

# Pest Resistance Management

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## Table of Contents

Editorial.....	2	Working Groups .....	12
A proposal for future directions of WRCC-66:.....	2	United Kingdom Weed Research Action Group (WRAG). 12	
<b>Features</b>		<b>Resistance Around the Globe</b>	
Behavioral of pyrethroid-susceptible and resistant <i>Heliothis virescens</i> larvae on cotton treated with insecticides .....	3	Monitoring insecticide resistance in <i>Myzus persicae</i> .....	12
Pesticide resistance management--challenges for industry .....	5	Role of Glutathione S-Transferase in methyl parathion/parathion resistance in Diamondback moth .....	13
<b>Legislative Highlights</b> .....	7	Detoxifying enzymes of selected insect species with chewing and sucking habits .....	14
Food safety, IPM and pest resistance.....	7	Research on the management of insecticide resistance pests in Taiwan.....	15
Summary of proposal H.R. 3153: The pesticide regulatory reform amendments of 1989 .....	8	Resistance management strategies for New Zealand .....	15
<b>News/Reviews</b> .....	9	Herbicide resistant weeds in Australia .....	16
ESCOPE resistance management brochure .....	9	Weed resistance to triazine herbicides in Poland.....	16
Arthropod biological control agents and pesticides .....	9	Development of resistance to <i>Bacillus thuringiensis</i> in field populations of <i>Plutella xylostella</i> in Hawaii .....	17
<b>Meetings/Symposia</b> .....	10	Resistance monitoring methods and strategies for resistance management in insect and mite pests of fruit crops .....	18
Colorado potato beetle resistance symposium.....	10	Pyrethroid resistance stability in horn flies--Kentucky .....	21
Molecular strategies for crop improvement .....	10	Insecticide resistance in Colorado potato beetles--Pennsylvania .....	22
Seventh International Congress of Pesticide Chemistry .....	10	Monitoring of pesticide resistance in pear psylla, <i>Psylla pyricola</i> , in Western Michigan.....	23
Achievements and developments in combating pesticide resistance .....	10	Ecological factors influencing distribution of O-P resistance in predaceous mites in apple.....	23
Protection of tropical crops is theme of Caribbean meeting in Puerto Rico in 1990.....	11	Change for the Australian resistance strategy .....	25
Summary of IRAC-U.S. cotton committee meeting.....	11	The nature and characteristics of herbicide resistance in Hungary.....	24
International Pest Resistance Management Congress.....	11	<b>Research Abstracts</b> .....	27
		Report to WRCC-60 on herbicide resistance.....	27
		An update of pyrethroid resistance in tobacco budworm and bollworm in Louisiana .....	32
		Enhanced metabolism and knockdown resistance in a field vs a laboratory strain of the soybean looper .....	32
		Pyrethroid resistance and the tobacco budworm .....	32

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Pyrethroid resistance in pear Psylla in Western North America .....	33	copper-resistant strains of <i>Xanthomonas campestris</i> PV <i>Vesicatoria</i> and <i>Erwinia herbicola</i> .....	40
Behavioral responses of <i>Plutella xylostella</i> populations to permethrin deposits .....	36	Variations in tolerance to benomyl among <i>colletotrichum cloeosporioides</i> isolates from Mango .....	41
Evaluating resistance to permethrin in <i>Plutella xylostella</i> populations using uniformly sized droplets .....	36	Small, conjugatable plasmid in copper-resistant strains of <i>Xanthomonas campestris</i> PV <i>Vesicatoria</i> <i>V. Dittapongpitch</i> .....	41
Fungal resistance to sterol demethylation inhibitors molecular mechanisms and baseline sensitivities .....	37	Fungicide resistance in <i>Botrytis cinerea</i> isolates from Pennsylvania greenhouses .....	41
Bioassay for resistance in pear psylla .....	37	Diagnostic media for the detection of fungi ( <i>Botrytis cinerea</i> ) resistant to vinclozolin and benomyl .....	41
Geographical and seasonal variation in pesticide resistance in cotton aphid, <i>Aphis gossypii</i> , in California cotton .....	38	WRCC-60 research progress report--Hawaii .....	42
Parasitoid resistance in California .....	38	Resistance to soil insecticides widespread in New York populations of the Colorado potato beetle .....	42
Tobacco budworm resistance update .....	39	Farmer practicable procedure for detection of soil insecticide resistance in CPB .....	43
Genetics of insecticide resistance in <i>Heliothis virescens</i> from cotton and tobacco .....	39	Susceptibility of <i>Heliothis</i> spp. to pyrethroids in Missouri during 1988 and 1989. ....	44
Cloning of the $\beta$ -tubulin gene from benomyl-sensitive and benomyl-resistant field strains of <i>Venturia inaequalis</i> .....	40	Insecticide resistance in western flower thrips in Missouri .....	44
The expression of resistance of <i>Ustilago avenae</i> to triadimenol is an induced response .....	40	Recent developments in resistance detection in <i>Anopheline</i> vectors of malaria .....	45
Baseline-sensitivity of three populations of <i>Venturia inaequalis</i> to flusilazole .....	40		
Homology between the copper resistance operon of <i>Pseudomonas syringae</i> PV <i>tomato</i> and plasmids in			
		<b>Bibliography of Resistance .....</b>	<b>46</b>

## Editorial

### A PROPOSAL FOR FUTURE DIRECTIONS OF WRCC-60: BROADENING THE OBJECTIVES OF THE COORDINATING COMMITTEE FOR SUSTAINABILITY

Since WRCC-60 was formed as a Regional USDA/ESCAP Coordinating Committee in 1985, we have almost exclusively focused on means to limit pest resistance to conventional chemical pesticides. However, it is time to broaden our perspective. Many changes have come in agriculture and environmental research, both in funding and technology. In addition, biotechnology has expanded the strategies and tactics of pest management.

With continued food surpluses, support for agricultural research has declined, but there has been some funding reallocation. Biotechnology and low-input sustainable agriculture (LISA) has received increasing support. Global ozone depletion, warming, acid rain and pollution are of heightened interest. Fortunately, funding for environmental research has not declined due to increased public awareness and demand for greater attention to this area of science. The fate of pesticides, food safety and biotechnology products are

perceived as key elements for environmental research. Integrated pest management (IPM) often leads to environmental improvement and pesticide resistance management (PRM) is a key strategy of IPM.

Both IPM and PRM research have been affected by a down turn in funding, policy and public awareness. For example, IPM researchers are concerned about implementation problems, limited interdisciplinary research, the need for systems integration and lack of management of linkages between systems. Biological control specialists to aid in IPM, have sought support from agricultural institutions with little success, despite widespread public interest. As a result of public awareness and funding, IPM workers have been focusing on environmental research and to a lesser extent plant protection and PRM. Yet there remains an increasingly critical need for maintenance research of IPM systems and expanded resources to fund PRM programs.

The development of resistant host plants containing genes cloned from a microbial biological control agents and the pending registration of genetically-improved *Bacillus thuringiensis*, has made PRM work even more critical. It has been demonstrated that these agents will be susceptible to the evolution of resistant pests (see the two B.t. resistance reports in this issue). While biotechnology will likely result in many new tools for pest control, particularly host plant resistance, their use may have environmental impacts and pests may adapt to them readily, if used unilaterally.

For the past few decades, IPM specialists have emphasized the design of pest control systems based primarily on the ecology of pests and associated species. The implementation of these more diversified systems has been slow and implementation less than satisfactory, but societal forces dictating adoption of IPM were never stronger than in 1989. We believe this trend will continue and even intensify. Yet today, most people involved in pest control are oriented toward production of new tactics or products of pest control rather than to the organisms or management systems of pest control. A case in point is the number of people involved in discovery, production and use of pesticides and engineered agents versus those studying management, deployment of IPM systems and the ecological attributes of pests.

In light of these trends, there is a need for the development and reestablishment of IPM systems that allow for sustained use of many of the older, more conventional tactics integrated with the new "engineered" agents of pest control. It is helpful that new engineered agents and plants are less degrading to the environment. Through integrated systems of pest control that increasingly focus on environmentally safe use while stabilizing the evolution of resistance through reduction of selection pressure we can sustain our production system into the twenty-first century. This approach can meet the challenges by developing effective economic pest control, while keeping pace with social demands and technological changes.

A broadened emphasis on building sustainable IPM systems for all pest management strategies and tactics is necessary today. It is also possible because many of the principles of resistance management apply to even engineered plants and biotic agents. Diversifying the genotypic and phenotypic basis of a pest control mechanism, limiting the intensity of selection, adjusting selection to match the fitness of the organism to the selection agents, managing susceptibility genes by providing refugia and matching the use of all control measures to the ecology of the target pest(s) are the common principles necessary for sustaining any pest control system. Monitoring the state of the system is also a key to adjusting tactics through timely delivery systems. The sociological process of gathering critical information and of feed back mechanism are also part of the foundation of a well conceived sustainable IPM system.

The focus of IPM should be on both the object(s) and the method(s) of pest control. Moreover, biotechnological developments emphasize the need for more sustainable systems of management around these new tactics. Such new tools take considerable time, capital and personnel resources to develop. They should be viewed much like nonrenewable resources, since finding new ones is becoming increasingly difficult and expensive. We believe that WRCC-60 can help in this process by expanding our coordination and communicational function in the following areas: 1) application of resistance management principles to the deployment of classical host plant resistance and novel genetically engineered plants and biotic agents, and 2) increasing our emphasis on the understanding of pest ecology and the linkages between pest systems.

Your comments and input on this proposal are appreciated. The *NEWSLETTER* editors will compile your responses and present them at the 1991 WRCC-60 meeting.  
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## Feature

### BEHAVIOR OF PYRETHROID-SUSCEPTIBLE AND-RESISTANT *HELIOTHIS* *VIRESCENS* LARVAE ON COTTON TREATED WITH INSECTICIDES.

#### INTRODUCTION

Resistance to pyrethroids in lepidoptera larvae is apparently due to increased rates of metabolic degradation and reduced sensitivity of the nervous system. However, an insecticide-resistant strain of insect may also exhibit different behavioral responses to a toxin than an insecticide-susceptible strain. Georgiou (1972) suggested that a decrease in irritability may be associated with an increase in ability of an insect to detoxify insecticides. Surprisingly, no research has been done to determine the way in which pyrethroid-susceptible and -resistant *Heliothis virescens* larvae behave on pyrethroid-treated cotton plants. A study conducted by us demonstrated that behavior of insecticide-susceptible larvae could be altered (i.e., they became more irritable) with residues of a synergist, chlordimeform, on the plant. Scientists have shown that the amount of pesticide pick-up depends on the behavior of the larvae (e.g. walking vs. resting). We hypothesize that diminished lethal effects of pyrethroids on pyrethroid-resistant larvae may be due, in part, to changes in behavior that result in reduced pick-up of pyrethroids by the larvae from treated plant surfaces (i.e., in addition to physiological mechanisms of resistance).

#### OBJECTIVE

To quantify the behavior of pyrethroid-susceptible and -resistant *Heliothis virescens* larvae on cotton plants that are treated with a sublethal dose of:

- A pyrethroid insecticide, cypermethrin,
- A formamidine insecticide, chlordimeform, and
- A mixture of both cypermethrin and chlordimeform.

## METHODS

### Insects:

The pyrethroid-susceptible and -resistant strains of *H. virescens* used in this study were obtained from a laboratory colony maintained at the ICI Americas, Inc. Biological Research Center at Pikeville, NC.

### Test Procedures:

Upon hatching, larvae were placed on pinto bean diet in 29.5-ml plastic cups and were held in an incubator at 27°C with 14:10 L:D cycle until they reached the third-instar. Newly enclosed third-instar larvae were then placed on cotton flowerbuds in plastic cups, and were held for 24 hr in the incubator before they were used in the study.

Before starting the test, cotton plants were individually grown in 20.0-L pots, until they began to bloom. Aqueous dilutions of each insecticide were then applied to the plants. One hr following application of the treatments (ca. two hr after sunrise), the plants from each treatment were placed in the greenhouse where larval behaviors on the plants were observed.

### Measurement of Behavior

A one-day-old third-instar larva was placed at the third mainstem intermode below the shoot terminal of each plant. Larval activities recorded were feeding (ingestion of food and/or sampling of plant surfaces with mandibles), resting, locomotion (crawling on plant surfaces), and spin-down (suspension from the plant surface by a silken thread). Plant structures recorded were shoot-terminal, square, leaf blade and stem (including leaf petiole). The number of the mainstem intermodes (i.e. counted down from the shoot-terminal) from which each plant structure arises were recorded. Activity and plant location were recorded for each larva at 15-min intervals for a maximum period of six hr, or until the larva abandoned the plant. These methods are similar to Treacy *et al.* (1987a, 1987b).

The pyrethroid, cypermethrin, was applied to plants at a dosage rate that caused ca. 10 percent larval mortality (LC10) in the susceptible strain of *H. virescens* (0.0302 µg/cm). The chlordimeform was applied at the LC15 (2.8 µg/cm) for the susceptible strain.

Each treatment was replicated 20 times. Data for frequency of occurrence of behaviors or locations were analyzed by converting numbers of observations of each behavior to percentages and then subjecting them to statistical analyses using Contrast ( $P \leq 0.05$ ).

## CONCLUSIONS

Behavior studies of *H. virescens* third-instar larvae on treated and untreated plants showed that larvae from the pyrethroid-resistant populations responded differently than the larvae from the susceptible population; and that plants

treated with different insecticides stimulated different insect behavior (Tables 1-2; Fig. 1).

Specific conclusions are that:

- Feeding on flower buds and time spent on flower buds was significantly reduced for both larval populations on chlordimeform treated compared to untreated plants.
- Resting was significantly reduced for both larval populations on chlordimeform treated compared to untreated plants.
- Locomotion on leaf blades, stems and petiole was significantly increased for both larval populations on chlordimeform treated compared to untreated plants.
- Spin-down was significantly increased for both larval populations on chlordimeform treated compared to untreated plants.
- Resistant larvae showed no significant change in behavior on cypermethrin treated plants compared to resistant larvae on untreated plants, although there was a noticeable trend for increased time spent on flower buds and reduced time on leaf blades, stems and petioles.
- Susceptible larvae on cypermethrin treated plants showed a significant reduction in feeding on flower buds, and a significant increase in time spent resting on leaf blades compared to susceptible larvae on untreated plants.
- Resistant larvae on plants treated with a mixture of cypermethrin and chlordimeform showed a significant reduction in feeding and resting on flower buds, and a significant increase in spin-down, locomotion, and time spent on leaf blades, stems and petioles compared to resistant larvae on untreated or cypermethrin treated plants.

Table 1. Time-activity budgets of pyrethroid-resistant (R) and -susceptible (S) *H. virescens* third instars on insecticide treated cotton plants.

Treatment	Insect	Time spent in activity <sup>a</sup>			
		Feeding	Resting	Loco- motion	Spin-down
Untreated	S	55a	34b	11b	0.2c
Untreated	R	59a	35b	6b	0.0c
Cypermethrin	S	28b	47a	21b	4.2bc
Cypermethrin	R	59a	32b	9	0.0c
Chlordimeform	S	21b	6cd	61a	11.6b
Chlordimeform	R	22b	5d	51a	22.5a
Cypermethrin + Chlordimeform	S	30b	16c	48a	6.1bc
Cypermethrin + Chlordimeform	R	28b	6cd	53a	13.1ab

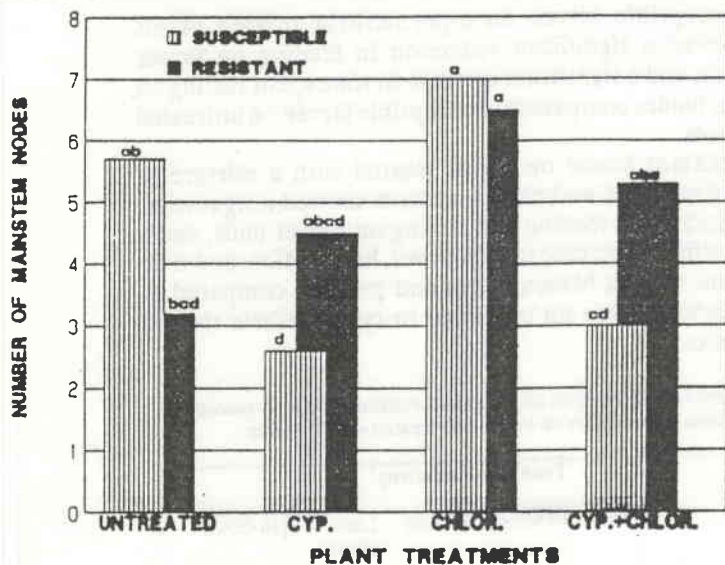
<sup>a</sup>Means within columns followed by different letters are significantly different ( $P \leq 0.05$ ) based on Contrast (SAS Institute, 1985). All analyses of variance were significant at 0.0006 or less (SAS Institute, 1985.)

