, df = 1,5, *P* = 0.08.
- 1995: bee type F = 3.96, df = 1,9, *P* = 0.10; treatment F = 9.03, df = 2,16, *P* = 0.002; interaction F = 0.00, df = 2, 16, *P* = 1.00.

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Diamondback Moth Resistance to *Bacillus thuringiensis* Toxin Cry1C in the Field

Introduction:

Many field populations of diamondback moth (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae), have evolved resistance to *Bacillus thuringiensis* subsp. *kurstaki* (*Btk*) (Tabashnik 1994). Resistance to *Btk* in DBM does not cause cross-resistance to Cry1C, a major toxin in *Bacillus thuringiensis* subsp. *aizawai* (*Bta*) (Tabashnik *et al.* 1993, Tang *et al.* 1996). In laboratory selection studies, several insects have evolved resistance to Cry1C (Gould *et al.* 1992, McGaughey & Johnson 1994, Muller-Cohn 1994, Moar *et al.* 1995). Although low-level resistance to *Bta* was found in some field populations of DBM (Perez *et al.* 1995, Shelton *et al*. 1993), no previous cases of resistance to Cry1C have been reported from the field. Recently, we found that a *Btk*-resistant DBM field population in Hawaii evolved >20-fold resistance to Cry1C toxin less than two years after *Bta* products were used (Liu *et al.* in press).

Materials and Methods:

We used five DBM colonies in our study. Colony LAB-P was susceptible to *B.t.* Colonies NO-QA, NO-93 and NO-95 were derived from the NO field population in 1989, 1993 and 1995, respectively. The NO population was resistant to *Btk* in 1989 (Tabashnik *et al.* 1990) and first treated with *Bta* in 1992. NO-QA was selected with *Btk* repeatedly in the laboratory. NO-93 and NO-95 were not exposed to *B.t.* in the laboratory. Colony SO-95 was derived in 1995 from a field population. SO was resistant to *Btk* in 1989 (Tabashnik *et al.* 1990) and has not been treated with *Bta*. All colonies were reared on cabbage plants.

We used Mycogen formulations of Cry1C and Cry1Ab and the *Bta* spore-crystal formulation XenTari (Abbott Laboratories, North Chicago, IL, USA). We tested 3rd instars from each colony with leaf disk bioassays (Liu *et al.* 1995). Probit analysis estimated LC_{50} values and resistance ratios were calculated as the LC_{50} for a colony divided by the LC_{50} for LAB-P. We analyzed mortality data with ANOVA for Cry1C and Cry1Ab at a single concentration against NO-QA, NO-95 and LAB-P.

Results and Discussion:

The resistance ratios to Cry1C toxin at five days were 3.0 for NO-QA, 23 for NO-93, 22 for NO-95 and 2.0 for SO-95 (Fig. 1). These results indicate resistance evolution to Cry1C in the NO population treated with *Bta*. No significant increase in LC_{50} to Cry1C was found in the NO-QA or SO-95 colonies resistant to *Btk*, but not exposed to *Bta*. Resistance ratios to *Bta* for NO-95 were only 2.0-3.0 (Fig. 2). NO-QA and NO-95 showed opposite patterns of responses to Cry1Ab and Cry1C toxins (Fig. 3). This suggests that resistance to Cry1C and Cry1Ab toxins were conferred by separate genes.

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Figure 1.

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Figure 2.

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Figure 3.

Our results suggest that *Btk*-resistant DBM populations can evolve resistance to Cry1C in the field in less than two years. In the NO population, resistance to Cry1C apparently evolved faster than to *Bta*. Several factors might cause the difference: (1) spores in *Bta*, (2) toxins in *Bta* other than Cry1C, (3) *Bta* materials other than spores and toxins, or (4) formulation ingredients. The difference in resistance of DBM between Cry1C toxin and a *Bta* spore-crystal formulation suggests that spore-crystal formulations may be more durable than single toxins. Our data, however, do not address the more difficult issue of whether it is best to combine toxins or to deploy them sequentially.

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Early Detection of Resistance to *Bacillus thuringiensis* in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in China

Since the late 1980s, cotton bollworm, *Helicoverpa armigera*, an important pest insect at the squareboll period of cotton developed high resistance levels to some pyrethroid insecticides such as fenvalerate and deltamethrin in the cotton growing areas of North China (Shen *et al.* 1993). At least 2-4 sprays of commercial formulated conventional *B.t.* are applied per season to control this insect in North China. The *B.t.*s are important and indispensable biopesticides that serve as alternatives to chemical insecticides for cotton bollworm control. This extensive usage is a potential resistance threat to the future of cotton bollworm control. In 1995, a bioassay (neonates exposed to a treated diet) generated baseline date in susceptible strain SUS_1 (held in our laboratory for 42 generations without exposure to *B.t.*) and a discriminating dose for the detection of *B.t.* resistance in the field populations of *H. armigera*. Our laboratory monitored for *B.t.* resistance in this insect at six locations in Shandong, Hebei, Henan, Anhui and Jiangsu provinces in China. The results indicated *H. armigera* collected from Yanggu (Shandong), Handan (Hebei), Xinxiang (Henan), Xiaoxian (Anhui) and Fengxiang (Jiangsu) in 1995 contained a proportion of *B.t.* resistant individuals. At each site, neonate mortality at the discriminating dose (2.0 mg *B.t.*/ml diet) was 5-10%. At the Dongtai site (*B.t.* sprays rarely applied), only 2%of *H. armigera* in the cotton survived the discriminating dose (Table 1).

Table 1. Resistance levels to *B.t.* in the *Helicoverpa armigera* neonate in 1995

Location	L-DP line	LC_{50} (mg/ml) (95% FL)	% of resistant individuals
S strain	y = 6.77 + 1.91x	0.12 (0.08 ~ 0.15)	1
Yanggu	y = 6.33 + 1.33x	0.10 (0.07 ~ 0.13)	9
Handan	y = 5.80 + 1.21x	0.22 (0.16 ~ 1.06)	9-10
Xinxiang	y = 6.18 + 1.31x	0.12 (0.09 ~ 0.17)	6
Xiaoxian	y = 6.24 + 1.44x	0.14 (0.10 ~ 0.19)	7
Fengxiang	y = 6.23 + 1.35x	0.12 (0.09 ~ 0.17)	5
Dongtai			2

Neonate survivorship collected from Yanggu, Shandong province and Xinxiang, Hebei province was determined. Mortality in the XinXiang population was about 16-30% less 5 days after treatment compared to the susceptible strains (SUS_1 and SUS_2) (Table 2). Based on this study, we feel that it is absolutely necessary to restrict the use of conventional *B.t.* to a maximum of two sprays per season in North China as a precaution against *B.t.* resistance development in *H. armigera*.

Table 2. Comparison of effect of transgenic cotton expressing *B.t.* toxin on neonates from the Yanggu and Xingxiang field population and susceptible strain

Transgenic cotton	Population	Cotton No.	No. of larvae	Mean Mortality (%)	
				3 days	5 days
R_{19}	SUS_1	10	300	78.7 ± 11.36	90.0 ± 5.21
	Xingxiang	5	150	69.3 ± 22.52	74.0 ±23.99
R_{16}	SUS_2	10	300	39.7 ± 25.12	53.0 ± 21.45
	Yanggu	18	540	10.38 ± 8.64	24.61 ± 15.32

* Field Yanggu population was collected from Yanggu county, Shandong province, and Xinxiang, Hebei province, at field second generation (laboratory first generation) in 1995
R_{19} and R_{16} were the transgenic cotton expressing *B.t.* toxin (subspecies *kurstaki*)

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Tobacco Budworm Populations in Cotton: Heterogeniety of Response to Anticholinesterase Inhibitors

The tobacco budworm attacks cotton in the Lower Rio Grande Valley of Texas and Mexico. Two facts are known about this pest. Inconsistency in population size in fields in this area is consistent. Inconsistency in population response to any anticholinesterase insecticide in this area is also consistent. Many factors are responsible for these inconsistencies. I will discuss these inconsistencies as they influence budworm control by anticholinesterase insecticides.

The biggest problem faced by each producer is the presence of large budworm populations in each field. I suggest that there are anticholinesterase insecticides available to suppress >50% of these populations when correctly used. Budworm populations must be treated as young larvae and the insect must contact or ingest the insecticide. Heterogenous populations are comprised of both adults and progeny present within this defined geographic area at a specific time. Because these adults are capable of movement, they may be present in the area for milliseconds. Whereas progeny of these adults only move short distances.