Table 2. Spatial distribution patterns of pyrethroid-resistant (R) and -susceptible (S) *H. virescens* third instars on insecticide treated cotton plants.

Treatment	Insect	Time spent in activity*			
		Terminal	Flower bud	Leaf blade	Stem/petiole
Untreated	S	16a	61a	16b	6.2cd
Untreated	R	16ab	65a	12b	7.1cd
Cypermethrin	S	13abc	31b	55a	1.8d
Cypermethrin	R	17a	74a	6b	3.3d
Chlordimeform	S	7abc	24bc	52a	17.2bcd
Chlordimeform	R	9abc	15bc	56a	20.4abc
Cypermethrin + Chlordimeform	S	4c	25bc	46a	25.9ab
Cypermethrin + Chlordimeform	R	5bc	11c	48a	36.0a

\*Means within columns followed by different letters are significantly different (P 0.05) based on Contrast (SAS Institute, 1985). All analyses of variance were significant at 0.0006 or less (SAS Institute, 1985.)

FIG. 1. NUMBER OF MAINSTEM NODES TRAVERSED



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## PESTICIDE RESISTANCE MANAGEMENT--CHALLENGES FOR INDUSTRY

Many pesticides once available to agriculture are no longer available for use due to EPA cancellation or industry's decision not to support costs required for

reregistration. Currently, the cost of development for a new compound is in the neighborhood of 50 million dollars, and if a new production facility is needed for the compound, another 100 million dollars may be required. Only the largest markets would justify such a development cost. And under a constantly changing governmental regulation climate, new product development is risky business. More than anytime in their history, the agri-chemical industries are concerned about resistance causing an early demise of established products. Therefore, support for pesticide resistance management (RM) has become a priority concern within industry.

Although the concern for resistance among industry, academia and the end users is universal, there are, and will be, disagreements on how to deal with the problem. The present discussion focuses on the general problems of communication and consensus among groups both within industry, and between industry and other groups involved in pesticide RM. Also, some of the challenges that specifically face industry research in the development of new compounds are discussed.

**Industry Research -- Management.** Research in industry is conducted each year within a fixed budget. Today, more than in the past, toxicological and environmental studies required by EPA & state organizations monopolizes this budget, leaving proportionally less to spend on basic or "discretionary" research. In our ever-changing regulatory climate, these costs continue to escalate. Consequently, only when management is convinced that research for RM is essential to the life of the product and to the benefit of the customers, are they willing to commit significant funds to support such research.

**Industry Research Sales.** Traditionally, the connection between research and sales has been the product label. Sales groups promote the product in accordance with the label information which is developed by research. To be supported uniformly by Industry Sales, resistance management statements should be incorporated on the product label. Very specific statements regarding resistance are often difficult to put on the label because a particular RM strategy may apply only to a particular geographic area, insect or crop. Often a statement such as "check your local extension office for use in areas where resistance may occur" will appear on the label.

Sales groups readily promote established RM strategies, once resistance becomes a problem or is an imminent threat to their customers. But our emphasis now is shifting toward delaying or preventing resistance development. Consequently, in those areas where resistance is not a significant problem, sales must be convinced that a particular RM strategy is essential to the life of the product to give their full support in promoting that strategy. Dealing with resistance before it becomes a field problem will be a new experience for most Industry Sales groups.

**Industry Sales -- Dealer/Distributor -- Customer.** The Agri-chemical business is not a direct sell business. In many cases, the customer (farmer) gets product use information primarily from the dealer/distributor (the

middle man in the business). The dealer/distributor must be educated and ultimately convinced that a RM strategy that may adversely effect his short term bottom-line is indeed beneficial to his customers and his long-term business health. Once convinced, the dealer/distributors must then pass the information to the farmer. The farmer must be convinced because RM may require the use of alternative products that are more expensive and less efficacious, or the farmer may be required to adopt a practice that will reduce year end profits. In many cases, profit margins for farmers are thin and maximum short-term profits are necessary to remain in business.

**Company -- Company.** RM strategies normally involve groups or classes of pesticides. Success of the strategies will require cooperation from all companies who market a product in that group. Communication and consensus among the companies involved is imperative for effective action. The Insecticide Resistance Action Group Committee (IRAC), Pyrethroid Efficacy Group (PEG) and other similar industry organizations have provided a forum for this effort.

**Industry -- Government (Legislative, EPA).** Compliance to resistance management in the US is voluntary. There is no governmental group through which RM can be enforced. In most cases RM requires unanimous adoption to be truly effective, but there is no legislative way this can be enforced. A significant problem we face with some RM strategies that require the use of multiple products is the price discrepancy among the products, and the constant availability of all products involved. In some countries price and availability can be set (usually by the government) to force compliance to government supported RM. If only a single compound is involved, a company may encourage compliance through pricing or availability of that product. Current antitrust laws prohibit companies from collectively maneuvering price or availability of groups of compounds to encourage compliance.

Optimal RM strategies may conflict with environmental concerns. RM strategies that would prolong the life of one group of compounds may require an increase in the use of alternative compounds that are considered more environmentally hazardous. In another case good RM may necessitate higher rates, more frequent applications, mixtures, etc., that would contradict EPA's effort to minimize pesticide use. The risk of losing the compound to resistance may need to be balanced with the risks associated with environmental concerns.

**Industry -- Academic or Government Research and Extension.** Principally stated, optimal IPM should include good RM of pesticides. But optimal RM strategies may conflict with current IPM strategies. Consider a hypothetical example where insecticide RM dictates applications made on the egg stage because resistance to field application rates is not expressed in the egg, but current IPM dictates that beneficials often reduce egg populations below an economic threshold so applications should be made based on the presence of later (but possibly resistant) stages. Just as RM will have to be developed in the light of environmental

concerns, risks of effective RM vs current IPM when in conflict will have to be balanced.

**Industry Public.** Funding for RM research and implementation must come from both the private and public sectors. But public monies to support efforts to maintain the use of a particular pesticide may not receive much political favor due to the public's general fear of pesticides. The public, therefore, must be convinced of the safety of the compounds to be managed, and the necessity of those compounds for the welfare of the agricultural community and the general economy. This may be difficult in our chemophobic society. It is a challenge to both industry and academia to confront the risks of pesticide use on a scientific basis and educate the public accordingly.

## Resistance Management and New Compound Development

**C**urrent research by industry on established products includes tests for cross-resistance, evaluations of resistance monitoring techniques and support for monitoring resistance earlier in the life of a product. But in the light of the increasing threat resistance imposes on agriculture and agri-business, there are new challenges to be met by industry researchers involved in the development of new products. High registration costs have dictated that compounds must be developed for large or high profit markets. Traditionally, large market compounds which have simple structures (easily or cheaply produced), are broad spectrum in use and activity, have extended residual activity, and are acutely toxic to the pest species. But these characteristics (generally, but not always) make the use of the compounds more prone to the development of resistance. The cost of registering new compounds will almost certainly increase in the 1990's so the "large market" emphasis will continue to influence the type of compounds developed. To develop a pro-active approach for RM of new products industry should consider the following:

- Develop resistance risk assessment procedures for newly developed compounds.
- Determine structure - activity relationship between pesticides and pests where resistance has not occurred even after extended use of the compound. This type of information could aid in the development of "resistant-proof" or "low risk of resistance" compounds.
- Develop improved screening methods for compounds that are not directly or acutely toxic to the pest, but still provide broad spectrum plant protection.
- Further explore the use of product mixtures to prevent or delay resistance.
- Promote regular forums with university and government agencies to discuss resistance concerns and to form policies.
- Evaluate risks vs benefits (efficacy, environment, economics, etc.) for RM tactics.
- Begin the RM educational process for the product end-user concurrently with the introduction of the product into the market place.

As someone has said "Susceptibility is a natural resource that must be conserved", and I, for one, believe it is the collective responsibility of industry, academia, government and the farmer to preserve this resource. Optimal cooperation among these groups for this endeavor can occur only when each group understands the special challenges or problems confronted by the other groups.

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## Legislative Highlights:

### FOOD SAFETY, IPM AND PEST RESISTANCE

In 1988, the Congress passed the first major reauthorization and reform of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) in a decade. That legislation established an accelerated timetable for the reregistration of approximately 600 pesticide active ingredients used in the more than 40,000 pesticide products.

In 1989, the Congress once again found itself focused on making "reforms" to FIFRA, this time under the guise of "food safety." This most recent debate will continue in 1990 as the Congress sorts through several proposals designed to streamline the process for removing pesticides from the marketplace, replace the zero risk "Delaney Clause" with a more reasoned policy of "negligible risk," and reconsiders the merits of the risk/benefit standard in FIFRA.

For those whose livelihood depends on the availability of necessary crop protection tools, these changes, and their effects, should be watched closely. Many of these individual issues have merit, and on the surface are viewed as good government amendments. Most people realize that the Delaney Clause is not good public policy and should be replaced with a policy that is more pragmatic. Similarly, everyone agrees that EPA should move expeditiously to reregister older pesticides according to current data standards, and cancel those that do not meet the test of safety.

However, it is critical that careful thought be given to the cumulative effects of these and other activities on the future availability of pesticides. What is the impact of these changes on integrated pest management programs? Will it exacerbate resistance problems? How will minor crops be impacted?

To illustrate the potential problem, we need only look at some of the activities currently underway at EPA. According to Linda Fisher, Assistant Administrator for Pesticides and Toxic Substances at EPA, the reregistration requirements in FIFRA will likely result in voluntary cancellation of 15,000-20,000 pesticide use registrations. Some of these uses are

simply paper registrations for which the product was discontinued long ago. Some involve products for which there are legitimate health and safety problems that would not meet current standards. The majority, however, involve voluntary cancellations for purely economic reasons. In other words, the manufacturer simply chose not to go to the expense to generate new health, safety and environmental fate data because of the lack of adequate economic return.

Similarly, the process of applying a negligible risk standard to active ingredients suspected of being oncogenic will further reduce the options of farmers when planning their spray programs. Last year a number of the uses of the fungicide Captan were cancelled in order to bring the overall dietary risk down to a negligible level (no additional risk of cancer greater than one in a million). This year an even more widely used fungicide, the EBDC group including Mancozeb and Maneb, are being subject to the same risk reduction effort. The result is that some growers will have no viable alternatives to protect their crops. Others have seen their options significantly curtailed and will be forced to rely on only one or two products, thus increasing the likelihood of resistance problems. The problem is particularly acute for minor crops and minor uses on major crops.

Clearly there is a need for policy makers to adopt a much broader perspective on this issue and to explore innovative solutions before the problems become unmanageable. There is a tremendous lack of understanding of agriculture and its needs among policy makers. I am often asked why farmers need more than one or two fungicides, insecticides or herbicides, or why farmers cannot simply stop using chemicals. Explaining the complexities of pest management strategies as they relate to soil, climate, planting decisions, available tools, and other variables is a slow process that requires a participant who is willing to be informed.

Farm Bureau believes that there are several things that can be done to respond to this problem.

First, with regard to the problems of minor use chemicals encountered by reregistration, Congress should adopt a series of amendments with the objective of preserving those pesticide uses identified as critical to continued production of minor use crops. We have submitted the following suggestions to the Congress:

- **Voluntary Cancellation**--Before a registrants' request for voluntary cancellation can be approved, the Administrator of EPA shall notify growers through the Federal Register and allow a period of 90 days for the registrant to arrange a transfer of the registration to a willing grower group. If the transfer occurs, no further regulatory action will take place for 180 days.
- **Data Standards**--We have suggested that the Administrator be required to consult with USDA regarding data waivers for minor use crops and be required to consider the economic effects upon minor users of a failure to modify data requirements.
- **Waiver of Liability**--This amendment would permit grower groups and registrants to agree to waive liability for crop damage that might occur on crops. This could



eliminate a potential impediment for manufacturers to seek minor crop registrations.

- **USDA Authority**--Under this amendment, USDA would establish a Minor Use Registration and Support Program in the Office of the Assistant Secretary for Science and Education. This office would be authorized to gather data to support existing registrations for minor uses, seek tolerances and tolerance exemptions, and gather data to support new tolerances. It would work cooperatively with the IR-4 program.
- **Waiver of Fees**--With this amendment, the Administrator would be required to waive all fees for any minor use registration.
- **Geographically Limited Data**--This would allow the Administrator to require residue data in support of a tolerance only from those geographical areas where the registration of the products allows such use.

Second, with regard to the implementation of a negligible risk policy, we have recommended that EPA use a "cropwide approach" rather than a chemical by chemical approach. For example, by looking at all of the fungicides used on tomatoes, risk reduction can be accomplished in a systematic manner where those fungicides posing the greatest oncogenic risk can be removed and those that pose the least oncogenic risk retained. According to the National Academy of Sciences, this approach would eliminate 90 percent of the theoretical dietary risk with the least adverse impact on farmers.

In contrast, the method used by EPA now is one of reviewing all of the uses of each chemical independently, and with no particular rhyme or reason eliminating enough uses to achieve an overall risk in the negligible range. Using this approach for example, could result in all fungicides for a particular crop being eliminated, while another usually higher value crop may retain several uses.

In testimony before the Waxman subcommittee, Farm Bureau testified, "We recommend that H.R. 1725 (Waxman/Kennedy) be amended so that a cropwide approach is used to reduce risk in this second phase. EPA can then weigh the benefits of each pesticide against the other in making the risk reduction goals."

The third and final area that should be emphasized is that of Integrated Pest Management. Farm Bureau has recommended that IPM be a national priority and that Congress significantly increase funding to the \$50 million level for research, demonstration, training, and information delivery systems. We have submitted a 9 point program to the Congress for consideration.

In closing, the problems of resistance, IPM and minor crop production all have in common, a need to preserve a broad menu of chemical tools for farmers to choose from. There is at this juncture in the public policy debate an opportunity to make necessary changes that will work to ensure that those tools and options are available.

Mark A. Maslyn, Assistant Director  
National Affairs  
American Farm Bureau Federal Affairs

## SUMMARY OF PROPOSAL H.R. 3153: THE PESTICIDE REGULATORY REFORM AMENDMENTS OF 1989

This legislation proposes four major changes to current law. The central purpose is to change Section 6 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to expedite the process and adjust the standards for the cancellation, suspension, and emergency suspension of a pesticide registration. This legislation also imposes a nine-year sunset on pesticide registration, phased-in with the current process of reregistration contained in the 1988 FIFRA amendments. In addition, the legislation proposes a new category of temporary state pesticide registration to allow agricultural producers severely impacted by a pesticide suspension decision to phase out their use of a suspended pesticide. Finally, the legislation proposes that the Department of Agriculture (USDA) compile and provide the Environmental Protection Agency (EPA) with a detailed accounting of pest control measures currently available, by pest and crop, and indicate research underway to deal with shortages of pest control measures. EPA and USDA will also engage in joint development of Integrated Pest Management (IPM) systems and methods and guidelines to be used in the calculation of benefits provided by the use of pesticides.

### Cancellation - Amendment to Section 6(b) of FIFRA

EPA may propose to cancel, deny an application, or change the clarification of a pesticide by using a notice and comment process in the Federal Register. When a pesticide has "a reasonable probability of causing unreasonable adverse effects on the environment when used in accordance with its labeling or in accordance with actual practice," EPA will consult with USDA and the Department of Health and Human Services (HHS) before issuing a proposed cancellation order. The proposed order will be sent to the registrant, to the Scientific Advisory Panel (SAP), and published in the Federal Register. A 60-day comment period will be allowed. At the end of that period, EPA can propose a final cancellation order which will be effective upon publication in the Federal Register. If EPA has established conditions and terms as an alternative to cancellation, 30 days will be allowed for the registrant to take those actions or the cancellation will be final. The cancellation is reviewable in Federal Court and can be overturned if it is "arbitrary, capricious, abuse of discretion, or not in accordance with the law." The burden of proof will be on the registrant to show that the standard for cancellation is not met. EPA can allow the use of existing stocks of the pesticide after a final cancellation order and the pesticide can be marketed during the pendency of the cancellation process.

## Suspension - Amendment to Section 6(c) of FIFRA

If EPA finds that the use of a pesticide "generally causes an unreasonable adverse effect on the environment," they may suspend the registration of a pesticide. EPA shall consult with USDA and HHS prior to issuing the suspension order. EPA shall notify the registrant and publish the order in the Federal Register. The order will become effective upon publication or upon receipt of the notice by the registration, whichever is first. The suspension will expire in 180 days unless EPA moves to cancel the pesticide. The order is subject to Federal Court review and can be overturned for the same reasons as the cancellation.

## Emergency Suspension - Amendment to Section 6(d) of FIFRA

If EPA finds that the use of a pesticide is "likely to result in an imminent hazard (defined as 'a situation in which the use of a pesticide poses a significant risk to human health')" EPA can order the suspension of a pesticide without prior notification of the registrant or consultation with other federal agencies. The order will expire within 180 days unless EPA moves to cancel the pesticide. The order is subject to Federal Court review and can be overturned for the same reasons as cancellation.

## Sunset - Amendment to Section 6(a) (1)

After initial phase-in and synchronization with the ongoing Areregistration program, a pesticide registration will automatically expire on a nine-year cycle. One year prior to the registration expiration (or six months prior for a formulator), EPA will notify the registrant of the upcoming expiration of registration. The registrant will have to apply for a renewal of its registration before the nine-year period expires. The application for renewal will be denied unless the registrant, 1) makes a timely application which complies with the requirements for registration renewal, 2) agrees for delivery of information needed for renewal, including a time table for delivery, and 3) makes a good faith effort to comply with the delivery schedule.

## Continuing Registration - New Section 24(d) of FIFRA

Within 60 days of a suspension order, a State may apply for the continued use of a suspended pesticide if: 1) severe economic dislocation will result, 2) no known alternatives to the suspended pesticide are available, 3) conditions for use of the pesticide, including plans for reducing the adverse effects are provided, 4) the estimated volume of pesticide to be applied and acreage treated are provided, 5) pesticide sale and use reporting provisions are required, and 6) a listing of research into alternative pest control is provided. EPA will have 120 days to act on the State's application and may approve it as long as the

continued use does not pose any "excessive risk." The registration will last one year, with two additional renewals possible, and will expire at the end of the approved period or upon final cancellation, whichever comes first.

## Cooperation with USDA - Amends Section 28 of FIFRA

USDA will help EPA identify pests to be brought under control and identify the pest control measures available for that control. USDA will provide EPA with an annual update of this information and identify those pests for which there is concern about the limited number of control methods available, or where pest resistance has been identified. USDA will also provide a description of research and extension efforts underway to deal with the areas of concern and on alternative control methods for pesticides approved for a continuing registration under the new Section 24(d). In addition, USDA and EPA will jointly develop IPM pest control methods, and jointly develop methods for calculating the benefits coming from pesticide use. Guidelines for benefits calculations will be published in the Federal Register.

Farm Bureau

## News/Reviews

### ESCOP Resistance Management Brochure

ESCOP (Experiment Station Committee on Policy) Subcommittee on Resistance Management has printed a short brochure entitled: "Management of Resistance to Pest Control Agents: A Plan for Action". Copies are available from

Dr. E. H. Glass  
Department of Entomology  
New York State Agriculture Experiment Station  
Geneva, NY 14456

### Arthropod Biological Control Agents and Pesticides

Arthropod Biological Control Agents and Pesticides, Dr. Brian Croft, Environmental Science and Technology: A Wiley-Interscience Series of Texts and Monographs, is the most comprehensive treatment of the subject ever published. It integrates research findings from numerous fields that focus on the interaction of pesticides with entomophagous arthropods, emphasizing those

characteristics that make natural enemies unique in their responses to chemical toxins.

This volume documents the direct and indirect toxic effects of pesticides on entomophagous arthropods (among them insect predators and parasitoids), including the mode of uptake of pesticides, lethal and sublethal effects, ecological effects, selectivity, and resistance and resistance management. It discusses conservation of natural enemies through the use of pesticides in selective ways and physiologically selective pesticides.

Complete with case studies of insecticide selectivity to arthropod natural enemies over their prey or host, *Arthropod Biological Control Agents and Pesticides* is an essential reference for all entomologists and pest control managers who seek to control pests with pesticides and other chemicals.

Brian Croft and  
Environmental Science & Technology  
A Wiley-Interscience Series of Texts and Monographs

## Meetings and Symposia

### Colorado Potato Beetle Resistance Symposium

A Colorado Potato Beetle (CPB) Resistance Symposium, will be held in conjunction with the 8th Annual Pesticide Research Center Conference

April 11-12, 1990  
Michigan State University  
East Lansing, MI 48824

Invited topic presentations will be followed by discussion:

Major topics are:

- CPB field resistance monitoring and analysis
- CPB insecticide resistance mechanisms and inheritance
- CPB *Bacillus thuringiensis* endotoxin resistance selection and characterization.

Contact: Dr. Bob Hollingworth, Mark Whalon  
or Ed Grafius  
Pesticide Research Center  
Michigan State University  
East Lansing, MI 48824-1311  
FAX (517) 353-5598, Phone: (517) 353-9430

### Molecular Strategies for Crop Improvement

Molecular strategies for crop improvement is the theme of the UCLA Symposium to be held in Keystone, Colorado, 16-23 April 1990. Contact:

Secretary UCLA Symposia  
2032 Armacost Ave.  
Los Angeles, CA 90025  
U.S.A.

### SEVENTH INTERNATIONAL CONGRESS OF PESTICIDE CHEMISTRY

The Seventh International Congress of Pesticide Chemistry (IUPAC) will be held in Hamburg, Germany on August 5-10th of 1990. With this congress there will be a poster session and workshop on "Mechanisms of Tolerance and Resistance". Additional information can be obtained from the Conference Secretariat: Gesellschaft Deutscher Chemiker, Abteilung Tagungen, Varrentrappstr. 40-42, P.O. Box 90 04 40, D-6000 Frankfurt/Main 90, Federal Republic of Germany.

A. L. Devonshire  
Rothamsted Experimental Station  
Harpenden, Hert AL5 2JQ  
United Kingdom

### ACHIEVEMENTS AND DEVELOPMENTS IN COMBATING PESTICIDE RESISTANCE

A conference organized by the Pesticides Group of the Society of Chemical Industry in collaboration with the British Crop Protection Council.

This major international conference will review recent progress in the various disciplines required to understand and tackle resistance problems. It will also provide an early opportunity to examine how rapid advances in molecular biology can be integrated with established chemical and biological approaches to provide effective, environmentally-safe ways of combating resistance to pesticides.

Suggestions are invited for relevant subject matter especially in the following areas:

- Diagnostics and monitoring
- Resistance problems and management strategies

- Simulation and prediction
- Mechanisms of resistance
- Future trends

If you would like to offer suggestions and/or receive a Second Circular, please request one from:

Dr. B. P. S. Chambray  
AFRC Institute of Arable Crops Research  
Rothamsted Experimental Station  
Harpenden  
Herts, AL5 2JQ  
UNITED KINGDOM

A. L. Devonshire  
Rothamsted Experimental Station  
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UNITED KINGDOM

## Protection of Tropical Crops is theme of Caribbean meeting in Puerto Rico in 1990.

The Caribbean Division of the American Phytopathological Society is holding its 30th meeting in Mayaguez, Puerto Rico, 27-31 May 1990. Speakers include Julio Bird, on virus diseases; Charles Delp, on fungicide resistance, R. Rodriguez-Kabana and N. Acosta, on nematode management and biocontrol; Paul R. Hepperly, on disease resistance and hybrid vigor; and Karl Maramorosch, on mollicutes in tropical crops. In addition there will be paper sessions and tours. For more information, write:

Dr. Julia S. Mignucci  
Depto. de Proteccion de Cultivos  
Colegio de Ciencias Agricolas  
Recinto Universitario de Mayaguez  
P.O. Box 5000  
Mayaguez, Puerto Rico 00709-5000

## SUMMARY OF IRAC - US COTTON COMMITTEE MEETING MINUTES

The IRAC - Cotton - U.S. committee met August 23-24, 1989 in Memphis, Tennessee. Topics discussed were: 1) S. Riley from DuPont summarized the last IRAC meeting held in April 1989 in Basle, Switzerland. S. Riley has published an IRAC organization and goals article in a recent issue of *Pesticide Science*; 2) there was considerable discussion on test protocols, once resistance monitoring protocols are agreed upon, publishing them may be considered; 3) the consolidated table of activity of non-pyrethroid products will be expanded to two tables. These will include a) activity of field strains of *Heliothis* resistant to pyrethroids, and b) activity of field strains of *Heliothis* susceptible to pyrethroids. These will be updated frequently; 4) committee members were asked to question

University staff in an effort to obtain more information on wider availability and resistant factors so a list of resistant strains can be prepared; 6) the B.t. resistance group has been meeting several times each year, their aim is to have a reporting relationship direct to IRAC international; 7) more documentation is needed of specific cases of resistance to all compounds; 8) an organization paper will be presented by the IRAC - U.S. - Cotton committee at the next Beltwide Cotton meeting; 9) future meetings should consider including consultants, extension and grower groups. Discussion and review of insect and pyrethroid resistance among states attended followed.

The next meeting at Beltwide Conference is January 10, 1990.

Don V. Allemann  
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Greensboro, NC 27419

## INTERNATIONAL PEST RESISTANCE MANAGEMENT CONGRESS: WORKING GROUPS PREPARE FOR NOVEMBER 1991 MEETINGS

An international organization for the implementation of pest resistance management will meet in Washington, D. C., January, 1990. Working Group Co-chairs will start the process of developing recommendations for adoption at the 1st Congress meeting in 1991. Membership on the Working Groups and attendance at the Congress meeting will be by invitation, and the planners are now soliciting nominations, ideas, help, and financial support from a broad base.

The Congress organization will identify practical approaches to encourage and coordinate the implementation of local resistance management programs on an international scale. This will be accomplished by bringing together key members of the public and private sectors to foster institutional policy, organization, and action to promote the implementation of resistance management. An international management group will be organized to ensure proper follow-up of Congressional recommendations and continuation of coordinated activity.

The Agricultural Research Institute (ARI) has agreed to host the first INTERNATIONAL PEST RESISTANCE MANAGEMENT CONGRESS FOR IMPLEMENTATION to be held at the National Academy of Sciences in Washington, D.C. in November 1991. Support is coming from government, industry and foundations. The thirty six member Host Nation Planning Committee and co-chairs of Working Groups represent academic, environment, government, and industry interests, and will involve broad international participation. Both scientists and policy makers at senior decision-making levels will be invited to the Congress meeting.

Working Group co-chairs and the Planning Committee at ARI Headquarters in Washington, D.C. on January 9 and 10, 1989 to launch the Congress and start the selection of working group members. Co-chairs of the working group are:

**Congressional Charter:**

Dr. Keith J. Brent (Long Ashton, UK)  
Dr. Bernard C. Smale (EPA, US)

**Communications and Data Management:**

Dr. John Metcalfe (CAB International, UK)  
Dr. Stuart H. Gage (MSU, US)

**Implementation Constraints:**

Dr. Lyndon Hawkins (CA Dept. Food & Ag., US)  
Dr. Van der Graaff (UNFAO, Rome) (pending)

**Insect Resistance Management**

Dr. Raymond E. Frisbie (Texas A & M, US)  
Dr. Geoffrey J. Jackson (WB/IRAC/GIFAP, UK)

**Plant Pathogen Resistance Management**

Dr. Heinfried Laufersweiler (FRAC, Germany)  
Dr. John Northover (Ag. Canada)

**Weed Resistance Management**

Dr. Johathan Gressel (Israel)  
Dr. Homer M. LeBaron (HRAC, US)

During 1990 and 1991, Working Groups will prepare options and recommendations on specific issues to discuss and adopt by the Congress at its first meeting in November 1991.

For additional information write to:

Dr. Bernard C. Smale, General Chairman  
International Pest Resistance Management Congress  
Host Nation Planning Committee  
P.O. Box 15760  
Arlington, VA 22215-0760, U.S.A.  
FAX (703) 557-1884

## Working Groups

### UNITED KINGDOM WEED RESEARCH ACTION GROUP (WRAG)

A Weed Resistance Action Group has recently been established in the United Kingdom. This comprises representatives from government research establishments, from the advisory service (ADAS), from the Pesticide Safety Division (Ministry of Agriculture) and from the chemical industry. The main aims of the group are to:

- Provide a forum for information exchange between people actively involved in research into herbicide resistance.
- Define research needs.
- Discuss strategies to avoid resistance or to manage resistant populations.
- Discuss test methodology and agree on standards if possible.
- Agree on statements for the media.
- Maintain communications with similar groups which have been established successfully in other countries.

Newsletters will be produced and meetings held when necessary. The inaugural meeting was held on 14 September 1989 at the 11th Long Ashton International Symposium, which had the appropriate title of 'Herbicide Resistance in Weeds and Crops.'

Further information can be obtained from:

Stephen Moss  
WRAG Secretary  
Long Ashton Research Station  
Long Ashton, Bristol BS18 9AF  
United Kingdom  
FAX (0278) 394007



## Resistance Around the Globe

### MONITORING INSECTICIDE RESISTANCE IN *MYZUS PERSICAE*

Insecticide resistance in the peach-potato aphid, *Myzus persicae*, is now widespread and has led to increasing difficulties in control over the last 15 years. Resistance is conferred by elevated levels of one of two closely related carboxylesterase enzymes, E4 or FE4, which both degrade insecticidal esters by hydrolysis and neutralize them by sequestration. For convenience, aphids are broadly classified by the amount of E4 present: susceptible (S) moderately resistant (R1) very resistant (R2) and extremely resistant (Rs). There is a four-fold increase in the amount of enzyme between these variants caused by a corresponding amplification of esterase genes. Qualitative estimations of E4/FE4 content are made with electrophoresis but a rapid and sensitive immunoassay technique allows a precise quantitative analysis of a large number of individuals.

A survey of the national distribution of resistance in unsprayed populations throughout the U.K. has shown that moderate levels of resistance (R1) predominate but the more resistant types, R2 and R3, which were extremely rare

in the field a decade ago, now account for approximately 25% of the population. Consequently, multiple sprays are becoming increasingly necessary, which in turn dramatically increases the frequency of very resistant variants.

The immunoassay has been used extensively to estimate resistant levels in large numbers of single insects. However, extremely resistant (R3) aphids, which have been common in glass houses for many years, can spontaneously lose their elevated esterase levels in the absence of insecticide while retaining the associated esterase gene amplification. Consequently, the true identity of these 'revertants' cannot be determined by measurement of esterase levels alone. It has been shown that loss of resistance occurs because the amplified esterase genes are no longer expressed in revertant aphids, and such individuals can therefore be distinguished from truly susceptible aphids by DNA probing. Instability in esterase levels has only been observed in aphids which have amplified genes for the esterase E4, while those with amplified genes for FE4 show stable esterase levels. It is important to identify revertant aphids since a small proportion of their offspring have high levels of esterase which can then be reselected by insecticides. This is particularly important as the proportion of R3 aphids is increasing in field populations and consequently revertants are also likely to become more abundant.

The loss of transcription in revertant aphids correlates with low levels of DNA methylation, whereas the amplified esterase genes in resistant aphids are highly methylated. It is therefore desirable to determine not only esterase levels and degree of amplification of esterase genes but also the type of gene and the extent of methylation. This is not possible for single aphids but can be done using DNA extracted from 20 of their clonal offspring, which is digested with the restriction enzyme MspI or HpaII. These enzymes recognize the same sites (CCGG) but only MspI will cut when the internal cytosine is methylated. Thus southern blots probed with E4 cDNA give different restriction patterns for the two enzymes if the esterase DNA is methylated. Furthermore, the restriction patterns identify the type of esterase gene present (E4 or FE4) and by comparing the amount of probe binding with standards the esterase gene content can be classified as high or low. So far, approximately 50 samples have been studied from 1989 field populations, confirming that either amplified E4 or FE4 genes are present with various degrees of DNA methylation, including unmethylated amplified E4 genes accompanying low levels of E4 (i.e. revertants). Thus, a much more detailed picture of the resistance status of aphid field populations should be provided by combining the immunoassay and DNA diagnostic.

### References:

- Field, L. M. Devonshire, A. L., French-Constant, R. H. and Forde, B. G. 1989. The combined use of immunoassay and a DNA diagnostic technique to identify insecticide-resistant genotypes in the peach-potato aphid, *Myzus persicae* (Sulz.). *Pestic. Biochem. Physiol.* 34:174-178.

Field, L. M. Devonshire, A. L., French-Constant, R. H. and Forde, B. G. 1989. Changes in DNA methylation are associated with loss of insecticide resistance in the peach-potato aphid *Myzus persicae* (Sulz.). *FEBS Letts.* 2:323-327.

S. D. J. Smith, L. M. Field and A. L. Devonshire  
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## Role of Glutathione S-Transferase in Methyl Parathion/Parathion Resistance in Diamondback Moth

Except for a few chlorinated hydrocarbons that are now banned, organophosphorus (OP) insecticides have had the longest history of use in the control of diamondback moth, *Plutella xylostella*. Although the subsequently developed pyrethroids and benzoyl phenylureas showed high potency against this insect in the beginning of their introduction, OP compounds remain the group farmers can turn to when resistance to other types of compounds should occur. In an attempt to clarify the role of glutathione S-transferase in OP resistance, we measured its activities towards model substrate, 1,2-dichloro-4-nitrobenzene (DCNB), and two OP compounds, methyl parathion and parathion, in a susceptible (FS), methylparathion-selected (MPA), parathion-selected (PA), and a field (LC) strains of diamondback moth larvae. The susceptibility to methyl parathion and parathion of these strains is given in Table 1.