Since the 1950s, anticholinesterase insecticides were applied to control budworms. Methyl parathion was applied for control in this area since 1951. Profenofos, suprofos and methomyl (including the pro-insecticide thiodicarb) were applied since the 1970s. All anticholinesterase insecticides are foliar sprays and should be applied to 1- to 3-day-old larvae for maximum control. I suggest that resistance may be evident if larval populations are not depressed by two sample dates following the application of an anticholinesterase insecticide.

Cotton producers must relate to the population size in each field. Plants within fields can have completely different population sizes. Adjacent fields (within 10m) can have totally different budworm populations. However, budworm populations in less than 3% of Rio Grande fields are sampled and documented each season.

What should be done about this problem in the Lower Rio Grande Valley of Texas and Mexico? First, the size of the larval population must be estimated in each field in one day. This is the stage to control and larvae may or may not respond alike to each anticholinesterase insecticide. Producers rarely know how many adults and pupae there are in each field.

Larval response to methyl parathion in the Lower Rio Grande Valley was variable from 1967 to 1981 (Wolfenbarger *et al.* 1984). Then, Vargas-C. & Wolfenbarger (1992 and 1994), Vargas *et al.* (1993), Norman *et al.* (1994) showed that the LD_{50} values in 1991 to 1993 were equal or lower than LD_{50}s shown in 1980-1981. A rise in LD_{50}s from late 1960s to 1973 was followed by a fall from 1974 to 1993. But what is the response to profenofos and sulprofos by budworms in this area? No resistance to these insecticides has been shown. There is a linkage of response levels to males as well as a continuum of response to methyl parathion (Wolfenbarger *et al.* 1984) and inconsistency of response to methomyl for a decade was consistent (Wolfenbarger *et al.* 1987).

We have to consider the inheritance complexities of biochemical, biological and environmental responses to any anticholinesterase insecticide by each larva within the population in each field or fields in this area. This is because the response among individuals in any population to these anticholinesterase insecticides is a continuum. A continuum indicates that at any one time or place larvae respond differently and some proportion of susceptible larvae are present in each population. The range of LD_{50}s can vary many fold and they can only be determined by treating progeny of single pairs or by sub-dividing a large population and treating each with a dose series of the desired toxicant. Thus, response by this insect to anticholinesterase insecticides is not an all or none scenario.

Anticholinesterase insecticides comprise more than 41% of insecticides applied to cotton to control budworm. There may be more than one resistant tobacco budworm someplace in this area. Only by sampling and determining the response level of individuals in each field or group of fields will we know how many truly exist.

I like the resistance definition by Winteringham & Hewlett (1964). They state that "resistance to insecticides is the development of an ability in a strain of insects (in a field or group of fields) to tolerate (=survive) doses of toxicant(s) which would prove lethal to the majority of individuals in a normal population of the same species". I suggest that greater than 51% of the larvae in any strain exhibit some degree of susceptibility. Hopefully, results from DNA analysis will allow us to gain insights into proteins that directly or indirectly affect efficacy of anticholinesterase insecticides. When males of a strain selected with methyl parathion were hybridized with non-selected females, their LD_{50}s and their range were greater than when females were selected and hybridized with non-selected males (Wolfenbarger *et al.* 1982). This indicates that gender also plays a role in the heterogeneity of response to these insecticides. Thus, genes that cause resistance to methyl parathion do not appear to be fixed in our populations. Does the same resistance mechanism affect each anticholinesterase insecticide in the same fashion? What modifiers of any gene may exist that affect the efficacy of anticholinesterase insecticides used today and what are the gene linkages that cause this resistance to other genes that contribute to biological parameters? And then, how do these genes respond to environmental parameters?

Even if a population that contained more than 50% resistant larvae (determined by some method) was present in a certain field on one day would this population exhibit reduced fitness? Would this be at the same level if we were to sample another larval population from the same field the next day? Would there be inbreeding depression of surviving larvae in a field that are resistant from one generation to the next? Would inbreeding depression be present in one population but not the next? What are the boundaries of these populations? What method should be used to determine these responses? What proportion of these populations have genetic or non-genetic physiological mechanisms that cause or induce resistance? What is the relationship of genes that cause or induce resistance to anticholinesterase insecticides in adult versus larval populations? These questions are posed to stimulate research on methods to understand, prevent or manage resistance budworm.

Today, the anticholinesterase insecticides applied to control the tobacco budworm are effective but some applications are not as effective as they should be against some populations in some fields during some years. However, I suggest that the

proportion of the populations that may be resistant prior to an anticholinesterase insecticide application cannot be predicted accurately.

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Whitefly Control in Arizona: Developing a Resistance Management Program for Imidacloprid

Abstract:

In 1995 we initiated a resistance management program to sustain the efficacy of imidacloprid. This paper delineates the groundwork for the program, describes methodology and conceptual advances toward our goal. Bioassay methods developed for adult whitefly consist of a one-day hydroponic uptake procedure by cotton seedlings. A reliable mortality criterion was established. Results from a statewide survey suggest slight geographic variation in whitefly susceptibility to imidacloprid. Long-term studies will 1) evaluate the risk of resistance to whitefly populations in commercial greenhouses and relate this to field populations, and 2) characterize the development of resistance in relation to cropping systems and spatial dynamics of whitefly. The overall objective of these investigations was to determine if a sustainable use strategy can be identified for imidacloprid.

Introduction:

Imidacloprid is a new chloronicotinyl insecticide that exhibits both systemic and contact activity primarily against sucking insects (Mullins 1993). It has a novel mode of action, binding to the nictinergic acetycholine receptor in the post-synaptic region of the insect nerve. Imidacloprid plays an important role in whitefly management in a broad range of crops (Mullins & Christie 1995, Palumbo 1994a, Palumbo *et al.* 1994, Palumbo 1995). Additionally, it has relatively low mammalian toxicity (rat dermal $LD_{50} > 5000$ mg/kg). Trade names in the United States include Gaucho®, Admire®, Provado®, Marathon®, and Merit®.

Imidacloprid was introduced to Arizona agriculture in 1993 as Admire®, under a Section 18 registration. This was necessary because pest managers were unable to control *Bemisia* on lettuce, cole crops, and melons in southern Arizona. Since then, imidacloprid was granted full registration by the EPA and continues to provide critical control of whitefly on vegetables and melons. It is essential that we sustain the long term effectiveness of this compound. Studies in California show that resistance to imidacloprid can be selected relatively rapidly in whiteflies (Prabhaker *et al.* 1995). Also, widespread resistance to imidacloprid in field populations was documented in the Almeria region of Spain (Cahill *et al.* 1996).

In response to this threat, we initiated a resistance management program for imidacloprid in 1995. The ultimate goal was to sustain imidacloprid efficacy against *Bemisia*. We present the following building blocks for this program.

- Development of bioassay methods
- Statewide survey of baseline susceptibility
- Isolation and characterization of homozygous resistant strains
- Ecosystem study of resistance dynamics
- Monitoring susceptibility in commercial greenhouses

Development of Bioassay Methods:

Efficient and reliable bioassay methods are a prerequisite for an effective resistance management program. Considering the systemic action of imidacloprid, we developed a bioassay that exposed whitefly to the chemical through voluntary feeding on leaf tissue. In a sequential manner, we evaluated 1) host type, 2) interval of hydroponic uptake, and 3) mortality criterion on whitefly susceptibility to imidacloprid.

Results indicated that host type had no influence on whitefly susceptibility ($P>0.05$) (Fig. 1a). Therefore, cotton was used in subsequent trials, since it was the more preferred host. Interval of imidacloprid uptake did not significantly affect whitefly mortality ($P>0.05$) (Fig. 1b). Though not significant, control mortality increased with duration of the uptake interval. This trend was probably due to a reduction of host quality as the interval of uptake increased. Also, phytotoxic effects were observed at the two- and five-day intervals for the highest imidacloprid concentration tested (1,000 μg/ml). These results indicate that a one-day interval of hydroponic uptake is appropriate for cotton. Results from trials comparing mortality criteria were similar at low concentrations; but at high concentrations, the criterion requiring normal locomotion led to greater mortality (Fig. 1c). Recovery of 'dead' individuals was most common with one-body-length criterion. Therefore in subsequent trials, repetitive movement was used as the mortality criterion.