Glutathione S-transferase activities measured in DCNB conjugation did not vary much among the four strains of diamondback moth with ca. 400- to 2000-fold resistance to methyl parathion and parathion (Table 2). Yet, resistant strains possessed significantly higher rates of methyl parathion/parathion degradation as compared with the susceptible strain, and this degrading ability appeared to correlate with the resistance levels. Parallel work did not show any evidence of the involvement of microsomal monooxygenases or hydrolases in diamondback moth resistance to these two OP compounds.

Our data indicate clearly that glutathione S-transferase activity measured with some standard substrates does not always reveal fully the contribution of this detoxifying enzyme to OP resistance observed in insects. Degradation of OP compounds *per se* should be determined. Current findings of the close relationship between glutathione S-transferase degradation and OP resistance offers an explanation to as why farmers can turn to OP

Insecticides for the control of diamondback moth when this pest becomes resistant to pyrethroids or benzoxyphenyl

ureas. Resistance to the latter in diamondback moth has been attributed to enhanced microsomal oxidation.

**References**

- Kao, C. H., C. F. Hung and C. N. Sun. 1989. Parathion and methyl parathion resistance in diamondback moth (Lepidoptera:Plutellidae) larvae. *J. Econ. Entomol.* 82:1299-1304.
- Hung, C. F. and C. N. Sun. 1989. Microsomal monooxygenases in diamondback moth larvae resistant to fenvalerate and piperonyl butoxide. *Pestic. Biochem. Physiol.* 33:168-175.
- Lin, J. G., C. F. Hung and C. N. Sun. 1989. Teflubenzuron resistance and microsomal monooxygenases in larvae of the diamondback moth. *Pestic. Biochem. Physiol.* 35:20-25.

Table 1. Susceptibility to methyl parathion and parathion of a susceptible (FS), methyl parathion-selected (MPA), parathion-selected (PA), and a field (LC) strains of diamondback moth larvae

Strain	Methyl parathion		Parathion	
	LC <sub>50</sub> mg/ml	RR <sup>a</sup>	LC <sub>50</sub> mg/ml	RR
FS	0.023	--	0.048	--
MPA	61.1	2657	28.5	594
PA	23.9	1039	50.2	1046
LC	10.3	448	18.7	390

<sup>a</sup>Resistance ratio.

Table 2. Glutathione S-transferase activities of a susceptible (FS), methyl parathion-selected (MPA), parathion-selected (PA), and a field (LC) strains of diamondback moth larvae

Strain	DCNB <sup>a</sup>	MPA <sup>b</sup>	PA <sup>c</sup>
	nmol/min/mg protein		
FS	34.2	10.5	10.1
MPA	55.6	158	118
PA	38.3	110	139
LC	66.6	66.8	63.4

<sup>a</sup>1,2-dichloro-4-nitrobenzene.

<sup>b</sup>Methyl parathion.

<sup>c</sup>Parathion.

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**Detoxifying Enzymes of Selected Insect Species with Chewing and Sucking Habits**

Levels of major detoxifying enzymes, glutathione S-transferase, carboxylesterase and microsomal monooxygenases, have been measured in two lepidopterous species, i.e., diamondback moth (*Plutella xylostella*) and Asian corn borer (*Ostrinia furnacalis*), and five homopterous species, i.e., brown planthopper (*Nilaparvata lugens*), small brown planthopper (*Laodelphax striatellus*), rice green leafhopper (*Nephotettix cincticeps*), turnip aphid (*Hyadaphis erysimi*), and green peach aphid (*Myzus persicae*).

Different profiles of detoxifying systems were observed between the chewing and sucking insects (Table 1).

Glutathione S-transferase activities in term of 1,2-dichloro-4-nitrobenzene conjugation were generally low in the species studied, and could not be detected in the two planthoppers, *N. lugens* and *L. striatellus*. The three rice-feeders possessed much higher carboxylesterase activity than the rest. The microsomal monooxygenase activity, in term of O-demethylation of methoxyresorufin, was 50-100 fold higher for the two chewing lepidopterans than the five sucking homopterans. This fundamental difference in the makeup of detoxifying enzymes may be related to the feeding habits of these insects.

Sucking insects contact only sap in the vascular tissues of the plants. Over 90% of the materials translocated in phloem consists of water-soluble compounds, e.g., carbohydrates, amino acids, organic acids and inorganic ions. Yet, chewing insects ingest plant foliage containing large quantities of various lipophilic secondary substances. This may account for the observation that very high levels of microsomal monooxygenases, which shows a unique preference for lipophilic xenobiotics, existed in the two lepidopterous species.

The observed different detoxifying enzyme makeup may also be related to the mechanisms of insecticide resistance identified in these insects. Microsomal monooxygenase detoxication has been related to diamondback moth resistance to pyrethroids and some benzoylphenyl ureas, while this oxidative mechanism has not been found of major contribution to the insecticide resistance in the five sucking insects. On the other hand, hydrolytic degradation has been reported as the primary cause of resistance to organophosphorus, carbamate and pyrethroid insecticides in the rice hoppers and the green peach aphid.

Future development of compounds for the control of these insect pests should take this difference of detoxifying enzymes into consideration.

**Reference**

- Hung, C. F., C. H. Kao, C. C. Liu, J. G. Lin and C. N. Sun. 1990. Detoxifying enzymes of selected insect species with chewing and sucking habits. *J. Econ. Entomol.* (in press)

Table 1. Activities of glutathione *S*-transferase (GT), carboxylesterase (CE) and microsomal monooxygenase (MMO) of selected insect species

Insect	GT <sup>a</sup>	CE <sup>b</sup>	MMO <sup>c</sup>
<i>P. xylostella</i>	44	2.2	160
<i>O. furnacalis</i>	25	2.0	298
<i>N. lugens</i>	ND <sup>d</sup>	40	2.9
<i>L. striatellus</i>	ND	66	ND
<i>N. cincticeps</i>	20	20	ND
<i>H. erysimi</i>	21	0.9	2.5
<i>M. persicae</i>	15	1.8	0.7

<sup>a</sup>nmol 1,2-dichloro-4-nitrobenzene conjugated/min/mg protein.

<sup>b</sup>μmol 1-naphthyl acetate hydrolyzed/min/mg protein.

<sup>c</sup>pmol methoxyresorufin *O*-demethylated/min/mg protein.

<sup>d</sup>Non-detectable.

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## RESEARCH ON THE MANAGEMENT OF INSECTICIDE RESISTANT PESTS IN TAIWAN

After more than 30 years of insecticide usage in Taiwan, several pests have developed resistance to pesticides. Among them, the brown planthopper on rice, the diamondback moth (DBM) on cruciferous vegetables and several mites on fruit plants are notorious. The council of Agriculture decided to organize a team to manage the problem in 1989 and funded US \$1.0 million to support the DBM research project. Dr. Edward Y. Cheng, Taiwan Agricultural Research Institute, serves as the project coordinator. Nine researchers from 5 research institutes are participating in this project. The objectives are to (1) establish the homozygous resistant strains, (2) develop biochemical detection techniques for resistance, and ultimately to (3) develop an IPM system by integrating both chemicals and biologically-based control measures.

According to Dr. Cheng's previous studies and field monitoring, the DBM, rooted from the mixed function oxidases (MFOs), has developed resistance to carbamates, synthetic pyrethroids and newly introduced insect growth regulators registered so far in Taiwan. Resistance can reach from hundreds to a thousand fold. Separate MFOs are involved in different groups of insecticide and no cross resistance among them has been detected. However, the resistance ratio for organophosphorous insecticides is usually in the range of 5 to less than one hundred fold and is multi-factorial. Qualitative differences in esterase activity,

enhancement of carboxylesterase, decrease in AchE sensitivity and higher glutathione-s-transferase activity are also involved. Three types of OP resistance according to their stability have been characterized for different OP insecticides. Compounds such as cartap causing only unstable resistance still can be used to control the DBM on an alternated basis. So far no evidence of resistance to *Bacillus thuringiensis* has been reported.

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## RESISTANCE MANAGEMENT STRATEGIES FOR NEW ZEALAND

In April 1987 the New Zealand committee on Pesticide Resistance (NZCPR) was formed to address pressing problems on pesticide resistance, particularly among pests of horticultural crops. Rapid growth in the horticultural sector has been accompanied by increased use of pesticides as producers and exporters strive to meet the quarantine requirements of importing countries. However, because there is a wide variation in the acceptability of different residue in different markets, New Zealand producers are increasingly dependent on a narrow range of pesticides that are acceptable in all export markets. Therefore selection pressure on some pests by certain pesticides is very high.

Currently the NZCPR has two Task Groups (Insecticides and Fungicides) with representatives from the agricultural industry, government agencies and universities (Elliott et al. 1987). The function of these groups is to pool relevant information on resistance, recommend monitoring methods, verify reports of resistance, encourage resistance research, identify pests and pesticides subject to high risk of resistance and develop resistance management strategies aimed at prolonging the useful life of pesticides.

Resistance management strategies are reported annually at the New Zealand Weed and Pest Control Society conferences and published in the conference proceedings. To date, resistance management strategies have been developed for:

- dicarboximide fungicides (Elliott et al. 1988)
- phenylamide fungicides (Elliott et al. 1988)
- demethylation inhibitor fungicides (Prince et al. 1989)
- spider mites (Prince et al. 1989)
- leafrollers (Prince et al. 1989)

Further strategies are being developed for greenhouse whitefly, green peach aphid and diamondback moth. Further information can be obtained from the author.



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## HERBICIDE RESISTANT WEEDS IN AUSTRALIA

This review considers the development of herbicide resistant weed biotypes in Australia. Biotypes of the important annual weed species, capeweed, wall barley, and hare barley are resistant to the bipyridylum herbicides paraquat and diquat. These resistant biotypes developed on a small number of alfalfa fields that have a long history of paraquat/diquat use within a distinct geographical area in central western Victoria. The resistant biotypes are controlled by alternative herbicides and pose little practical concern. Some populations of wild oat are resistant to diclofop-methyl. Of greatest concern is the development of cross resistance in biotypes of annual ryegrass to aryloxyphenoxypropionate, cyclohexanedione, sulfonylurea, and dinitroaniline herbicides. The cross resistant annual ryegrass infests crops and pastures at widely divergent locales throughout the cropping zones of southern Australia. The options for control of cross resistant annual ryegrass by herbicides are limited. A biotype of annual ryegrass on railway tracks treated for 10 yrs with an amitrole-atrazine mixture has resistance to amitrole and atrazine and other triazine, triazinone and phenylurea herbicides. Management tactics for cross resistance are discussed. Nomenclature: amitrole, 1 H-1,2,4-triazol-3-amine; atrazine, 6-chloro-N-ethyl-N<sup>o</sup>-(1-methylethyl)-1,3,5-triazine-2,4-diamine; diclofop, (+)-2-[4-(2,4-dichlorophenoxy)phenoxy] propanoic acid; diquat, 6,7-dihydrodipyrido[1,2-@:2', 1"-c] pyrazinedium ion; paraquat, 1,1"-dimethyl-4,4'-bipyridinium ion; annual ryegrass, *Lolium rigidum*; Gaud.#3; capeweed, *Arctotheca calendula*; (L.) Levyns. #; hare barley, *Hordeum leporinum*; Link. # HORLE; wall barley *Hordeum glaucum*; Steud. # HORMC; wild oat, *Avena fatua* L #AVEFA. Additional index words. Cross resistance; *Arctotheca calendula*; *Avena fatua*; *Hordeum glaucum*; *Hordeum leporinum*; *Lolium rigidum*.

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<sup>3</sup>Letters following this symposia are a WSSA-approved computer code from Composite List of Weeds, Weed Sci. 32. Suppl. 2. Available from WSSA, 309 Clark St., Champaign, IL 61820.

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## WEED RESISTANCE TO TRIAZINE HERBICIDES IN POLAND

Triazine herbicides, mainly simazine and atrazine, have been used in commercial orchards, bush fruit plantations and fruit tree nurseries in Poland since the early 60s. First report on the appearance of weeds resistant to these herbicides has been published by Lipecki and Stanek (1983) concerning *Erigeron canadensis* L. and Gawronski and Lipinska (1984) concerning *Echinochloa crus-galli* (L.) P.B. Recent publications dealing with this problem pointed out that the resistance has also appeared inside the species *Chenopodium album* L. and *Amaranthus retroflexus* L. in maize plantations in western Poland (Rola 1988) and in *Capsella bursa-pastoris* (L.) Med, in orchards in eastern Poland (Lipecki 1988). These data come out from the field and pot experiments and the resistance of weeds has not been checked by physical methods. However, the fact that *Capsella bursa-pastoris* (L.) Med plants survived the use of 10 kg ha<sup>-1</sup> of active ingredient of simazine in pot experiments points out that they are resistant, in enzymatic or chloroplastic way.

Observations carried out in commercial orchards in eastern Poland in the years 1984-1989 showed the presence of many other species of weeds in herbicide strips, part of which is probably also resistant to triazine herbicides, as it was shown in other countries (LeBaron 1988). Most frequent were (in alphabetical order): *Atriplex patulum* L., *Chenopodium album* L., *Digitaria sanguinalis* (L.) Scop., *Epilobium* sp (probably *ciliatum* L.), *Echinochloa crus-galli* (L.) P.B., *Poa annua* L., *Senecio vulgaris* L., *Setaria glauca* L. and *Stellaria media* Vill. The occurrence of these plants in triazine-treated areas was confirmed in many experiments done in other parts of Poland, according to private information.

The occurrence of species in which the resistance to triazines has not been found up to now was also observed in some orchards with *Erodium cicutarium* (L.) L'Herit, *Viola arvensis* Murr., *Geranium pisillum* L. and *Lamium purpureum* L. being the most common. Their occurrence in

herbicide strips seems to prove that they achieved some level of resistance to triazines and that further selection in this direction is possible. Especially sharp increases was observed in frequency of *Erodium cicutarium* (L.) L'Herit in 1989.

Some changes in the occurrence of weeds in orchards were observed in the years 1981-1989. examples are given in Tables 1 and 2.

Table 1. The percentage of orchards in which the species of weeds were observed in the years 1984-1989 (commercial apple orchards, eastern Poland).

Species	1984	1985	1986	1987	1988	1989
<i>C. bursa-pastoris</i>	25	36	39	73	79	90
<i>E. canadensis</i>	65	86	72	73	79	40
<i>D. sanguinalis</i>	25	0	44	36	50	70
<i>E. crus-galli</i>	75	71	89	100	93	100

Table 2. The percentage of the interrow covered by some weeds (experimental) apple orchards. AES Felin near Lublin over-all herbicides)

Species	1981	1982	1983	1984	1985	1986	1987	1988	1989
<i>A. retroflexus</i>	0.2	1.1	6.7	12.1	3.5	0.9	7.4	4.2	10.7
<i>C. bursa-pastoris</i>	1.7	2.2	0.3	3.8	0.2	0.0	0.0	2.5	8.3
<i>E. canadensis</i>	0.0	0.5	0.7	7.4	58.6	73.2	36.7	24.7	6.4
<i>E. crus-galli</i>	5.6	7.1	7.6	9.1	0.9	0.4	1.0	2.5	12.7

Preliminary attempts made in the last two years showed that biomass and probably also seed production of the resistant biotypes of several weeds increased under high nitrogen fertilization, shadow and lowered pH of the soil to a higher degree than the sensitive forms. This would explain why they become so popular in herbicide strips in orchards where they find favorable conditions.

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## DEVELOPMENT OF RESISTANCE TO *BACILLUS THURINGIENSIS* IN FIELD POPULATIONS OF *PLUTELLA XYLOSTELLA* IN HAWAII

Some field populations of diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), in Hawaii have developed resistance to Dipel, a commercial formulation of the HD-1 strain of *Bacillus thuringiensis* subsp. *kurstaki* (*Bt*). Leaf residue bioassays showed that the LC<sub>50</sub>'s of late instar larvae from two farms that had been treated repeatedly with *Bt* were significantly higher than the LC<sub>50</sub>'s of two susceptible laboratory strains and several minimally treated field populations.

LC<sub>50</sub>'s (all stated in mg [AI]/liter with 95% FL) of the two heavily treated populations were 63.9 (46.1 - 89.0) and 24.1 (17.7 - 32.3). LC<sub>50</sub>'s of the two laboratory strains ranged from 1.76 (1.05 - 2.89) to 2.57 (1.48 - 4.28). LC<sub>50</sub>'s of six minimally treated field populations ranged from 1.56 (0.89 - 2.57) to 11.9 (7.16 - 20.0). The LC<sub>50</sub> of the most resistant population was 36 times greater than the LC<sub>50</sub> of the most susceptible laboratory strain and 41 times greater than the LC<sub>50</sub> of the most susceptible field strain.

At a concentration of 25.6 mg [AI] per liter, which is comparable to the recommended field application rate, mortality at 48 hours after treatment was 90-100% in the laboratory strains, 60-90% in the minimally treated field populations, and only 34-35% in the two resistant field populations.