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Figure 1a.

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Figure 1b.

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Figure 1c.

Figures 1a, 1b & 1c. Comparison of host plant (a), interval of uptake (b), and mortality criteria (c) for development of bioassay methods (mean mortality + SD).

Our results indicate that a reliable bioassay procedure for assessing the susceptibility of adult whitefly to imidacloprid was a one-day hydroponic uptake by cotton seedlings and the above-mentioned mortality criterion.

Statewide Survey of Baseline Susceptibility:

Statewide assessment of whitefly susceptibilities to imidacloprid will allow us to detect resistance once it appears in Arizona, and will provide a foundation for managing resistance once it appears. Approximately 8,000 whiteflies were collected from each of eight locations throughout the cotton regions of Arizona during the 1995 field season. Bioassays with the hydroponic uptake method described above were conducted approximately 36 hours after field collection.

Results illustrated a high degree of similarity in susceptibility to imidacloprid within Arizona populations (Fig. 2a). Whitefly populations from four of eight locations sustained 100% mortality at 1,000 μg/ml. However at concentrations of 10 and 100 μg/ml, only the population from the Yuma Valley Agricultural Center sustained 100% mortality. Differences were greatest at 1 μg/ml. For example, Casa Grande exhibited 40% mortality and Yuma >95% mortality at 1 μg/ml. These results parallel those from a related survey indicated that whitefly from western Arizona, *e.g.* Yuma Valley Agricultural Center, were more susceptible to pyrethroid-organophosphate mixtures than populations elsewhere in the state (Dennehy *et al.* 1996). We hypothesize that this may be attributed to a higher proportion of unsprayed hosts, *e.g.* alfalfa, in western Arizona than in other parts of the state. These unsprayed hosts may act as a buffer to the resistance development.

The state wide survey provided growers with information on the imidacloprid resistance among specific whitefly populations. In 1995, a pest manager contacted our laboratory to report a whitefly control failure in an autumn melon crop treated with imidacloprid. In the past, imidacloprid provided the manager with good control of whitefly, even in fields like this one, surrounded by cotton. Subsequent bioassays showed no difference in susceptibility between the field-collected population (suspected as resistant) and a population never exposed to imidacloprid (pristine population) (Fig. 2b). Intensive flights of whitefly in late summer from cotton to melons can occur as cotton plants deteriorate and become less suitable whitefly hosts (Byrne *et al.* 1990). Therefore, it is possible that the 'control failure' was due to whitefly movement from cotton into melons and not resistance to imidacloprid.

This 'pseudo-resistance episode' illustrates the interdependence between vegetable/melon and cotton production in Arizona. Cotton growers depend on vegetable/melon growers for timely plowdown that limits whitefly dispersal into their crop. Similarly, in autumn, vegetable/melon growers rely on cotton growers to limit whitefly movement from cotton by making necessary treatments, timely defoliation and plowdown of cotton. This interdependence between the cotton and vegetable/melon production in Arizona points to the benefits gained from cross-commodity cooperation for production and resistance management.

Isolation and Characterization of Homozygous Resistant Strains:

Selection of resistant strains is an essential prerequisite for studies to characterize resistance mechanisms, cross-resistance, and develop then validate resistance detection methods. We are selecting for imidacloprid resistance in Arizona whitefly populations. We determined the baseline susceptibility of many Arizona field populations, including some never exposed to imidacloprid (Fig. 2a). We consistently observed a 'plateau' in the population response where approximately 10% survival occurred at high imidacloprid concentrations (Fig. 2c). This data strongly suggests a polymorphism that confers reduced susceptibility to imidacloprid among Arizona populations. Selection experiments underway should confirm or reject this hypothesis. If resistance is selected in the laboratory, future work will focus on the stability of imidacloprid resistance and cross-resistance characterization.

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Figure 2a.

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Figure 2b.

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Figure 2c.

Figures 2a, 2b & 2c. Statewide susceptibility of whitefly to imidacloprid, 1995 (a), diagnosis of a pseudo-resistance episode (b), baseline susceptibility of a whitefly population never exposed to imidacloprid (c) (mean mortality + SD).

Ecosystem Study of Resistance Dynamics:

Interactions between insecticide resistance and the spatial dynamics of whiteflies, hosts and cropping systems represent an ecological approach to resistance management. Such studies allow the concept of 'sustainable efficacy' to be evaluated on a regional scale and interface with the area-wide pest programs in Arizona.

We know that resistance of whiteflies is less severe in Yuma, Arizona and the Imperial Valley, California, than in central Arizona. We hypothesize that this phenomenon is predicated on the prevalence of untreated hosts, such as alfalfa and weeds, where there is little or no selection for resistance. Such hosts act as reservoirs of susceptibility in whiteflies.

In 1995, we initiated a long-term study exploring these interactions in southwestern Arizona. Whitefly susceptibility to imidacloprid will be monitored at five sites throughout the year. Each site was characterized by production of lettuce, cole crops, alfalfa, cotton, and melons. By contrasting resistance development in treated crops and its impact on nearby untreated crops, we hope to elucidate use patterns and refuge conditions that sustain imidacloprid efficacy.

Monitoring Susceptibility in Commercial Greenhouses:

Imidacloprid (Marathon®) was registered for commercial greenhouses in Arizona in August 1995. We expect many greenhouse populations of *Bemisia* to be subjected to intensive selection pressure by imidacloprid because 1) this chemical is very stable in the soil, and 2) low tolerances for cosmetic damage in ornamental plants promote frequent application in greenhouses.

Monitoring greenhouse populations will help us anticipate the time-course of resistance development in field populations. We hypothesize that imidacloprid use in Arizona greenhouses will substantially increase the rate at which resistance will occur in field populations. Once selected in greenhouses, resistant whiteflies will be transported throughout Arizona (and elsewhere) on ornamental plants delivered to garden shops. Consumers will further disseminate the whiteflies into urban areas adjacent to agricultural fields. Thus, imidacloprid use in commercial greenhouses will serve as an effective mechanism for selecting and distributing imidacloprid-resistant whitefly throughout Arizona. Documenting whitefly resistance to imidacloprid in greenhouses will help assess the validity of this hypothesis.

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Resistance to Maize Weevil in Quality Protein Maize Lines and Commercial Corn Hybrids

The maize weevil, *Sitophilus zeamais*, is an important pest in stored corn grain, especially in the tropics. The discovery of a corn type with the Opaque-2 (O-2) mutant gene that changes the protein composition and increases the lysine and tryptophan content, offered new perspectives for corn use in human nutrition. However, the softness of the endosperm caused the O-2 maize to be very attractive to the stored grain pest. To solve this problem, the O-2 gene was transferred to corn with normal kernels. This new type of maize was called Quality Protein Maize (QPM). Research with the BR-45, a QPM line (Santos 1992) showed that it was less preferred by the maize weevil than the corn with the O-2 gene, but it was still preferred by this pest over some available commercial hybrids. Thus, it is necessary to improve the genetic resistance to maize weevil in the QPM lines. This study proposed to evaluate a selected group of QPM experimental lines plus commercial hybrids for genetic resistance to the maize weevil.

Material and Methods:

This research was conducted at the CNPMS, an EMBRAPA/ Ministry of Agriculture Institution, located in Sete Lagoas, MG, Brazil. Two entries, Cateto-SL and IAC-I O2 IV, were obtained from the germplasm bank, and 23 entries with the QPM genetic background came from the CNPMS breeding program. Three entries, BR-201 (hybrid), BR-106 and BR-451 (lines) were material released by EMBRAPA/CNPMS for commercial planting, and the AG-122, AG-510 and C-805 were hybrids sold by private companies. The QPM tested were all experimental material except the BR-451. Based on previous work (Santos 1977), the entries Cateto-SL and the IAC-I O2 IV were considered resistant and susceptible,

respectively. These entries together with commercial hybrids and the QPM material were compared based on damage caused by insects during storage. The identification of all genotypes tested are shown in Table 1.

A *S. zeamais* laboratory culture was maintained in controlled environment (70% R.H. and 27 ± 0.5°C). Species identification was confirmed according to the aedegus characteristics. Seed samples never treated with insecticide were placed in the freezer (-15°C) for five days to eliminate any previous insect infestation. Neonate to 5-day-old insects were placed in a small sample of each tested entry to allow them to adjust to the new food source prior to oviposition. Maize susceptibility was measured following methodology reported by Dobie (1974).