We tested one of the heavily treated populations for resistance in 1986 and then again in 1989. During the interval between tests, this population had been treated 15 times with Javelin, a commercial formulation of the NRD-12 strain of *Bt*. Bioassays showed a significant increase in resistance during this interval. The LC<sub>50</sub> in 1986 was 10.2 (5.70 - 16.9) compared with 24.1 (17.7 - 32.3) in 1989. Similar tests showed no increase in resistance between 1986 and 1989 in two untreated laboratory strains and a minimally treated field population.

Our results suggest that repeated field applications of *Bt* caused resistance to *Bt* in field populations of diamondback moth. If relatively transient foliar applications of *Bt* can cause resistance development in pests, as our data suggest, then persistent production of *Bt* in genetically engineered crop cultivars may select intensely for *Bt* resistance.

We think that concerns about the potential for development of resistance to *Bt*, as expressed previously in this newsletter and elsewhere, are well-founded. Applications of concepts from resistance management and integrated pest management may help to prolong the efficacy of *Bt* and other biopesticides

**Reference**

Tabashnik, B. E., N. L. Cushing, N. Finson and M. W. Johnson. 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.*: in press.  
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**RESISTANCE MONITORING  
 METHODS AND STRATEGIES FOR  
 RESISTANCE MANAGEMENT IN  
 INSECT AND MITE PESTS OF FRUIT  
 CROPS**

**ABSTRACT**

On the basis of a worldwide survey, the Fruit Crops Working Group of the Insecticide Resistance Action Committee (IRAC) has identified the major resistance problems in pests of fruit crops.

Recommended resistance monitoring methods have been developed for *Panonychus ulmi* and *Tetranychus* spp. (eggs and adults), *Psylla* spp. and *Myzus persicae*.

The merits of various resistance management strategies are discussed and a provisional approach to resistance management in spider mites on deciduous fruit crops is proposed.

**INTRODUCTION**

The establishment of the Insecticide Resistance Action Committee (IRAC) under the umbrella of the International Group of National Associations of Agrochemical Manufacturers (GIFAP) was described by Voss (1987). IRAC's task is to provide expert advice to GIFAP on all technical and scientific matters relating to insecticide and acaricide resistance, to coordinate industry's efforts to prolong the life of pesticides by defining appropriate technical strategies and to develop research relationships with non-industrial institutions.

IRAC has established a number of working groups based on crops or problems and this paper describes the progress made by the Fruit Crops Working Group since it was set up in 1985.

IRAC Fruit Crops Working Group.- members, 1988:

- R. W. Lemon, Schering Agrochemicals Limited (Chairman)
- C. Erdelen, Bayer AG
- A. St. J. Green, Merck Sharp & Dohme Research Laboratories
- A. C. Grosscurt, Duphar B.V.
- P. K. Leonard, Dow Chemical Company Limited

H. P. Streibert Ciba-Geigy AG  
 J. Tipton Shell International Chemical Company Limited  
 A. Waltersdorfer Hoechst AG

In order to set Priorities for future work, in 1985, IRAC initiated an extensive survey of resistance problems through its own member associations and companies. The results of this survey were analyzed by the Working Group and published by Voss (1988). The analysis was based on IRAC's definition of field resistance. For the term "resistant" to be applied, the following criteria must be met:

- The product for which resistance is being claimed carries a use recommendation against the particular pest mentioned, and has a history of successful performance.
- Product failure is not a consequence of incorrect storage, dilution or application, and is not due to unusual climatic or environmental conditions.
- The recommended dosages fail to suppress the pest population below the level of economic threshold.
- Failure in control is due to a heritable change in susceptibility of the pest population to the product.

The perceived problems were divided into three categories.

In the first category were grouped those cases where resistance rendered chemical control difficult or uneconomic in a number of countries. These were cases where involvement by industry had become essential.:

Pest	Crop	Resistance to Chemical class	Territories
<i>M. persicae</i>	peaches	OP's carbamates	Worldwide France, Italy Portugal, Australia
<i>Psyllia</i> spp.	pears	OP's, pyrethroids	Europe N.America
<i>A. aurantii</i>	citrus	OP's	Greece, mid-East S.Africa
<i>P. ulmi</i> & <i>Tetranychus</i> spp.	top fruit	various	Worldwide
<i>P. citri</i>	citrus	various	U.S.A, Japan, Italy

In the second category were those cases which have the potential of becoming more serious. Careful observation and initiation of monitoring programmes was recommended.

Pest	Crop	Resistance to Chemical Class	Territories
<i>E. lanigerum</i>	apples	OP's	Spain
<i>P. humuli</i>	hops	OP's carbamates	Europe
<i>C. pomonella</i>	pome fruit	OP's	Argentina
<i>L. scitella</i>	pome fruit	OP's	Italy
		benzoylureas	
<i>L. blancardella</i>	pome fruit	OP's	Greece
		pyrethroids	U.S.A.
<i>S. pilleriana</i>	grapes	OP's	Spain
<i>B. phoenicis</i>	citrus	various	Brazil
<i>E. carpini</i>	grapes	various	France
<i>D. theobroma</i>	cocoa	chlorinated	Ghana
<i>S. Singularis</i>		hydrocarbons	

In the third category were cases considered to be of low priority at this time, which will remain in IRAC's database but will not lead to action in the foreseeable future.

The problems identified in category 1 were ranked by the Fruit Crops Working Group into the following order of priority for development of monitoring methods and recommendations for resistance management.

- *Panonychus ulmi/Tetranychus* spp. - deciduous fruit
- *Psylla* spp. - pears
- *Myzus persicae* - peaches
- *Panonychus citri* - citrus
- *Aonidiella aurantii* - citrus

## MONITORING METHODS

An effective susceptibility monitoring programme to obtain baseline data and to detect early signs of resistance in field populations of insects and mites is an important component of any resistance management strategy.

Many companies undertake resistance monitoring programmes using their own test methods but standardization of these methods is seen as an important step in a cooperative approach to resistance management, particularly where different companies as well as non-industrial institutes are working with the same class of compound.

During the past three years, members of the IRAC Fruit Crops Working Group, in consultation with non-industry experts have developed and validated simple but reliable proposed methods for the following species:

- *Panonychus ulmi* and *Tetranychus* spp. eggs and adults
- *Psylla* spp. - nymphs
- *Myzus persicae* - adults

The methods are designed to be used by field personnel without sophisticated laboratory facilities and to simulate the field treatment conditions as closely as possible.

Descriptions of the methods are now available from GIFAP. It is emphasized that the methods have been validated for specific compounds or classes of compounds only and modifications may be required for compounds with different modes of action.

The following is a brief summary of each of the methods currently available.

### Spider mite adults

Slide-dip methods as recommended by FAO (Anon 1974) have frequently been used for spider mite resistance tests. The disadvantages of this type of test compared with residual bioassays were demonstrated by Dennehy, *et al* (1983).

The method adopted by IRAC is a whole leaf residual contact assay based on that described by Welty, *et al* (1987) in work on cyhexatin resistance in *P. ulmi*.

Apple or plum leaves are dipped for five seconds in selected dilutions of the test formulation and then placed top surface uppermost on a layer of moist cotton wool in a 9cm open petri dish. A strip of damp cotton wool 1 cm. in width is laid around the perimeter of the treated leaf, half over the leaf and half over the cotton wool bed.

Ten adult female mites are then placed on the surface of the treated leaf. After a recommended exposure period, the mortality is assessed using a binocular microscope or hand lens.

The method has been validated for bromopropylate, cyhexatin, dicofol, formetanate and propargite.

### Summer eggs of *P. ulmi* and eggs of *Tetranychis* spp.

The method adopted is similar to that recommended by FAO and described in Anonymous (1974). Sections of plum or apple leaf are placed top surface uppermost on a sheet of moist filter paper on moist cotton wool in open petri dishes. Ten-fifteen adult female mites collected from the field are placed on each leaf section and maintained at a minimum temperature of 20C., minimum photo period 16 hours and a high light intensity, but not in direct sunlight.

After a maximum of 48 hours, when sufficient eggs have been laid, the mites are removed. The leaf sections with eggs are then dipped in the test liquids for five seconds. The leaf sections are returned to the petri dishes and maintained in the conditions described above until hatch can be recorded.

The method has been validated for clofentezine, hexythiazox and tetradifon.

### Winter eggs of *P. ulmi*

Short pieces of twig bearing eggs are taken from the field. The twigs are split into two longitudinally and sections bearing a minimum of 25 eggs are dipped into the test liquids for five seconds. When dry, the twig sections are

placed on a film of petroleum jelly in a petri dish and egg numbers are counted. The dishes with lids replaced are stored outside but protected from rain and direct sunlight. When egg hatch is complete, numbers of hatched larvae are recorded.

The method has been validated for clofentezine and hexythiazox.

### Pear psylla

Shoots infested with immature stages are collected from the field. The best time is when 1st and 2nd instar nymphs of the second generation are present. It is important to treat before much honeydew is produced.

The shoots are placed in water and the number of live nymphs recorded. The shoots are dipped for ten seconds in the test liquid and then kept at room temperature for 24 hours before assessing numbers of surviving nymphs.

The method has been validated for organophosphates and amitraz.

### Myzus persicae

Uninfested peach tree leaves are dipped into the test liquids for ten seconds, allowed to dry and then placed lower surface uppermost individually in petri dishes. A small piece of damp cotton wool is placed around the petiole of each leaf. Each leaf is infested with 20 adult aphids collected from the field. Mortality is assessed after 24 hours by checking the aphids ability to show coordinated movement in response to a touch with a small brush.

The method has been validated for organophosphates and carbamates.

In addition to the conventional monitoring methods described above, biochemical methods are being considered where they can be conveniently used under the conditions described above.

## **STRATEGIES FOR RESISTANCE MANAGEMENT**

The ultimate objective of all IRAC Working Groups is to agree and recommend strategies aimed at preventing or delaying the onset of resistance in the field and the management of resistance where it already exists.

Ideally, such strategies should be based on an understanding of the resistance mechanisms involved and the inheritance of these mechanisms. However, such studies take time and when a product is first introduced, the company can only assess the risk of resistance and has to decide whether to recommend the compound in a way that will reduce that risk to a minimum.

Similarly, when resistance first occurs in the field, the manufacturer does not have time for detailed investigations before taking action in an attempt to manage the situation.

The first priority of the Fruit Crops Working Group of IRAC was to develop a recommended strategy for spider mite control in deciduous fruit, where there is a long history of resistance problems.

It was agreed that the strategy adopted should be based on consideration of all methods available for control of the

pest and the use of these methods in the best possible way to minimize the risk of resistance.

Chemical methods include the use of a variety of products, e.g. organotin, propargite, amitraz, dicofol, bromopropylate, flubenzimine, pyrethroids, tetradifon, clofentezine and hexythiazox and biological methods, the use of predatory mites (including OP-resistant *Typhlodromus*) and insects.

The published literature together with strategies implemented by the Fungicides Resistance Action Committee and by the Pyrethroid Efficacy Group were reviewed and the Fruit Crops Working Group concluded that the options available for spider mite resistance management were as follows:

- Use of mixtures of acaricides subject to different resistance mechanisms.
- Alternation/rotation of acaricides
- Moderation of use:
  - Reduced rates (in conjunction with biological control)
  - Less frequent application (linked with more use of threshold numbers and improved scouting)
  - Localized treatments

Mixtures applied as co-formulations, are from the company's point of view, easier to control than alternations/rotations. However, in addition to being subject to different resistance mechanisms, ideally the components of a mixture should have equal residual activity which can seldom be achieved (Curtis 1985). They should act on the same stage in the life cycle and in order to gain the full benefit they should be used at full rates which is seldom economic.

Furthermore, the build-up of resistance to one component of the mixture may be masked by the activity of the other component until it reaches a high level and is then more difficult to manage.

Rotation was therefore selected as the basis of the recommended strategy, but clearly compounds used in rotation like those in mixtures should not be subject to the same resistance mechanisms.

The acaricides available were therefore grouped according to known or expected cross-resistance patterns, although it was accepted that knowledge of cross-resistance patterns was incomplete and considerable research would be required to clarify the situation. The provisional list is as follows. As knowledge improves this will be revised.

- Group A Organotins (Edge & James, 1983) (Balevski, 1983)
- Group B Clofentezine, hexythiazox (Gough, 1987\*)
- Group C Bridged diphenyl compounds
- Group D Pyrethroids
- Group E Flubenzimine
- Group F Tetradifon
- Group G Amitraz
- Group H Propargite
- Group I Quinomethionate
- Group J Benzoximate
- Group K Dinobuton

\*Case referred to was on roses.

The following guidelines in the use of acaricides are based on the above groups:

- Not more than one compound from any one group should be applied to the same crop in the same season.
- Any one compound should be used only once per season on any one crop.\*\*
- Compounds from the same group must not be mixed.
- Compounds should be used in such a way that detrimental effects on predatory insects and mites are minimized.
- Use compounds only at manufacturer's recommended rates and timings.
- Monitoring should be conducted to detect early signs of resistance.

\*\*Because of specific activity against certain life stages, some compounds may be recommended for two successive applications to provide effective control.

Agreement on a proposed strategy is only the beginning. Implementation of that strategy will not be easy. It will require not only cooperation between the agrochemical companies but cooperation with advisers/extension personnel and most importantly, the growers themselves. The ways in which this will be achieved will be the subject of discussion at future meetings of the Working Group.

### PROPOSALS FOR FURTHER WORK

Work to establish cross-resistance patterns in spider mites will be funded by IRAC. A decision on where to place this project has not yet been made.

A high priority will be given to the implementation of the resistance management strategy for spider mite control in top fruit.

Monitoring methods will be developed for *Panonychus citri* and *Leucophaea scitella* but in view of a reduction in the use of broad-spectrum OP's on citrus, work on a method for *Aonidiella aurantii* has been postponed.

Resistance management strategies will be developed for pear psylla and for *Myzus persicae* control on peaches based on the same principles as those used in the recommendations for spider mite control.

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## PYRETHROID RESISTANCE STABILITY IN HORN FLIES--KENTUCKY

Pyrethroid resistant horn flies (an obligate blood feeding pest of cattle) were reared on individually stanchioned holstein bulls in separate screened indoor stalls during 1988. This study followed the stability of resistance to permethrin in the absence of additional insecticide selection, with and without susceptible horn fly immigration. The parental resistant population was composed of 5% RS and 95% RR individuals and was ca. 42-fold for the duration of the study (4 generations). One influx of susceptible horn flies at a ratio of 1:10 (resistant:susceptible) into another stall reduced the resistance ratio to 3-fold at the LC<sub>50</sub> level during the F1 generation. Although a drastic reduction in the LC<sub>50</sub> level occurred, the population was still composed of 32% RS and 26% RR individuals with the remainder being homozygous susceptible. Permethrin resistance ratios during the 4 generation periods ranged from 3 to 6.2-fold and never approached susceptible levels.

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## INSECTICIDE RESISTANCE IN COLORADO POTATO BEETLE:--PENNSYLVANIA

Insecticides provide the only economical method of controlling the Colorado potato beetle (CPB) in Pennsylvania. However, many insecticides are ineffective due to insect resistance or have variable effectiveness against CPB. To optimize and preserve the currently registered insecticides strategies must be developed that maintain insecticide efficacy. The limited efficacy data on available chemicals throughout Pennsylvania and improper timing of applications also diminishes our ability to control CPB. Thus, the degree of insecticide resistance of insecticides registered for CPB in Pennsylvania was characterized, and the influence of insecticide application strategies on development of resistance was studied.

CPB adults were collected from 12 counties in Pennsylvania during 1987-88 for characterization. Considerable variation was found within and between counties. The resistance ration (highest:lowest LD<sub>50</sub> found for each insecticide tested throughout the state) was 256 for Ambush, 41 for Asana, 1,985 for Pydrin, 85.4 for Thiodan, and 262 for Guthion. The LD<sub>50</sub>s for Sevin and Furadan were, 318 and 198 respectively, for all counties.

Two application strategies were tested. Sequential treatment of CPB with Pydrin for five generations, and rotating treatment, of which each generation was treated with a different class of insecticide. The first generation of CPB was treated with Pydrin (synthetic pyrethroid), the second with Guthion (organophosphorus), the third with Furadan (carbamate), the fourth with Thiodan (chlorinated hydrocarbon), and the 5th with Pydrin. A control was also included to which the population was not subjected to insecticide pressure. Additional CPB from the 1st and the 5th generations of each application strategy were treated with Guthion, Furadan and Thiodan for comparison purpose. The tests were performed on laboratory reared adults.

Results of the sequential applications of Pydrin for five generations are contained in Figure 1. A 2.5 fold increase in the LD<sub>50</sub> was recorded by the 2nd generation, and approximately 30-fold increase for the 3rd and 4th generations. The LD<sub>50</sub> by the 5th generations was 65 times greater than that of the 1st generation. Additional dose response tests on the 6th and the 8th generations resulted in LD<sub>50</sub>s 177.5 and 1700 times greater than that of the 1st generation. The Pydrin LD<sub>50</sub> for the Rock Spring field population from 1987 to 1989 varied from 0.021 to 0.09. No changes occurred for the Furadan LD<sub>50</sub> between the 1st and 5th generations. The LD<sub>50</sub>s for the Guthion decreased from 23.7 to 13 between the 1st to the 5th generation. While the LD<sub>50</sub>s for the Thiodan increased from 5.33 to 7.67.

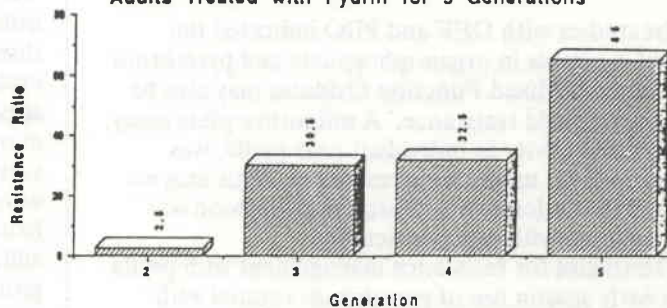
The Pydrin LD<sub>50</sub>s for the 1st and 5th generations of CPB subjected to a rotating insecticide regime were 0.02 and 0.04, respectively. The LD<sub>50</sub>s for Guthion were 23.7 for the 1st

generation and 16.3 for the 5th generation. No differences were detected for Furadan between the 1st and the 5th generations, where as Thiodan increased from 5.33 to 16.5.

There was little change in LD<sub>50</sub>s for the untreated CPB population. No changes were found for Furadan between the 1st and 5th generations. Pydrin increased 2.5 fold from the 1st to the 5th generation, while Thiodan and Guthion decreased slightly.

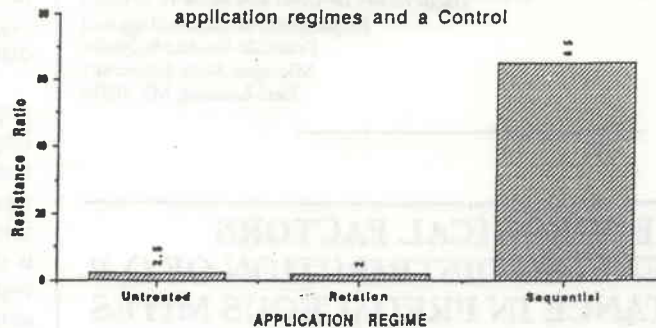
In conclusion, CPB under insecticide selection pressure develops resistance rapidly. In this study the resistant ratio for CPB treated with Pydrin for 5 generations increased 65 folds, while CPB treated with rotating classes of insecticides and no insecticide increased 2 and 2.5 folds, respectively (Figure 2). Rotating classes of insecticides appear to slow the development of insecticide resistance in CPB.

Figure 1. Increase In Resistance Ratios\* for CPB Adults Treated with Pydrin for 5 Generations



\* Ratio = LD50 of each generation / 1st generation

Figure 2. Resistance Ratios\* for Pydrin LD50s to CPB Adults After 5 Generations for 2 application regimes and a Control



\* Ratio=LD50 of 5th generation / 1st generation

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## MONITORING OF PESTICIDE RESISTANCE IN PEAR PSYLLA, *PSYLLA PYRICOLA*, IN WESTERN MICHIGAN

Populations of pear psylla, *Psylla pyricola* Foerster, were monitored for pesticide resistance in western Michigan in 1989. Ten orchards were sampled including commercial and abandoned orchards. Using a slide-dip toxicity bioassay the efficacy of Guthion, Parathion, Pydrin, Dithan M-45, Morestan, and Thiodan was tested on adult psylla. Dithan and Morestan were not effective as adulticides. Psylla were tolerant to Guthion and Parathion, whereas Pydrin and Thiodan were effective even at low concentrations tested. Winter-form psylla (September) were more tolerant to pesticides than summer forms (June/July). Levels of tolerance and susceptibility were regional and not orchard specific.

Synergist studies with DEF and PBO indicated the importance of esterases in organophosphate and pyrethroid resistance, although Mixed Function Oxidases may also be important in pyrethroid resistance. A microtitre plate assay, detecting esterase activity in individual pear psylla, was evaluated as a tool for monitoring resistance. This enzyme assay indicated that tolerance to Pydrin and Guthion was positively correlated with esterase activity.

Future strategies for resistance management with psylla may include early season use of pyrethroids rotated with other compounds such as Mitac later in the season, use of previously used compounds such as Thiodan that are still effective, use mixtures of pesticides and synergists. However, long-term solutions for resistance management are more likely if natural enemies of pear psylla are included in more selective pesticide management programs.

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## ECOLOGICAL FACTORS INFLUENCING DISTRIBUTION OF O-P RESISTANCE IN PREDACEOUS MITES ON APPLE

*Typhlodromus pyri* Scheuten and *Metaseiulus occidentalis* Nesbitt are the two primary predatory mite species found in apples in the Pacific Northwest. Both species have become effective biological control agents of spider mites, largely because of the development of resistance to pesticides used in commercial apple production. While the two phytoseiid species are closely related, differences in

natural history traits influences the potential for their use in IPM and resistance management programs. Dispersal ability is one such trait. We have previously shown that *T. pyri* is relatively sedentary, rarely moving distances of over 10 m in a season. Alternatively, *M. occidentalis* is highly dispersive, moving distances greater than 100 m within weeks. These characteristics of migration lead to the hypothesis that resistance in *T. pyri* should be more patchy and localized, while a more regional homogeneity in resistance levels would be found in *M. occidentalis*. However, the influence of phytoseiid immigration from unsprayed surrounding habitat is unknown. To assess the impact of surrounding vegetation on the evolution and maintenance of pesticide resistance, a study was initiated in 1989 to examine the distribution and dynamics of organophosphate resistance in *T. pyri* and *M. occidentalis* populations in two distinct apple growing regions of Oregon, the Hood River and Willamette Valleys.

For the first year of study, six experimental locations were selected in both the Hood River and Willamette Valleys. Within each valley, three sites were classified as isolated, i.e. surrounded primarily by native vegetation, and three were classified as intense, i.e. surrounded by other orchards. Three samples were made over the growing season along transects from the surrounding vegetation 100 m outside the orchard, through the orchard, and into the surrounding vegetation on the other side. Leaf samples were taken 100 m and 10 m away from the orchard, and from the outside edge and center of the orchard. In addition, corresponding samples were taken from the groundcover within the orchard. Adult female predatory mites were exposed to a diagnostic dose of 0.10% a.i. azinphosmethyl using the slide-dip method. Mortality was measured at 48 hours and sample percent mortality was compared for the treatments. Treatments included: region (Willamette Valley, Hood River Valley); orchard locality (isolated or surrounded primarily by other orchards); habitat type (orchard, groundcover, surrounding vegetation); sample time (early, mid, or late season); and distance from the orchard center.

*T. pyri* in both valleys had large differences between populations within orchards and outside orchards. Populations from within orchards were all resistant, with some variation in their level of resistance. Those at 100 m were all susceptible, and edge populations were either intermediate or susceptible. An example from Hood River is shown in Figure 1. For *M. occidentalis*, levels of resistance were approximately equal at all orchard and surrounding vegetation locations in the Hood River Valley. In the Willamette Valley, there was more variation in the level of resistance from site to site, but the population densities of *M. occidentalis* were too low to reach any conclusions.

Further studies will be conducted to examine the distribution of OP resistance, and will also examine isozymic variation between populations inside and outside of the orchards. This will help determine the amount of gene flow occurring into and out of orchards, and indicate



whether constant selection pressure will be necessary to maintain pesticide resistance in the predatory mites. Figure 1. Resistance levels found in *T. pyri* along a transect through the McCarty orchards, Hood River, OR.

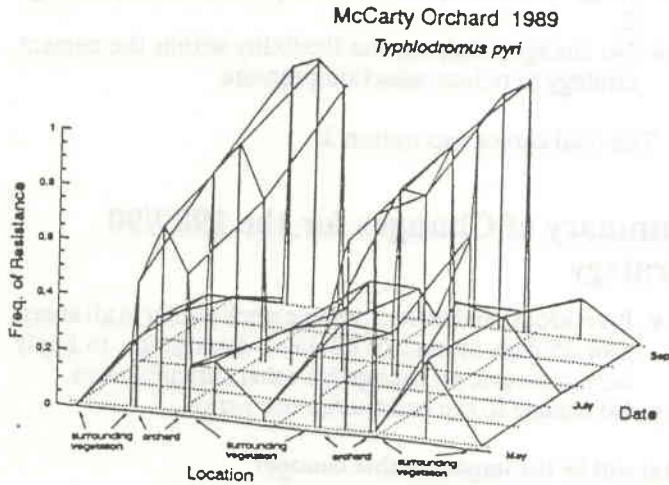


Figure 1. Resistance levels found in *T. pyri* along a transect through the McCarty orchards, Hood River, OR.

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## THE NATURE AND CHARACTERISTICS OF HERBICIDE RESISTANCE IN HUNGARY

Resistance to herbicides has evolved in different localities in Hungary. The most widespread resistances are to s-triazine herbicides but others have been appearing. All cases of herbicide resistance have occurred in Hungary, were used repeatedly mono-herbicides (e.g. corn-monocultures, orchards, roadsides and railroads). Hungary has the world's largest laboratory on herbicide resistance selection--a whole nation.

Atrazine resistant weeds are resistant to triazines (*A. retro flexus*, *A. bouchonii*, *A. hybridus*, *C. album* and *C. canadensis*), phenylureas (the mentioned species also), uracils *A. retroflexus*, *C. canadensis*), bipyridyliums (*C. canadensis*) and a carbamate: phenmedipham (*C. album*) (Table 1). In Hungary there is a case of atrazine/chloridazon co-resistance in *C. album* in fields with crop rotation of corn (with atrazine) and sugar-beet with chloridazon. It should be presumed that each mutation was an independent event and the frequency of each different resistant chloroplast biotype should have been the same. Thus, if it took eight years to obtain populations of triazine resistant biotypes, it should take another eight to obtain resistance to each of the PSII herbicides used as a replacement. Triazine resistance became a fact throughout the Hungarian monoculture corn growing areas within 8-12 years of use after corn and atrazine were co-introduced. Thus, there are recently evolved *C. album* biotypes that are

Table 1. Herbicide resistant weed biotypes and their characteristics in Hungary

Species	Primary resistance by selection	2nd resistance by selection	Tertiary resistance without selection	Cross or Co.	Multiple
<i>A. retroflexus</i> <i>A. hybridus</i>	atrazine-R		chlorbromuron-R lenacil-R linuron-R metribuzin-R phenmedipham-R	+	
<i>A. retroflexus</i> <i>A. bouchonii</i> <i>C. album</i>	diuron-Ra atrazine-R atrazine-R	diuron-R	phenmedipham-R fenuron-R pyrazon-R Pyridate-R	+	+
<i>C. canadensis</i>	atrazine-R paraquat-R diuron-R	paraquat-R	chlorbromuron-R diquat-R linuron-R metribuzin-R terbutryn-R terbutylazin-R	+	+
<i>C. arvense</i>	phenoxy acid (2,4-D,MCPA)-R				

resistant to atrazine; atrazine and chloridazon; atrazine and fenuron; atrazine, chloridazon and pyridate. In all cases resistance is at the chloroplast level, as with atrazine resistance. The plastom mutator gene (psbA) frequently should be much higher in triazine-resistant populations than in wild-type populations. This higher frequency of the mutator genes would facilitate a much more rapid sequential evolution of secondary and tertiary resistance to PSII (and other) herbicides, and has obvious implications in designing strategies to prevent resistance.

Paraquat-resistant *C. canadensis* was found (1984) in some vineyards in Hungary. Paraquat resistance is due to a single dominant gene that pleiotropically controls elevated levels of at least three enzymes that participate in the detoxification of the active oxygen generated by paraquat: superoxid-dismutase, ascorbate reductase and glutathion-reductase. In 1987, we have found atrazine-paraquat co-resistant *C. canadensis* populations along the local train line of Budapest. This biotype of *C. canadensis* has been known so far only from the vineyards of Kecskemet and this is the first time that co-resistant populations of *C. canadensis* were found in uncultivated land. The fact that populations are resistant to paraquat had appeared 3 years after the application and this indicates that earlier atrazine treatments may promote the appearance of the resistance to paraquat, contrary to literature data mentioning a period of 6 years.

In 1987, we observed that the *C. arvensis* has a resistance to phenoxy-herbicides (2,4-D and MCPA). These populations appeared in winter wheat stands of the counties Hajdu and Pest. In the mentioned counties were used 2,4-D and MCPA herbicides over 15-20 years, continuously. The appearance of the resistant population are the result of a long selection process.

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## CHANGE FOR THE AUSTRALIAN RESISTANCE STRATEGY

The Australian Field Crops Insecticide Resistance Management Strategy has been in place now for six seasons. Over this period, there have been excellent industry acceptance of this voluntary strategy. There have been very few minor changes to the strategy in that time. However, what appears to be a slow but gradual deterioration in the pyrethroid resistance situation has prompted a tightening up of the strategy. A number of possible options were discussed over the winter:

- Reduce stage 2 window by 1 week (finish Feb. 13)
- Move stage 2 window forward 10 days (start Jan. 1, finish Feb. 10)

- Reduce pyrethroid window by 1 week (finish Feb. 13) but allow endosulfan to finish still on Feb. 20.
- Reduce the number of pyrethroid sprays in stage 2, from 3 to 2.
- Remove pyrethroids altogether for a season.
- Double the rates of pyrethroids used.
- Put pressure on companies to reduce the price of stage 3 chemicals.
- Make the inclusion of one OP spray mandatory in stage 2.
- No change. Rely on the flexibility within the current strategy to reduce selection pressure.

The final choice was option 3.

## Summary of Changes for the 1989/90 Strategy

- Pyrethroid window to close one week earlier in all areas, now 35 days instead of 42 days. Restriction to apply only in cotton, no change for other summer crops.
- No change to the endosulfan use period.

### What will be the impact of this change?

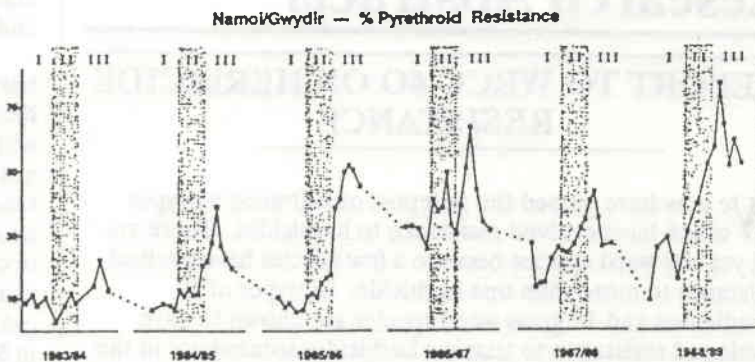
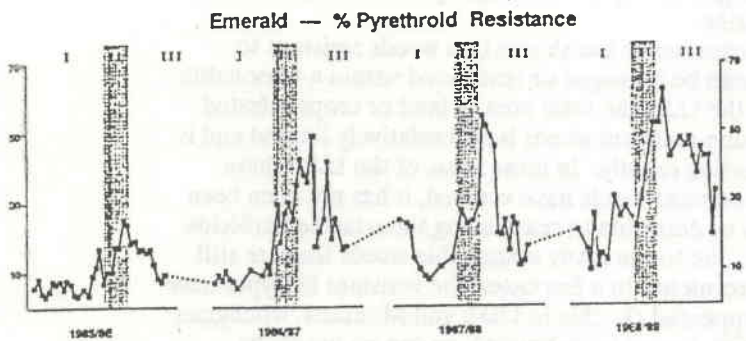
This is very hard to predict. Hopefully, it will buy more time for the pyrethroids which are needed to reduce the increasing selection pressure on the alternative chemicals, particularly endosulfan. We need more time to evaluate new initiatives such as light stable synergists (e.g. piperonyl butoxide) and perhaps even in the longer term, resistance breaking pyrethroids.