Table 1. Index of Susceptibility (IS) of some Quality Protein Maize (QPM) and some commercial corn, tested for genetic resistance to maize weevil.

Order	Name	Index of Susceptibility (IS)[1]
01	93 HT-18*	10.73 A
02	IAC-1 O2IV***	10.50 A B
03	93 HT-11*	10.31 A B C
04	93 HD-12*	10.29 A B C D
05	93 HD-06*	10.10 A B C D E
06	93 HT-24*	09.88 A B C D E F
07	CA-805**	09.84 A B C D E F
08	CMS 455C*	09.67 B C D E F G
09	93 HR-30*	09.66 B C D E F G
10	93 HT-12*	09.50 C D E F G H
11	93 HD-03*	09.38 D E F G H I
12	93 HD-01*	09.30 E F G H I
13	BR-106**	09.28 E F G H I
14	93 HT-17*	09.06 F G H I J
15	SYNTHETIC*	09.03 F G H I J K
16	93 HT-23*	08.93 G H I J K L
17	93 HD-20*	08.90 G H I J K L
18	BR-201**	08.86 G H I J K L M
19	BE-451*	08.75 H I J K L M N
20	AG-510**	08.74 H I J K L M N
21	93 HD-26*	08.57 I J K L M N
22	HT-2X*	08.48 I J K L M N O
23	93 HT-28*	08.48 I J K L M N O
24	93 HD-14*	08.35 J K L M N O
25	AG-122**	08.33 J K L M N O
26	92 HD-04*	08.12 K L M N O
27	CMS-455*	08.06 L M N O
28	93 HD-05*	07.98 M N O
29	93 HD-01*	07.94 N O
30	93 HT-27*	07.67 O
31	CATETO-SL****	

* QPM
** Commercial
*** Susceptible check
**** Resistant check
[1] Means followed by the same letter do not differ significantly at $\alpha = 0.05$, according to the Least Significant Difference Test.

Results and Discussion:

The Index of Susceptibility (IS), described by Dobie (1974), was found to be correlated with important genetic resistance factors like grain weight loss, number of F1 progeny, grain hardness and rate of insect increase (Classen *et al.* 1990, Gomes *et al.* 1992).

The antibiosis and nonpreference were observed acting together as resistance mechanisms to the maize weevil in corn grain (Santos & Foster 1983) They both may be responsible for the variation of the IS value.

The mean number of F1 progeny ranked from 60.4 for the genotype HT-27 to 137.1 for the 93 HT-11. Some QPM genotypes produced equivalent insect numbers as the resistant check, the Cateto-SL; however, some QPM genotypes produced as many insects as the susceptible check, the IAC-I 02 IV. The mean number of insects in the commercial hybrids stayed around the overall mean. The greater the F1 progeny, the heavier the infestation and the more susceptible the genotype. The Mean Development Period (MDP) from egg to adult ranked from 45.6 for the genotype 93 HT-18 to 54.2 days for the Cateto-SL. Some QPM genotypes had the MDP equivalent to the susceptible check and some to the resistant check. The MDP for the commercial hybrids, in general, stayed around the overall mean. A shorter MDP results in more generations per year and therefore greater susceptibility of the genotype.

Table 1 lists the IS to maize weevil for all genotypes tested. The higher the IS, the more susceptible the genotype. Entries followed by the same letter are not different from one another at the α= 0.05 significance level according to Least Significant Difference Test (LSD) (C.V.= 6.16%, Sx= 0.32, LSD= 0.908). The IS value ranked from 7.5 for the Cateto-SL, (resistant check), to 10.7 for the 93 HT-18, with the IS for the sustainable check 10.5. Eight QPM lines did not significantly differ from the resistance check, but the other five QPM had an IS did not differ from the susceptible check. One commercial hybrid, the AG-122, did not differ from the resistance check, but another, the CA-805, did not differ from the susceptible check.

Some QPM lines had an IS very close to the resistant control (a very hard flint endosperm maize). This indicates that some QPM corn hybrids, in addition to carrying genes for enhanced protein quality, also carry genes for resistance to maize weevil. Eight QPM lines had an IS mean lower than that from the commercial hybrids; however, another group of QPM lines had an IS similar to the susceptible check. The mean IS for the commercial hybrids stayed between the resistant and susceptible checks. Some QPM lines had an IS very close the susceptible check (a soft endosperm grain corn) providing evidence that there is genetic variability with in QPM genotypes for insect resistance. It may be possible to increase insect resistance by means of specific selection, since this trait was not selected for while breeding for the QPM hybrids. We believe we can develop QPM commercial hybrids with resistance to stored grain pests with levels compatible with the normal endosperm commercial hybrids.

Our studies agree with Arnason *et al.* (1992) who studied one group of QPM genotypes in relation to maize weevil resistance. QPM corn is not necessarily more or less susceptible to weevil damage than the normal endosperm type corn. We have discovered a group of QPM corn hybrids and lines that carry genes for resistance to the maize weevil, *S. zeamais*.

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Horn Fly Control with Pyrethroids in Argentina

Pyrethroid efficacy on several Argentina horn fly populations was evaluated by observations of the flies on treated cattle and by bioassays with fenvalerate-treated filter papers (Sheppard & Hinkle 1987).

Farmers control horn flies based on the husbandry and production practice of their region. In Northern and Northeastern Argentina, horn fly populations are abundant during a long season (October-March) so dips, sprays and pour-ons are the usual control methods. During last summer, the efficacy of the three methods fell appreciably. Dips gave good fix control for about fifteen days, but now they protect animals for much shorter periods ranging from zero to six days. The period of spray efficacy fell from about 30 to 20 days and to 5 days in some areas (Misones and Corrientes Provinces). Pour-ons used to keep flies off for about 45-60 days, but this summer pour-ons of deltamethrin, cypermethrin, cyhalothrin or cyfluthrin did not control the infestations for more than four weeks. Resistance is also reflected in *in vitro* assays when horn flies are exposed to filter papers impregnated with fenvalerate. Lethal doses of fenvalerate for horn flies in some areas have increased significantly. Details of these toxicological studies are reported in a manuscript submitted to the Journal of Medical Entomology.

In the other areas of Argentina, horn flies are more easily controlled. South of Buenos Aires Province and La Pampa, pyrethroid pour-ons (decamethrin, cypermethrin, cyflothrin) are the usual way to control horn flies. In general, pour-ons keep horn fly populations below 200 flies per animal for about 45 days.

In central Argentina, Santa Fe, Santiago del Estero and Cordoba Provinces, the efficacy of insecticide treatments are acceptable. Although farmers in some areas complain about the reduced efficacy of the treatments, poor control is not attributed to resistance. Sprays and pour-ons are generally used to control horn flies in this area. Dairy cattle are treated more frequently than beef cattle. The increased treatment frequencies against horn fly populations may be one of the factors responsible for the development of pyrethroid resistance in horn flies in the Northern regions of Argentina.

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Laboratory Selection of *Lucillia cuprina* Larvae with Ivermectin

On Australian sheep, blowfly strike is the most important ectoparasitic disease causing wool loss, reduction of wool quality, and sheep death. The Australian sheep blowfly, *Lucilia cuprina* Wiedemann, is the major myiasis fly, initiating around 90% of all strikes. *L. cuprina* rapidly developed resistance to the cyclodiene, organophosphate, and carbamate insecticides used to control fly strike (Hughes & McKenzie 1987). Laboratory selection resulted in high levels of resistance to deltamethrin, butacarb and diflubenzuron (Kotze & Sales 1994). Selection for resistance in *L. cuprina* may be enhanced by exposure to residues following treatment for other parasites. To determine if ivermectin resistance was present in the field or could be generated in the laboratory, a composite field strain of *L. cuprina* was selected for study. This strain was examined with metabolic inhibitors and phenobarbital induction of monooxygenases in selected and nonselected larvae. The responses of individual strains to ivermectin were examined to assess the range of resistance levels.

Insects:

CFS89 was a pooled field strain derived from larvae collected from over 30 separate field strikes during the 1989-90 blowfly season. LS was a laboratory-susceptible strain. Field strains were cultured from larvae collected from struck sheep during the 1992-94 seasons.