### How much time will it give?

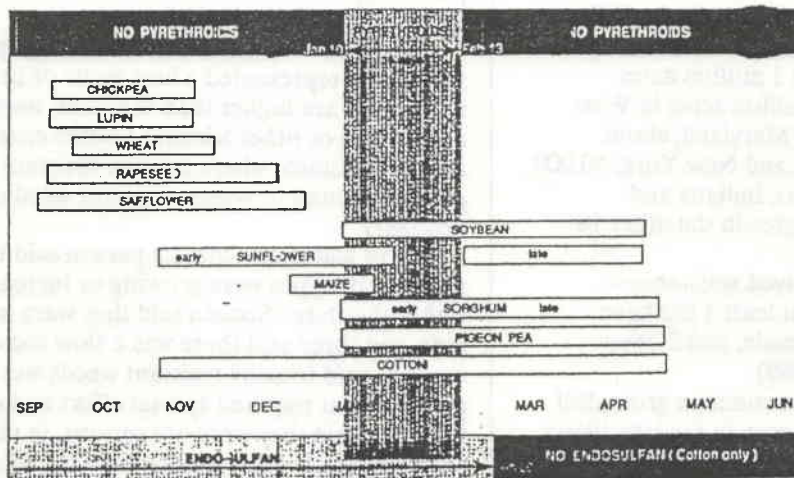
Optimistically, the revised strategy could contain pyrethroid resistance levels for perhaps another ten years or so. Successful field control will depend mainly on the *Heliothis armigera* pressure, with low pressure seasons masking high resistance (as was the case last season). However, a return to even moderate pressure seasons, could see field control failures, particularly in the *armigera* prone eastern cropping areas.

## Revised Strategy Guidelines

- Do not re-spray a suspected pyrethroid failure with a pyrethroid.
- In multiple spray crops, use at least three of the five available chemical groups.
- The use of ovicides is encouraged when egg pressures warrant.
- Pyrethroids should only be targeted on small larvae (less than 5mm). Applications on larger resistant larvae will be ineffective and will increase levels of pyrethroid resistance. Regular and thorough scouting is essential to achieve this objective.



**SUMMER CROP  
RESISTANCE MANAGEMENT STRATEGY**



- Avoid pyrethroids when there is high *H. armigera* pressure.
- If pyrethroid is used to control sorghum midge, do not follow up with a pyrethroid for *Heliothis* control as the midge spray will have already selected for pyrethroid resistant *Heliothis*.
- Minimize the use of endosulfan in ALL crops where reasonable cost effective alternatives exist.
- Cotton crop residues should be thoroughly cultivated to minimize survival of overwintering pupae.
- Cotton growers should avoid growing December flowering crops (mainly early sown maize and sunflowers) in predominantly cotton areas. These act as resistance

nursery crops producing large numbers of *H. Armigera* for the stage 2 pyrethroid window.

- Avoid consecutive sprays of pyrethroids where *H. armigera* emerging from neighboring maize, sorghum or sunflower fields as resistance levels will be exacerbated by selection of moths prior to mating.
- Aim to avoid using a pyrethroid as the last spray for the season on cotton.

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# Research Abstracts

## REPORT TO WRCC-60 ON HERBICIDE RESISTANCE

We now have passed the milepost of 100 weed biotypes which have evolved resistance to herbicides. There are not yet 100 weed species because a few species have evolved resistance to more than one herbicide. Biotypes of 40 broadleaves and 17 grass weed species are known to have developed resistance to triazine herbicides somewhere in the world. A total of 45 weed biotypes (27 broadleaves and 18 grasses) have evolved resistance to 14 other types of classes of herbicides. Only 22 of the triazine-resistant biotypes and 16 biotypes resistant to other herbicides have been found in the U.S., but one or more of these resistant biotypes have invaded 31 states, 4 provinces of Canada and 26 other countries.

The distribution and spread of herbicide resistant weeds are increasing. A recent survey of each state in the U.S. shows the total areas infested with triazine-resistant weeds exceed 2 1/2 million acres, with about 1 million acres estimated for Wisconsin, about 1/4 million acres in West Virginia, Virginia, Pennsylvania, and Maryland, about 100,000 acres in Colorado, Michigan, and New York, 50,000 or more in Oregon, Washington, Ohio, Indiana and Delaware, and from a few to 20,000 acres in the other 18 states (see Tables 1 and 2).

Of the 6 weed species having evolved resistance to sulfonyleurea and related herbicides, at least 1 has been reported in 9 states, 1 province of Canada, and 2 other countries (no update since March, 1989)

The discovery of triazine-resistant common groundsel (*Senecio vulgaris*) in Western Washington in the late 1960's, and the subsequent widespread and frequent occurrence of other triazine-resistant weeds over the past 20 years, have made triazine herbicide resistance the best known and most studied case of herbicide resistance. Triazine resistance has also been of greatest interest because of the importance and extensive use of this group of herbicides.

According to my recent survey, biotypes of 40 broadleaves and 15 grass weed species are known to have developed resistance to triazine herbicides somewhere in the world. Within the U.S., however, only 21 of these triazine-resistant biotypes have been reported in 33 of the 50 states.

This includes all triazine herbicides in all crops and uses. Generally, if a weed evolves resistance to one triazine herbicide, it is relatively resistance to all of them. Most of these resistant biotypes have evolved resistance in corn following frequent and continuous use of atrazine and/or simazine. A few biotypes, including those first occurring in Washington and Oregon (i.e., common in groundsel, pigweed, and lambsquarter) evolved resistance originally to simazine in nurseries and perennial tree crops. A few of the

biotypes (e.g., kochia, downy brome grass, and witchgrass) first evolved resistance in noncrop areas (e.g., railroad beds, highway right-of-ways) following repeated use of atrazine and simazine.

Past experience has shown that weeds resistant to triazines can be managed or restrained within a reasonable limit. In the U.S., the total area of land or crops infested with triazine-resistant weeds is still relatively limited and is not expanding rapidly. In most areas of the U.S. where triazine-resistant weeds have evolved, it has not even been necessary or desirable to cease using the triazine herbicide of choice, due to the many susceptible weeds that are still usually prevalent. In a few cases, the resistant biotypes have even disappeared (kochia in Utah and Montana, witchgrass in Michigan, foxtail in Nebraska), or can no longer be confirmed.

From a recent thorough survey conducted in every state, the total numbers of acres of all crops and land areas being treated with these herbicides where resistant biotypes exist or can be expected include about 2 1/2 million acres (see Table 1). These data were collected or confirmed within the past two months (April to June, 1989) by personally contacting the best informed scientist of extension specialist in each state.

The numbers given were not always precise but sometimes represented a best guess or estimate, and in many cases are higher than the "real" number. They include all the corn or other triazine-treated crops grown in the area of the states where triazine resistant biotypes are generally found or where resistant weed management is necessary.

In 14 states, the contact person said that the triazine resistant biotypes were growing or increasing in acreage within the state. Sixteen said they were not expanding in area, and three said there was a slow increase. Nine state contacts said triazine resistant weeds were a serious weed problem that required special effort and extra work. Eighteen said they were not serious, in that they were easily controlled with other herbicides and did not require special effort or expense. Six stated that they were a minor to moderate problem.

Every contact was asked if any farmers or users of herbicides had stopped using atrazine because of resistant weeds or for other reasons. Without exception, they all responded an emphatic, no. Virtually all users are still applying atrazine because they consider there are no adequate or economical alternatives. There are always many other susceptible weeds that are controlled, and they handle the resistant biotypes as they do other weeds which are not always easily controlled with atrazine (e.g., crabgrass, fall panicum, johnsongrass, shattercane). They select a combination partner (e.g., metolachlor, alachlor, butylate, pendimethalin) or subsequent postemergence herbicide (e.g., 2, 4-D, dicamba, bromozynil, MCPA) to best control the other weeds not controlled with atrazine.

For a complete list of all atrazine resistant weeds having been reported within the U.S., including the year when they were first confirmed, see Table 2.

Our knowledge about herbicide sites and modes of action has been essential in our research and understanding of herbicide resistance mechanisms. Herbicide resistant weeds have also been valuable scientific tools, contributing greatly to our understanding of herbicide modes of action, plant biochemical and physiological processes, molecular genetics, physical structure, and anatomy. However, it is interesting that the mechanisms of resistance developed by most of the weed biotypes to atrazine are different from the mechanisms of selectivity to the herbicide in crops.

Research to date indicates that most of the triazine-resistant biotypes are resistant because they do not have the normal triazine binding sites in their chloroplasts, whereas crop selectivity is due mainly to metabolism or translocation differences. Triazine-resistant velvetleaf in Maryland, and a few other biotypes in Europe, are exceptions in that resistance is due to enhanced herbicide metabolism by glutathione transferase activity.

Extensive research has shown that most, if not all, of the weed biotypes which have evolved resistance to atrazine have been inferior in vigor, competitiveness and fitness compared to the wild-type or susceptible weeds of the same species. This is apparently due to a less efficient photosynthesis mechanism or system in the resistant weeds. This lack of fitness in most triazine resistant weeds is a very important reason why they have been fairly easily controlled, and why more problems of cross-resistance or multiple resistance have not occurred where both a triazine and other types of herbicides have been used repeatedly together.

Also, within the U.S., the close cooperation and communication between industry, state and university research, extension service, and farmers have been very important in avoiding, delaying and controlling atrazine resistant weeds. With the first invasion of resistant weeds, prompt action is essential in order to avoid serious and more permanent problems. Preventive action to avoid herbicide resistant weeds from developing in the first place is definitely the best strategy. It is virtually essential in all cases of herbicide resistance to have other classes or types of herbicides, with alternate sites and mode of action, available. In some countries and situations, failure to respond promptly, the lack of suitable alternatives, or for other reasons, control of triazine-resistant weeds has not been successful, resulting in rapid invasion and almost total loss of these herbicides in the area.

It is worthy of note that resistant weeds are not limited to the triazine herbicides. More recently weed species resistant to the following herbicides among others have been reported in the literature: chlorsulfuron, diclofop, DSMA, MSMA, paraquat and trifluralin.

Some herbicides within the AHAS inhibitor class are presently being developed for weed control in corn, the primary use for atrazine. Inasmuch as resistant weeds have evolved in crops where at least some of these herbicides are currently registered, resistant weeds can be expected to occur when they are registered for use on corn. Considering this, atrazine will continue to be needed for control of a broad spectrum of weeds in corn that have not exhibited resistance to it during its use for approximately 30 years.

## Summary Status of Atrazine Resistant Weeds in the U.S.

- Resistant weeds have been reported in 33 states.
- These include almost all of the northern states (except North Dakota, Vermont and Missouri), plus California, Hawaii, and North Carolina.
- Many of the areas infested with atrazine resistant biotypes are not known with exactness and some of the confirmed cases reported have disappeared or cannot now be identified. Most of the known cases are small (e.g., one or few farms with less than 100 acres infested).
  
- Total of all areas where resistant weeds are prevalent within the U.S. is estimated to be about 2,500,000 acres.
- Resistant weeds have not been of major economic consequence in the U.S. Only in a few states (e.g., Maryland, Pennsylvania, Virginia, West Virginia and Wisconsin) are they of significant concern. Even here, they are being controlled in most cases.
- Before resistant weeds evolved, most corn and sorghum growers were using herbicide combinations and crop rotations for other reasons.
- Triazine resistant biotypes are generally less fit (less vigorous and competitive) and more easily controlled than susceptible biotypes.
- Industry (CIBA-GEIGY), state research, extension and farmers have been alert and have worked closely together to contain or eradicate resistant weeds.
- In continuous culturing of a single crop and in conservation tillage (e.g., no till), where a given herbicide is used repeatedly, we are most vulnerable to the occurrence and spread of herbicide resistant weeds. In this regard, we need to retain all possible herbicide options. This is extremely important in no till situations where the employment of mechanical tillage to assist in controlling weeds is precluded.
- At present, atrazine is essential to efficient corn and sorghum production, even where triazine resistant weeds exist. The cost to farmers to control these weeds range from no increase to \$10 per acre.
- The major methods of avoiding or managing atrazine resistant weeds include:
  - (a) Herbicide combinations.
  - (b) Sequential applications of other herbicides (e.g., postemergence - 2, 4-D, dicamba, etc.).
  - (c) Crop rotations.
  - (d) Herbicide rotations.
  - (e) Cultivation, mowing or other tillage.

TABLE 1  
RESULTS FROM A RECENT SURVEY ON THE DISTRIBUTION, ACREAGE AND  
CONTROL OF ATRAZINE-RESISTANT WEEDS IN U.S. (AS OF JUNE, 1989)

STATE	NUMBER OF R. WEED SPECIES <sup>1</sup>	YEAR 1ST CONFIRMED	INFESTED ACRES	GROWING	SERIOUS	REFERENCE <sup>2</sup>
California	3 (2b, 1g)	1976	4,000	No	No	J. Holt, UCR H. Agamalian, UC, Salinas
Colorado	3b	1977	100,000	Yes	No	P. Westra, CSU
Connecticut	3b	1980	10,000	Slowly	Moderate	J. Ahrens, CAES, Windsor
Delaware	1b	1977	30,000	Yes	Yes	F. J. Webb, UD, Georgetown
Hawaii	2g	1988	100	Yes	Moderate	L. Santo, HSPA
Idaho	1b	1976	500	No	No	R. Callihan, UI
Illinois	2b	1982	30	No	No	E. Knake, UI
Indiana	2b	1983	50,000	No	No	T. Bauman, Purdue
Iowa	3b	1980	100	No	No	M. Owen, ISU
Kansas	2 (1b, 1g)	1977	2,000	Yes	No	D. Marishita, KSU
Kentucky	1b	1985	10,000	Yes	No	M. Barrett, UK
Maine	2b	1984	100	No	No	M. McCormick, UM
Maryland	6 (3b, 3g)	1972	200,000	Yes	Yes	R. Ritter, UM
Massachusetts	2b	1978	50	No	No	P. C. Bhomick, UM
Michigan	4 (3b, 1g)	1975	100,000	Yes	Moderate	J. Kells, MSU
Minnesota	1b	1982	200	No	No	C. Kern, CIBA-GEIGY
Montana	2 (1b, 1g)	1977	0	No	No	P. Fay, MSU
Nebraska	4 (2b, 2g)	1976	20,000	Slowly	Moderate	A. Martin, UN
New Hampshire	1b	1984	2,000	Yes	Yes	J. R. Mitchell, UNH
New Jersey	1b	1985	100	No	No	J. A. Meade, Rutgers U.
New York	3b	1977	110,000	Yes	Yes	R. Hahn, Cornell U.
North Carolina	2b	1985	2,000	Yes	Yes	D. Worsham, NCSU
Ohio	3b	1981	50,000	Yes	Minor	M. Loux, OSU
Oregon	4 (3b, 1g)	1970	80,000	No	Moderate	A. Appleby, OSU
Pennsylvania	7 (6b, 1g)	1978	200,000	Yes	Yes	N. L. Hartwig, PSU
Rhode Island	1b	1983	50	No	No	R. C. Wakefield, URI
South Dakota	1b	1986	80	No	No	L. J. Wrage, SDSU
Utah	1b	1976	0	No	No	S. Devey, USU
Virginia	2b	1976	250,000	Yes	Yes	S. Hagood, VPI
Washington	5 (4b, 1g)	1968	40,000	No	Yes	W. Anliker C. Bucholtz, CIBA-GEIGY
West Virginia	3 (2b, 1g)	1980	300,000	Slowly	No	C. E. Sperow, WVU
Wisconsin	3b	1978	1,000,000	Yes	Yes	R. E. Doersch, UW
Wyoming	1b	1978	20	No	No	S. D. Miller, UW

Total = 31 states reporting a total of 2,571,330 acres (approximations).

<sup>1</sup> = number of weed species with resistant biotypes reported in each state: b = broadleaf species, g = grass species.

<sup>2</sup> = major contact(s) in each state providing estimates.