Bioassays:

Insecticides were diluted in methanol or acetone then one mL solution was applied to a 12 x 3 cm strip of blotting paper and allowed to dry. Individual strips were rolled lengthwise and placed in a 8 x 25 mm vial. One mL bovine serum fortified with yeast extract (2% w/v) and buffered with monobasic potassium orthophosphate (0.5% w/v) was added to each tube. Approximately 50 -100 neonate *L. cuprina* were added to the tube and plugged with cotton wool. Assay tubes were held at 27±2°C and 70% RH. Mortalities were assessed by counting dead and live maggots 48 and 24 hours after ivermectin and diazinon treatments, respectively.

Selection:

Neonates were selected with the bioassay system described above. More than 2,000 neonates were placed in 20 tubes containing blotting paper treated with a concentration expected to produce greater than 70% mortality. After 48 hours, larvae were removed from the tubes and allowed to complete development under standard culture conditions. Larvae were selected for over four years or 60 generations with ivermectin concentrations ranging from 10 to 50 µg/L (Fig. 1.). After the first selection, the LC_{50} increased 2-fold compared with that of the parental strain (CFS89). These responses were significantly different at LC_{50} based on the non-overlap of 95% fiducial limits. LC_{50} values increased gradually with subsequent selections and stabilized at a level approximately 8-fold higher than the parental strain. The selected strain reverted towards susceptibility fairly rapidly following relaxation of selection pressure. Within eight generations, the LC_{50} values dropped from 7-fold to 2-fold compared to the parental strain, but stabilized at this slightly elevated level for subsequent generations.

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Figure 1. Concentration responses to ivermectin of unselected (X) and ivermectin-selected (o) larvae of *Lucilia cuprina*.

Synergism/Induction:

CFS89 and selected larvae were pretreated with piperonyl butoxide (PBO, 0.1 mg/mL), triphenyl phosphate (TPP, 2.0 mg/mL), tridiphane (TRI, 1.0 mg/mL), phenobarbital (0.3 mg/mL) or acetone, in the bioassay system for 6 hours before they were transferred to standard concentration-response assays. The LS strain was also examined with phenobarbital because induction was expected to be greatest in the most susceptible strain.

PBO synergised both strains. This was most evident as a 60% reduction in LC_{50} of the selected strain. TPP and TRI had no apparent effect on CFS89, but a slight synergistic effect (~20% reduction in LC_{50} values) on the selected strain. However, all three synergists produced a similar reduction (~30%) in the selection ratio (LC_{50} selected/ LC_{50} nonselected) relative to non-synergised larvae.

The susceptible LS strain and CFS89 showed induced tolerance to diazinon. LC_{50} values in phenobarbital-treated larvae increased 5.2-fold and 1.8-fold in these two strains, respectively. The response slopes of both strains were similar in both induced and non-induced larvae indicating a true shift in tolerance to diazinon. By contrast, the induced selected strain gave a 3.5-fold increase in the LC_{50} for diazinon compared with the non-induced larvae, but had a markedly steeper response slope (~3-fold) indicating a lower actual increase in tolerance. The responses of non-induced and induced larvae to ivermectin were similar within each strain, although induced larvae in the LS strain had a 2.2-fold increase in tolerance (at LC_{50}) associated with a 2-fold increase in the slope of the response line.

Field strains/monooxygenases:

The responses to ivermectin were determined for seven organophosphate resistant field strains with known monooxygenase levels (Kotze & Sales 1995). Aldrin epoxidase activity was an indicator of relative monooxygenase levels in the selected and parental strains.

Larvae from all field strains had a similar response to ivermectin with LC_{50}s ranging from 0.7 to 1.2 times the CFS89 strain. These responses appeared to be independent of aldrin epoxidase levels, that were 1.3 - 4.2 times greater than CFS89. Aldrin epoxidase levels in the selected strain were higher (2.9-fold) than the CFS89 strain, indicating that selection with ivermectin resulted in increased levels of monooxygenases and these may have a role in the tolerance observed. However, the epoxidase activity in the selected strain was within the range of activities found in the field strains tested.

Discussion:

The low level of resistance induced after more than 60 generations of selection and the susceptible response of all field strains tested indicated that there was little if any ivermectin resistance in the field populations of *L. cuprina* tested. This suggests that the *L. cuprina* strain tested did not possess any avermectin-specific resistance mechanism since this would have been detected via laboratory selection. The responses found in the populations assayed agree with Hughes & Levot (1990) who assessed field populations collected prior to the commercial release of ivermectin as an anthelminthic in sheep. Thus, the susceptibility of field strains has not changed even though larvae potentially exposed to ivermectin residues since 1987.

Elevated aldrin epoxidase activity in the selected strain implicated monooxygenases in the low level resistance obtained. However, pretreatment with the monooxygenase inhibitor, PBO, resulted in only a slight decrease in tolerance to ivermectin. Also, treatment with phenobarbital apparently induced monooxygenases as evidenced by increased tolerance to diazinon, but had no effect on the toxicity of ivermectin to *L. cuprina*. Further, the field strains had widely varying levels of monooxygenase activity, but there were no apparent differences in their susceptibility to ivermectin. Thus, the observed increase in aldrin epoxidase activity may be a generalized response to the presence of a xenobiotic or to the selection method itself, and not a direct response to ivermectin. Similarly, the slight synergism of ivermectin by TPP in the selected strain could indicate some metabolism by carboxylesterases; however, the lack of any tolerance to ivermectin

in organophosphate-resistant field strains implies that the enzymes responsible for diazinon resistance are not effective in metabolizing ivermectin.

While it is not possible to ascribe a specific mechanism to the ivermectin tolerance selected in *L. cuprina* larvae, a number of factors besides enzymatic metabolism could be involved. The relatively small shift in susceptibility generated after more than 60 generations of constant selection pressure, and rapid reversion following relaxation of selection pressure are consistent with unstable, low level resistance due to multiple mechanisms typical of laboratory selection.

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Breeding Potato (*Solanum tuberosum* I.) for Resistance to Colorado Potato Beetle and Potato Tuber Moth in Italy

Introduction:

The Colorado potato beetle (CPB*) (Leptinotarsa decemlineata* Say) (Coleoptera: Chrysomelidae) represents one of the major causes of yield loss in cultivated potato in Italy. Adults and larvae feed on leaves and can quickly destroy a plant. In Italy, the potato tuber moth (PTM*) (Phthorimaea operculella* Zeller) (Lepidoptera: Gelechidae) affected the potato (*Solanum tuberosum* L.) crop during the last 6-7 years. The larvae feed on leaves and on tubers in the field and in storage. These infestations can cause yield loss between 20 and 100%.

At present, chemical insecticide applications are the main control practice in the field. In storage, low temperature can reduce the development of PTM infestation, but does not preserve the produce.

The problems and environmental risks associated with chemical control encourages the exploration of alternative control strategies including genetic (host-plant) resistance.

Sources of Resistance:

In *Solanum berthaultii*, a wild species originally from Bolivia, an effective resistance mechanism is conferred by the presence of two glandular trichomes on leaves and stems. The type A trichome, about 0.1 mm high, bears a tetralobate gland on the tip and secretes a viscous exudate that, in contact with air, hardens and darkens. The type B trichome, about 0.4 mm high, exudes a naked droplet composed mainly of sucrose esters of fatty acids from the tip.

The exudates of both trichome types entrap small arthropods, and limit movement and feeding of large insects.

Some cultivar traits, such as early maturity and deep tuberisation, can limit PTM infestation in the field. Sources of resistance in the tubers have been selected from primitive cultures and wild species at the International Potato Center at Lima, Peru (Ortiz *et al.* 1990). Evaluations in Italy with local PTM population did not produce satisfactory results (Arnone *et al.* 1993). Genetic analysis, based on the enzymatic polymorphism of adults, showed few differences normally attributed to inter-population variations in populations from Peru and Italy (Arnone *et al.* 1994).

Selection Procedures for CPB Resistance:

Tests performed on a set of 20 progeny evaluated for divergent characters such as trichome densities, enzymatic activity from exudates of trichome A (MEBA), and antibiosis on larvae were compared together with the results of field tests of antixenosis (Bacchetta & Sonnino 1994). The densities of trichomes of type A and B were not correlated. The density of type A trichomes was moderately correlated to the enzymatic activity of trichome exudates. There was a moderately negative correlation between defoliation levels in field and densities of both type A and B trichomes. The correlation between the field defoliation and MEBA was weak. The antibiosis test performed in laboratory, according to the methods of Mariani *et al.* (1987), gave weak results and did not correlate with any of the other tests.

Based on these findings, the density of trichomes appears to be the first criterion that could be used for mass selection. Since some progenies with high trichome density had a low quantity of exudates, this character should be evaluated in the advanced selections. The final validation of the resistance in the selections should be performed by challenging plants grown in the field with the pest.

Developing Varieties Resistant to Colorado Beetle:

A program aimed at incorporating the trichome-mediated insect resistance from *S. berthaultii* into the cultivated potato was initiated in 1977 at Cornell University. A tetraploid F1 progeny was produced by 4x X 2x crosses, making use of the unreduced gametes produced by the male parent. After three generations obtained by intercrossing selected progenies, back crosses to the cultivated potato were alternated with intermating of selected progenies (Kalazich 1989).

Several progenies belonging to four families and possessing 87.5% of *tuberosum* background (2 cycles of backcross to *tuberosum*), were evaluated in the field during 1988 and 1989. In the first year of the field trials, some progenies suffered less damage than the check cultivars (Bacchetta *et al.* 1989). During the second year, the 14 most resistant progenies were compared to cultivars in plots sprayed with insecticides and in plots not sprayed. The yield loss in the latter treatment was negligible for the progenies and three times higher in the check cultivars. Unfortunately, the tested progenies gave approximately half the yield of the controls.

For this reason, the best progenies were backcrossed to the most popular European potato varieties such as Spunta, Desirée, Jaerla, Nicola and Desital. The progeny performances were not very encouraging because when the yield significantly improved, trichomes were lost or drastically damaged.

A much wider population (3,916 lines belonging to 28 families) was obtained at CIP by intercrossing and backcrossing trichome material. A wide range of variability for both trichome characters and for morphological traits of plant and tubers was observed in greenhouse studies (Bacchetta *et al.* 1993). Due to the lack of correlation between trichome density and morphological traits, the number of progenies screened were reduced to one sixth the original number. The selected clones were tested for several years in the field under the presence of natural pest infestations.

Selection Procedures for PTM Resistance:

To find sources of leaf or tuber resistance to the Italian "strains" of PTM, antibiosis and antixenosis tests were carried out in the laboratory and the field. For the tuber resistance, a collection of wild *Solanum* species (*sparsipilum, spegazzini, commersonii, tarijense, berthaultii, sucrense, pinnatisectum*) were tested. For leaf resistance, the hybrids from the *S. tuberosum* x *S. berthaultii*, breeding program were evaluated. The characters evaluated included morphological (trichome densities), drop exudate levels and chemical (TLC for trichome B exudates, MEBA for trichome A exudates).

Conclusions:

S. berthaultii possesses multiple resistance mechanisms operating in complementary fashion (Yencho & Tingey 1994). The resistance mechanisms in this pest species are highly complex, very effective and potentially durable. Since each resistance mechanism is under control of different loci, the introgression of the whole resistance of *S. berthaultii* into potato background is complicated. In particular, some components of the resistance, such as the secretory activity of the type B trichomes, are difficult to transfer into cultivated potatoes, because of strong associations with poor yield, poor tuber appearance, late tuberization, etc. (Kalazich 1989). Genetic linkages, detected at the molecular level (Bonierbale *et al.* 1994), are responsible for these difficulties. Conversely, the type A trichome characteristics have been successfully transferred into lines with an acceptable horticultural performance (Plaisted *et al.* 1992). The breeding program, carried out in Italy, confirmed that some levels of resistance together with acceptable agronomic performance are selectable. Good agronomically performing clones that incorporate the full insect resistance mechanisms from *S. berthaultii* have not yet been obtained.

The presence of type B trichome enhances the resistance to CPB and provides resistance to small arthropods and fungal diseases. It is important to try to break the linkages and to introgress type B trichomes into cultivated potatoes. To facilitate this task, chromosomal alternations produced by the application of mutagenic treatments to *in vitro* growing plantlets (Sonnino *et al.* 1991) can be pursued.

S.pinnatisectum and *S. sparsipilum* lines showed a high to moderate antibiosis effect on PTM larval feeding in laboratory tests with leaves or tubers. *S. pinnatisectum* also had an antibiotic effect and both leaves and tubers exhibited high antibiosis effects. There is an opportunity to find sources of PTM resistance in potato tubers and leaves. Hybrids between *S. tuberosum* haploids x *S. sparsipilum* were obtained to attempt to transfer the resistance to cultivated potatoes.

Among the hybrids *S.tuberosum* x *S. berthaultii* and *S. berthaultii* lines, endowed with leaf glandular trichomes and a low level of tuber resistance, there was an antibiosis effect in the leaf towards the larval in the lines with a high density of type A trichomes with good exudant activity. There was also an antixenosis effect against oviposition in the laboratory, possibly due to the volatile components in the exudates of the leaf hairs, particularly type B trichomes. The variability in the response in field, the low correlation between leaf and tuber infestation, and highly mobile larvae suggests that many resistance factors combined in a unique genotype could be useful for controlling PTM infestation.

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MEETINGS/SYMPOSIA

HRAC Meeting Minutes

The second 1995 meeting of the Herbicide Resistance Action Committee (HRAC) was held on November 20 at the Royal Thistle Hotel, Brighton, England.

1. Cooperation between Groupement International des Associations Nationales de Fabricants de Produits Agrochimiques (GIFAP) and HRAC - K. Vlahodimos presented priorities for GIFAP. The HRAC provide an important technical resource for GIFAP. Key areas for cooperation with HRAC are:

- Development of a global or regional labeling plan to show herbicide modes of action
- Improved involvement of Japanese companies in HRAC
- Establishment of working groups in Latin America, SE Asia, Africa and Middle East.

2. Communications - HRAC has a new brochure entitled *Partnership in the Management of Resistance*. This will be available at Brighton and subsequent international meetings and is also on the Internet.

HRAC is looking for further ways to disseminate information and D. Nevill will look at the possibility of using Agrow, Resistant Pest Management Newsletter and Weed Technology to publish articles and minutes of meetings.

D. Nevill and D. Cornes will investigate the establishment of a WWW Homepage for HRAC.

Jim Graham will make a standard slide set available to members.

3. European Herbicide Resistance Working Groups (EHRWG) - Sam Howard (AgrEvo) will take over leadership of EHRWG from Helmut Walter (BASF).

EWRWG has three priority areas that were discussed in a workshop in Spring 1996. They are:

- Information exchange
- Standardization of testing procedures
- Practical implementation of resistance management strategies

This workshop will involve participation from academia and industry cooperators from the major European countries.

4. North America Herbicide Resistance Working Group (NAHRWG) - Dr. Tim Chicoine (DuPont) is the new chairman of NAHRWG which has been created through the fusion of the previous ALS, Triazine and Grass Herbicide Working Groups. The first meeting was held at the Weed Science Society of America (WSSA) conference on February 8, 1996.

5. Research Funding - Steve Powles (Adelaide, Australia) requested funding for two projects. A project aimed at evaluating point-mutations in ACC-ase that may confer resistance in wild oats was considered too basic and therefore outside the terms of reference of HRAC regarding funding. A second project on integrated weed management programs for resistance management of dicot weeds was funded at $ 10,000 for one year.

A proposal from Prof. Zanin (Padua, Italy) to fund a PhD project on monitoring and genetics of resistance in grasses was supported, in part. $5,000 was offered for one year to support the monitoring program.

6. HRAC Priorities - The following were discussed and set as primary goals for HRAC:

- Support the establishment and development of regional herbicide resistance working groups to implement programs for managing herbicide-resistant weeds.
- Dissemination of herbicide-resistant weed information to farmers, farm advisors, extension and others who are interested in managing weed resistance.
- Support programs to improve the management of herbicide-resistant weeds.
- Support for surveys on the occurrence and spread of herbicide-resistant weeds.
- Support programs to define the barriers which prevent farmers from adopting measures to manage resistance.
- Identify new improved solutions to specific resistant weed problems.
- Standardize techniques to identify resistant weeds.

The HRAC will provide resources for a limited number of projects that address our priorities. The intent of this funding is to demonstrate the value of these programs to grower, commodity and governmental agencies with the intent of securing longer term funding from these organizations.

7. Resistance Survey - HRAC agreed to support the WSSA resistance survey that was implemented by Ian Heap. We will meet his request for $1,800 to complete documentation for the first results of the survey and then provide a further $2,700 to support Internet availability for the first year.

8. New Chairman - Dale Shaner (Cyanamid) was elected as chairman to take over from Jim Graham in June 1996.

9. Next Meeting - Our next meeting will be on June 24, 1996 at the Sheraton Hotel, Copenhagen prior to the start of the International Weed Control Congress.

Note:
Many HRAC publications are available on our internet homepage at http://ipmwww.ncsu.edu/orgs/hrac/hrac.html.

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NAHRWG Meeting Minutes

The inaugural meeting of the North America Herbicide Resistance Working Group (NAHRWG) took place on February 8, 1996 in Norfolk, VA. Representatives from 14 different companies (Bayer, Valent, Zeneca, Nissan, Cyanamid, Ciba, Sandoz, Uniroyal, AgrEvo, FMC, DuPont, Monsanto, BASF, and Kumiai) attended the meeting.

The following topics were discussed at this meeting.

1. A short history of the pre-existing working groups was presented by various people: D. Shaner (Cyanamid) - AIRWG; F. Taylor (Cyanamid) - ACCRWG; B. Dill (Ciba) - TRWG; H. Wright (Ciba Canada) - Canadian HRAC; H. Walter (BASF) - European HRWG; and D. Shaner - HRAC.

2. J. Retzinger (Cyanamid) gave a short presentation on the classification of herbicides by mode of action that he has been working on for the past year for WSSA.

3. There was considerable discussion on the primary objectives of NAHRWG. These were decided to be as follows:

- Act as source of information for monitoring scope of resistance problem.
- Draft and review guidelines on resistance management.
- Support uniform classification of herbicides by mode of action based on HRAC/WSSA system.
- Promote proper terminology on herbicide resistance.
- Communicate positive aspects on managing resistant weeds in terms of what practices are effective based on experience in areas that have resistant weeds (e.g. triazine resistance in corn, ALS-inhibitor resistance in cereals, etc.).
- Act as the voice for industry on resistance management in North America.

4. The following people volunteered to be officers of NAHRWG:

Chairman:
Dr. Tim Chicoine - DuPont
Vice Chairman / Secretary:
Dr. Dale Loussaert - Monsanto
Treasurer:
Dr. Kevin Staska - AgrEvo

5. The next meeting will be held at the WSSA Congress in 1997.

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PUBLICATIONS AVAILABLE

Monitor

Monitor. A biannual Newsletter produced by the members of the IOBC/SEARS (International Organization for Biological Control / South and East Asian Regional Section) Working Group on IPM in Greenhouse Crops. The Working Group aims to encourage the adoption of IPM in greenhouse crops by regular exchange of information between its members (currently over 70 in 15 different countries). For more information contact Dr Stephen Goodwin, Horticultural Research and Advisory Station, PO Box 581, Gosford, NSW 2250 Australia. Phone: 61-43-481900, Fax: 61-43-481 910, Email: goodwis@agric.nsw.gov.au World Wide Web pages for the Working Group, including Monitor, can be found at http://www.dpi.qld.gov.au/iobc/wg.htm.

Weed Watch

Weed Watch is a tri-annual newsletter that highlights research from the Cooperative Research Center for Weed Management Systems. The CRC's research activities concentrate on weeds of temperate Australia and brings together expertise in areas such as herbicide technology, biological control, vegetation management, bioherbicides, population and economic modeling, decision support, weed ecology and population dynamics. There is no subscription fee and interested people can contact Mrs. Sharon Corey on via fax at 61 (6) 246 4177 or by E-mail at sharon.corey@ento.csiro.au.

Insecticide Resistance Management: Consider the Alternative

Farmers in China's cotton-growing regions are waging a fierce battle against resistant insects, most notably the bollworm (*Helicoverpa armigera*). With 30 percent of its cotton production lost to the cotton bollworm (China's cotton harvest reached 5.5m tonnes in 1991 and was reduced to 3.7m tonnes in 1993. Mainly due to losses caused by the cotton bollworm according to the Country's State Statistical Bureau), resistance has undoubtedly contributed to China's displacement as a world leader in the international cotton market.

On Long Island, New York, when resistance to pyrethroids and other synthetic pesticides raged out of control in 1991, growers spent up to $400 per acre on pesticides in attempts to control Colorado potato beetle. That year, some potato growers lost up to 50 percent of their crop to the pest.

Spider mites, bane of apple and pear producers, have a long history of developing resistance. The pests' short life cycle and rapid generation time accelerates the speed at which resistance develops. Already, more than a half dozen acaricides have been rendered ineffective by mite resistance, and only a few remain effective through strict implementation of resistance management strategies.

These tragic failures are not isolated incidences. Every major crop - cotton, rice, corn, fruits, vegetables and ornamentals - has one or more resistant pests. In total, more than 500 species of insects and other arthropods have already shown resistance to one or more classes of insecticides.

And the problem is not isolated to developing countries. Cotton producers in the United States, Australia, China, Turkey, Pakistan and India are battling resistance present in more than a dozen species of insects and mites; as are fruit growers in Europe, Canada, northwestern United States, Australia and Japan among others.

The price of insecticide resistance in lost yields and higher insect control costs is staggering - more than $1 billion in the United States from the budworm/bollworm complex alone. Once a crop protection product is rendered ineffective by resistance, it may be lost from your toolbox forever.

Facing facts:

In the past, if one class of chemicals showed resistance, a new one was sent in to save the day. The agrichemical industry's own ingenuity has contributed to growers' false sense of security.

The cold, hard truth is that we should not rely solely on industry to develop new chemistries that will take the place of what we have now. It takes from $40 to $80 million and an average of 10 years to take a new chemical product from conception to market. In the past decade, very few insecticides or acaricides with novel chemistry have been introduced.

The crop protection industry has made great strides in the development of biological pesticides in the past decades. These pesticides work better as supplemental tools to more traditional pesticides. Integrated Pest Management (IPM) and transgenic crops will play a bigger role in the future, but these and other new technologies developed will require extensive management to maintain their effectiveness. In short, chemical pesticides will be important for future crop protection, if they are still available.

Why resistance has developed:

Resistance development is simply a consequence of natural selection. A control agent (an insecticide or acaricide) prevents susceptible individual insects and mites from reproducing. A small percentage of the pest population may harbor resistance genes that allow them to survive, and these survivors pass the genes on to their offspring. The gene or genes that allow an individual pest to survive already may exist in a pest population or may arise through mutation. As crop protection products keep removing susceptible individuals, the balance of the population changes. The resistant ones continue to multiply and ultimately become predominant.
The rate at which resistance develops is determined by "selection pressure," and depends on three things: 1) the biology of the pest including reproductive rate, migration and host range, 2) the crop protection products' persistence and specificity, and 3) the intensity of product use including dose rate, number and timing of applications.

Resistance management is up to you:

Ultimately, only two players count in the struggle against insecticide resistance - the pest and the grower. The key to managing resistance is to reduce selection pressure. Consistent with IPM principles, the following five resistance guidelines can help keep our valuable protection products working effectively and help to keep grower costs down.

Consult with an agricultural advisor in your area for regional insecticide resistance and IPM strategies. Consider the pest management options available and map out a season-long plan to avoid unnecessary applications of insecticides.

Before planting - Consider the options for minimizing insecticide use by selecting early maturing varieties or varieties that are insect resistant and by managing the crop for early maturity.

In season - Regularly monitor fields to properly identify pests and natural enemies, estimate insect populations and track stage of development. Generally, insecticides and miticides should be used only if insect counts go over the local economic threshold - the point where economic losses exceed the cost of the insecticide plus application costs. Time applications against the most susceptible life stages to gain maximum benefit from the product.

Insecticide selection - When selecting crop protection products for use against specific pests, consider more than cost and effectiveness. Take into consideration:

- *Beneficial insects* -- What impact will the selected program have on existing beneficial insect populations? Maintenance of beneficials can keep pest populations below economic thresholds, thereby reducing the need for or number of applications.

- *Product class* -- Follow local recommendations for rotating or mixing products from different classes based on modes of action, not just different brands. When there are multiple applications per year, alternate products with different classes so that only one generation per year is exposed to a class. Rotate products from different classes from year to year if only one application is made to reduce selection pressure.

- *Rates and spray intervals* -- Use insecticides and acaricides at labeled rates and spray intervals. Do not reduce or increase rates from manufacturer recommendations. This can hasten resistance development. Monitor subsequent pest levels to gauge control.

- *Coverage* -- Calibrate equipment for accurate application. Use recommended spray volumes and pressures.

End of season - Remove crop residues as appropriate to eliminate food sources and overwintering habitats for pests.

If resistance is suspected:

Prevention is the best strategy; but if resistance is suspected, first eliminate other possible causes. In many instances, lack of control can be attributed to application error, equipment failure or less-than-optimal environmental conditions. If these possibilities have been eliminated, work with local agricultural advisors and the manufacturer to confirm resistance to the compound applied. In the event of a control failure due to resistance, do not respray with an insecticide of the same chemical class.

Protecting our options and profits:

With cooperation between growers, agrichemical suppliers and agricultural advisors, insect and mite resistance management can ensure continued access to valuable crop protection tools. Currently, a small area is consistently providing low-cost food and fiber for many. The loss of just a few crop protection tools could drastically change this. Resistance development can lead to increased treatments, higher cost, lower yields, additional land needs or even the inability to grow current crops. Follow resistance management strategies and encourage others to do so or costs to the producer, the environment and the consumer will increase.

About the Insecticide Resistance Action Committee:

The Insecticide Resistance Action Committee (IRAC) was formed in 1984 to provide a coordinated agrichemical industry response to the global development of resistance in insect and mite pests. IRAC has been instrumental alongside other groups in surveying product failures due to resistance, developing practical monitoring methods, publishing management guidelines and sponsoring fundamental and applied research in several countries. IRAC is now concentrating its resources on local implementation of resistance management strategies by growers, establishing the relationship between monitoring data and level of control in the field, and educating all involved in crop protection. IRAC Members: Abbott Laboratories, AgrEvo, American Cyanamid, Bayer, Inc., CIBA Crop Protection, Cotton Incorporated, DowElanco, DuPont, FMC Corporation, Gowan Company, Merck AgVet, National Cotton Council, Rhone-Poulenc, Rohm & Haas, Sandoz Agro, Inc., Uniroyal Chemical Company, Valent & Zeneca

For a copy of this brochure or more information, please contact:

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ABSTRACTS

Glutathione S-transferase activity in the maize weevil, *Sitophilus zeamais* (Motsch): Y.S. Naik, J.A. Hasler & D.P. Giga*, Department of Biochemicstry & *Department of Crop Science, University of Zimbabwe, Harare, Zimbabwe)

Abstract: Enzymes involved in pesticide detoxication include the cytochrome P-450 monooxygenase system, esterases and glutathione S-transferases (GST). These enzyme systems are responsible for insecticide resistance in many insect species. This study was aimed at characterizing the GST of the maize weevil, *Sitophilus zeamais* (Motsch), a major pest of maize in small farmers' stores in Zimbabwe's communal areas. The extensive use of grain protectants such as organophosphate insecticides (*e.g.* malathion, pirimiphos-methyl) has subjected insects to high selection pressures. Maize weevils were collected from Goromonai and Zwimba communal areas and their GST activities compared with a

strain that had been under continuous culture in the laboratory for several years. Enzyme activity was detectable with 1-chloro-2,4-dinitrobenzene (CDNB), the "global substrate" for GST and also with the 1,3-dichloronitrobenzene (GSM), a substrate for the mammalian class-μ enzyme. Km values of 0.73 mM and 0.25 mM respectively, for GSM and CDNB were found using laboratory strain. The field strains exhibited Km values ranging between 3.88 mM to 9.08 mM for GSM and 0.02 mM to 0.08 mM for CDNB.

Significantly higher Km values (CDNB) were recorded in the F_3 generation of the field strains reared in the laboratory after collection. The lower Km values of the field strains compared to those of susceptible laboratory strains indicate a higher affinity for xenobiotics such as CDNB in the field strains. Further work is presently underway in our laboratory to characterize the GST in other populations of *S. zeamais* and other insect species.

WORLD WIDE WEB ACCESS

The Resistant Pest Management (RPM) Newsletter is now available over the internet as a world-wide-web hypertext document. This version of the RPM newsletter retains most formatting elements of the hard copy version, as well as figures and tables. Hypertext links ease movement between the table of contents and individual articles. This version of the newsletter is available from the Mississippi State University web server (URL http://www.msstate.edu/Entomology/EntHome.html).

To view the newsletter, a WWW browser, such as Mosiac, Netscape, Lynx, MacWeb, or WebExplorer is required. It is not feasible in a short space to explain the use and installation of such programs (there are books dedicated to this purpose). The most commonly used program, Netscape, is available free (for educational institutions) for the Macintosh, Windows and Unix operating environments, and may be obtained by anonymous ftp from ftp.netscape.com. For those Windows 3.1 users willing to sort out the details, access to the internet over phone lines can be achieved with the trumpet winsock shareware package, available as winsock.zip from the ftp.ncsa.uiuc.eduor from sunsit.unc.edu (the directory at the latter site is pub/packages/infosystems/www/ in the socket directory. Several companies are producing all-in-one solutions for internet connections, while newer operating systems such as OS/2 V3.0 and Windows '95 have internet access built in.

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INTERNET ACCESS

Acquiring the RPM Newsletter via the Internet using FTP or e-mail.

In addition to Word Wide Web hypertext format, the electronic version of the RPM Newsletter is now available via other internet file transfer methods. If you have access to the World Wide Web (WWW), see Dr. Caprio's article "World Wide Web Access" in this issue. This is an excellent way to view the newsletter. However, if you are not fortunate enough to have direct access to the Word Wide Web you may use more traditional internet access methods to obtain a copy of RPM Newsletter files, so that you may view them on your computer.

As of this writing, the newsletter will be available from Michigan State University as follows:

File Name	File Type	Description

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There are two methods for obtaining these files: internet file transfer protocol (FTP) and electronic mail (e-mail). I must assume that you are familiar with the software of your particular system for using these methods, as there are too many variants of these programs to give accurate instructions. We can provide the necessary details for access but no description of how to use FTP or e-mail software. See your local computer whiz for help.

File Transfer Protocol (ftp):

The files listed above are accessible via our FTP host computer. The parameters for accessing this computer are as follows:

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Electronic Mail (e-mail):

If your only connection to the internet is e-mail, we are prepared to manually handle requests for RPM Newsletter files. You may send a message to us with your request and we will reply with a message containing the RPM news file you requested.

However, internet e-mail is limited to ASCII text, that is, binary files such as word processor formatted or image cannot be sent and received by e-mail directly. Hence, only the unformatted file "RPM-SP96.txt" may be sent directly. To send and receive these binary files, a coding scheme must be utilized, and the file is sent as a coded "attachment." We use the MIME standard for coding and sending non-text RPM Newsletter files. Many e-mail reading programs can translate these codes (for example Eudora by Qualcomm software, of which a free version is available at ftp.qualcomm.com). If you do not have such a program, a binary file sent to you (such as RPM-Sp96.doc) will arrive as encoded gibberish. At

that point, you must initiate the decoding process manually. Again, given the wide variety of programs and computers, please see your local computer whiz for help.

To receive a file from us, send a message as follows:

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The subject field of your e-mail message indicates which file you would like to receive. For this issue of the newsletter, the subject field should be as follows:

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A LETTER FROM THE COORDINATOR

As the Resistant Pest Management Newsletter grows, so does the cost of printing and mailing the Newsletter out. Please take a few minutes to fill out the Newsletter application. Even if the address is correct on your mailing label, please send the application back with "No changes" checked.

If you would like to submit an article to the Newsletter, the deadline for Volume 8, Number 2, will be November 8, 1996. Article submission can be done via disk (preferred) in nearly any of the popular word processing formats, or via any hard copy. Please keep your articles under 3 to 4 single-spaced (10 or 12 point) pages. Also, any graphics that go with your papers should be in black & white or gray tones since the Newsletter is not printed in color.

If you have any questions about submitting articles or anything else regarding the Newsletter, please feel free to contact me by phone at (517) 355-1768, by FAX at (517) 353-5598, or by e-mail at zieglerj@pilot.msu.edu.

Thank you all for your continued support of the Resistant Pest Management Newsletter.

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