Table 2

Distribution of Atrazine-Resistant Weeds by State Within the U.S.  
(as of June, 1989)

	<u>States</u>	<u>Species</u>	<u>Common Name</u>	
1.	California	Kochia scoparia Poa annua Senecio vulgaris	kochia annual bluegrass common groundsel	1984 1976 1977
2.	Colorado	Amaranthus arenicola Amaranthus hybridus Kochia scoparia	sandhills amaranth smooth pigweed kochia	1977 1985 1977
3.	Connecticut	Amaranthus hybridus Amaranthus retroflexus Chenopodium album	smooth pigweed redroot pigweed common lambsquarters	1980 1980 1983
4.	Delaware	Amaranthus hybridus	smooth pigweed	1977
5.	Hawaii	Chloris barbata Chloris radiata	swollen fingergrass plush grass	1988 1988
6.	Idaho	Kochia scoparia	kochia	1976
7.	Illinois	Amaranthus hybridus or retroflexus Chenopodium album	redroot pigweed common lambsquarters	1982 1985
8.	Indiana	Amaranthus retroflexus Chenopodium album	redroot pigweed common lambsquarters	1983 1985
9.	Iowa	Chenopodium album Kochia scoparia Polygonum pennsylvanicum	common lambsquarters kochia Pennsylvania smartweed	1986 1980 1988
10.	Kansas	Bromus tectorum Kochia scoparia	downy brome kochia	1977 1977
11.	Kentucky	Amaranthus hybridus	smooth pigweed	1985
12.	Maine	Amaranthus hybridus Chenopodium album	smooth pigweed common lambsquarters	1984 1984
13.	Maryland	Abutilon theophrasti Amaranthus hybridus Chenopodium album Echinochloa crus-galli Setaria faberi Setaria glauca	velvetleaf smooth pigweed common lambsquarters barnyardgrass giant foxtail yellow foxtail	1984 1972 1982 1978 1984 1984
14.	Massachusetts	Amaranthus hybridus Chenopodium album	smooth pigweed common lambsquarters	1978 1983
15.	Michigan	Amaranthus hybridus Chenopodium album Panicum capillare Solanum nigrum	smooth pigweed common lambsquarters witchgrass black nightshade	1981 1980 1975 1984
16.	Minnesota	Chenopodium album	common lambsquarters	1982
17.	Montana*	Bromus tectorum Kochia scoparia	downy brome kochia	1977 1979

	<u>States</u>	<u>Species</u>	<u>Common Name</u>	
18.	Nebraska	Amaranthus hybridus	smooth pigweed	1986
		Bromus tectorum	downy brome	1977
		Kochia scoparia	kochia	1976
		Setaria glauca	yellow foxtail	1980
19.	New Hampshire	Chenopodium album	common lambsquarters	1984
20.	New Jersey	Amaranthus hybridus	smooth pigweed	1985
21.	New York	Amaranthus hybridus	smooth pigweed	1978
		Amaranthus retroflexus	redroot pigweed	1982
		Chenopodium album	common lambsquarters	1977
22.	North Carolina	Amaranthus retroflexus	redroot pigweed	1986
		Chenopodium album	common lambsquarters	1985
23.	Ohio	Amaranthus retroflexus	redroot pigweed	1981
		Chenopodium album	common lambsquarters	1981
		Sicyos angulatus	burcucumber	1985
24.	Oregon	Amaranthus powellii	green pigweed	1970
		Bromus tectorum	downy brome	1978
		Kochia scoparia	kochia	1977
		Senecio vulgaris	common groundsel	1973
25.	Pennsylvania	Amaranthus hybridus	smooth pigweed	1978
		Ambrosia artemisiifolia	common ragweed	1984
		Chenopodium album	common lambsquarters	1983
		Chenopodium missouriense	lambsquarters	1978
		Physalis longifolia	longleaf groundcherry	1984
		Polygonum convolvulus	wild buckwheat	1984
		Setaria glauca	yellow foxtail	1984
26.	Rhode Island	Chenopodium album	common lambsquarters	1983
27.	South Dakota	Amaranthus retroflexus	redroot pigweed	1986
28.	Utah*	Kochia scoparia	kochia	1976
29.	Virginia	Amaranthus hybridus	smooth pigweed	1976
		Chenopodium album	common lambsquarters	1979
30.	Washington	Amaranthus powellii	green pigweed	1968
		Bromus tectorum	downy brome	1978
		Chenopodium album	common lambsquarters	1973
		Kochia scoparia	kochia	1980
		Senecio vulgaris	common groundsel	1968
31.	West Virginia	Amaranthus hybridus	smooth pigweed	1981
		Chenopodium album	common lambsquarters	1983
		Echinochloa crus-galli	barnyardgrass	1980
32.	Wisconsin	Amaranthus hybridus	smooth pigweed	1985
		Chenopodium album	common lambsquarters	1978
		Kochia scoparia	kochia	1985
33.	Wyoming	Kochia scoparia	kochia	1978

\*Both Montana and Utah report that these resistant weeds can no longer be located or confirmed.

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## AN UPDATE ON PYRETHROID RESISTANCE IN TOBACCO BUDWORM AND BOLLWORM IN LOUISIANA

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Over 5700 male tobacco budworm moths were bioassayed from May through September 1988 against 1, 5, 10 and 30 g/vial doses of cypermethrin. Examination of these data reveal that pyrethroid resistance in tobacco budworm varied with location and date of bioassay. Resistance levels decreased during May and June when pyrethroid use was low and increased dramatically during late July and August when pyrethroids were being used extensively. Overall pyrethroid resistance levels in May, June and early July were lower in 1988 than in 1987. However, due to the fact that the 1988 cotton crop matured later than the 1987 crop which resulted in greater use of pyrethroids in August and September) 1988 were higher than the levels recorded during those same months of 1987. Resistance levels were generally highest in areas of extensive cotton production (hence more extensive use of pyrethroids) and lowest in areas with little or no commercial cotton production. The responses of over 800 tobacco budworm moths to tralomethrin at doses of 1, 2.5, 5, 10 and 30 g/vial indicated a similar pattern of resistance as that observed with cypermethrin. These data support previous results that indicate that resistance to pyrethroid generally confers cross-resistance to other pyrethroids. The responses of over 1400 bollworm moths to cypermethrin at doses of 0.5, 1, 2 and 5 g/vial serve as baseline data for this pest. The data obtained reveal that bollworms are much more susceptible to cypermethrin than tobacco budworms. However, there was some variation in the tolerance level of bollworm moths based on location of collection. The most pyrethroid tolerant bollworm moths were collected from the same locations as the most resistant tobacco budworm moths. Overall all of the data collected suggest that the Tri-State (Mid-South) Pyrethroid Resistance Management Plan has been successful in delaying pyrethroid resistance development in *Heliothis*. However, low tobacco budworm populations during 1987 and 1988 have no doubt been responsible for the virtual absence of field control failures.

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## ENHANCED METABOLISM AND KNOCKDOWN RESISTANCE IN A FIELD VS A LABORATORY STRAIN OF THE SOYBEAN LOOPER (LEPIDOPTERA:NOCTUIDAE)

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Soybean looper, *Pseudoplusia includens* (Walker), larvae collected from a field where permethrin failed to provide adequate control and for which a reduction in susceptibility to permethrin had been demonstrated (3-fold, LC<sub>50</sub>) were compared with an established laboratory colony with respect to knockdown resistance (*kdr*) and *in vitro* metabolic capacity for a variety of substrates. The time necessary to achieve 50% knockdown of the field population (22.9 1.3 min) after the topical application of 1 mg permethrin was significantly greater than that required for the LSU laboratory colony (18.4 1.0 min.). Rates of metabolism for first generation larvae from the field population were significantly greater than for larvae from the laboratory culture for substrates of glutathione transferase (1-chloro-2,4-dinitrobenzene, 2.7-fold), monooxygenases (p-nitroanisole Q-demethylase, 1.8-fold), and hydrolases (alpha-naphthyl acetate (1.5-fold), p-nitrophenyl acetate (1.5-fold), and permethrin (1.5-fold)). Significant differences between populations were not observed for NADPH cytochrome c reductase nor acephate hydrolysis. Results of the study indicate that a combination of target site insensitivity and increased activity of several enzymes involved in insecticide metabolism including a *trans*-permethrin hydrolase may be contributing to the reduced susceptibility of the field population relative to the laboratory colony.

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## PYRETHROID RESISTANCE AND THE TOBACCO BUDWORM: INTERACTIONS WITH CHLORDIMEFORM AND MECHANISMS OF RESISTANCE

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The effect of chlordimeform (CDF) on permethrin and cyhalothrin-K uptake from a treated surface by third instar larvae of the tobacco budworm was determined. In general, CDF increased the uptake of both pyrethroids, especially in pyrethroid resistant (ICI-R) tobacco budworms. Compared to the susceptible (LSU-Lab) tobacco budworm larvae, the pyrethroid resistant strain

consistently picked up less insecticide. A hot-probe bioassay for knock-down resistance (*kdr*) (Bloomquist & Miller 1985) indicated the presence of *kdr* in the pyrethroid resistant strain. The pyrethroid resistant strain also had higher titers of *trans*-permethrin hydrolase activity. The role of metabolism in pyrethroid resistant tobacco budworm larvae was evaluated by topically treating third instar larvae with  $C^{14}$  radiolabeled permethrin or cyhalothrin-K. At 18 hr. posttreatment, the resistant larvae treated with permethrin had less total radiolabeled material internal, and less parent than did the susceptible larvae. However, for cyhalothrin-K treated larvae there was little difference between the two strains in the amount of total internal radiolabel or in the amount of parent. Thus, there appears to be some differences in how the resistant strain handles permethrin and cyhalothrin-K. The above studies suggest that *kdr*, and increased metabolic capability and, perhaps, a reduced level of larval activity in the presence of the pyrethroids all contribute to pyrethroid resistance in the ICI strain of the tobacco budworm.

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## PYRETHROID RESISTANCE IN PEAR PSYLLA IN WESTERN NORTH AMERICA

### SUMMARY

Adult pear psylla, *Cacopsylla pyricola* (Foerster), from commercial pear orchards near Wenatchee, Washington, were tested using a slide-dip technique for susceptibility to fenvalerate over a 5-year period from 1984-1988. Results were compared with those from similar tests using psyllids from an unexposed population near Corvallis, Oregon. During 5 years, resistance of adults increased by 4 to 136 fold at Wenatchee while that of the Corvallis population did not change. In 1988, tests with five pyrethroids and pyrethroid-piperonyl butoxide (pbo) combinations indicated that pear psylla from Wenatchee were also resistant to permethrin and flucythrinate but not to fenpropathrin or cyfluthrin. Pbo synergism was proportional to the level of resistance, indicating that resistance probably is due to increased mixed function oxidase activity. Fenvalerate resistance in pear psylla was monitored at 51 sites in Washington, Oregon, California and British Columbia during 1988. Resistance levels ranged from susceptible in an unsprayed orchard in the Willamette Valley, Oregon, and several commercial orchards near Placerville, California, to highly resistant (100-fold) at several sites in central Washington. Generally, resistance levels were greater in the north than the south. In the Wenatchee and Yakima, Washington areas pyrethroid resistance was areawide,

showing similar levels in both heavily treated and untreated orchards. In the willamette Valley, Oregon, pyrethroid resistance was local and more consistent with treatment histories of individual orchards. Reasons for high resistance in central Washington are not known, but this pattern is consistent with earlier patterns of insecticide resistance in pear psylla.

### INTRODUCTION

Pear psylla, *Cacopsylla pyricola* (Foerster), is a key pest of pear in western North America. A season-long program of several sprays are required each year to manage this pest at sub-economic density. Efforts to integrate and biological and chemical control have not significantly reduced dependence on chemical sprays. In the arid pear growing areas of North America there are few weed trees vigorous enough to support pear psylla populations; therefore, the entire population exists in commercial orchards and is exposed to intense pesticide selection. Currently populations in commercial pear growing areas of western North America are resistant to most classes of synthetic insecticides (Harries and Burts 1965; Westgard and Zwick 1972; Riedl *et al.* 1981; van de Baan 1988). Follett *et al.* (1985) reported a survey of pear psylla resistance and alluded to its regional nature in areas of concentrated pear production.

A key component of pear psylla control is a dormant spray directed at post-diapause adults applied when they begin to oviposit. During the past 12 years pyrethroids have been the material of choice for this spray. In spring of 1987 pear growers in north central Washington reported control failures with fenvalerate and permethrin. Investigations in several orchards showed that surviving adults exceeded the retreatment threshold (Burts and Brunner 1981) after two applications of either fenvalerate or permethrin. Because in the past, pear psylla has developed resistance to pyrethroids, rapid evolution of resistance to these compounds was anticipated. In order to delay resistance the following use strategy was recommended by public research and extension people in Washington and Oregon and followed by most growers: first, pyrethroid were limited to the prebloom period (dormant to clusterbud stages of tree development), and second, the minimum effective rate was used. Amitraz and mancozeb were used for post-bloom control. We think this use strategy resulted in longer effective life of pyrethroids against pear psylla.

Studies we report here include tracking of pyrethroid resistance intensification and spread through pear growing areas of western North America, the regional nature of that resistance, what we have learned about the mechanisms of pesticide resistance in pear psylla and finally some thoughts about resistance management in this species.

### MATERIALS AND METHODS

All tests were conducted using adult pear psylla collected from commercial pear orchards except those from a small unsprayed pear orchard on the campus of Oregon

State University, Corvallis, Oregon, which was considered to be susceptible to pyrethroids and served as a base-line for measuring resistance in other populations. Field-collected psyllids were anesthetized with CO<sub>2</sub> and mounted on glass microscope slides using the technique of Follett *et al.* (1985). Slides with psyllids were dipped for 5 x in water dilutions of pyrethroids or pyrethroid-piperonyl butoxide (pbo) combinations. All pyrethroids and pbo used were formulated as emulsifiable concentrations. In some cases 5 or 6 serial dilutions of each pesticide were tested in 12-36 replications of 10 psyllids per concentration. When mortality occurred in controls data were corrected by Abbott's formula (Abbott 1925). In the resistance survey in LC<sub>50</sub> values were estimated for low and moderately resistant populations based on an average slope value of 2.6 (from van de Baan 1988). For highly resistant populations in Washington, LC<sub>50</sub> values were calculated by probit analysis (Finney 1971) using data from six concentrations ranging from 11.3 to 360 mg fenvalerate (AI)/L. Resistance levels were calculated by dividing LC<sub>50</sub> values by that of a susceptible population at Oregon State University Entomology Farm.

**RESULTS AND DISCUSSION**

At the beginning of the study in 1984 Wenatchee Psyllids were already less susceptible to fenvalerate than Corvallis, Oregon, psyllids. Resistance present in Wenatchee psyllids in 1984 probably developed from selection with permethrin and fenvalerate during the previous five years or from cross resistance due to intense selection with synthetic pesticides in other classes over three decades, or from both. During the five years that pyrethroid resistance was monitored in the Wenatchee area the LC<sub>50</sub> for fenvalerate increased from about 4-fold in 1984 to 136-fold in 1989 over that of the susceptible Corvallis population (Figure 1). During the spring of 1987 when pyrethroid resistance at Wenatchee was about 75-fold, growers in that area began to experience control failures. In 1988, slide-dip tests with five pyrethroids and pyrethroid-piperonyl butoxide combinations indicated that pear psylla adults were also resistant to permethrin and flucythrinate but not to fenpropathrin or cyfluthrin (Table 1).

Piperonyl butoxide (pbo) is a synergist for natural pyrethrins and pyrethroids (Baillie and Wright 1985). Pbo can be used to make pesticides more effective against resistant pests or to lower rates needed to provide adequate control. In this study pbo significantly (p=0.05) synergized fenvalerate, permethrin and flucythrinate but not fenpropathrin or cyfluthrin (Table 1). The latter two pyrethroids contain cyano groups which reduce the ease with which they can be metabolized oxidatively. Mortality of pbo-pyrethroid combinations increased with increased concentration of pbo up to 75 mg AI/L for fenvalerate to 150 mg AI/L for permethrin. Although laboratory data showed pbo to be an effective synergist against pyrethroid resistant psyllids, grower applications of fenvalerate-pbo combinations did not provide satisfactory control in spring of 1988.

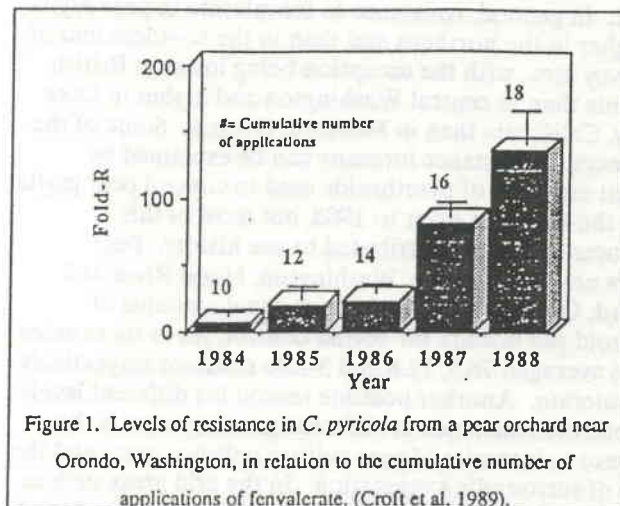


Figure 1. Levels of resistance in *C. pyricola* from a pear orchard near Orondo, Washington, in relation to the cumulative number of applications of fenvalerate. (Croft *et al.* 1989).

Table 1. Effects of piperonyl butoxide concentration on mortality of *C. pyricola* from Wenatchee five pyrethroids in slide-dip tests (Burts *et al.* 1989)

mg AI/L pbo	Mean percent mortality					
	pbo only	pbo plus listed mg AI/L pyrethroids				
		90 fenvalerate	90 permethrin	11.125 fenpropathrin	2.5 cyfluthrin	22.5 flucythrinate
1200	37.8	98.0	95.0	—	—	—
600	20.0	93.8	90.0	—	—	—
300	11.1	87.3	88.2	55.4	73.3	72.7
150	5.6	88.2	80.1	44.6	67.5	63.6
75	4.4	81.7	33.3	45.4	68.3	53.3
37.5	0.8	50.0	20.0	42.3	50.0	45.6
18.8	0.0	51.0	20.0	42.3	50.0	23.3
0.0	—	39.0	12.3	37.7	55.0	29.1
control	2.4	2.7	2.0	2.3	3.0	4.5

In the Wenatchee area cyfluthrin was labeled for emergency use against pear psylla in spring on 1988 and 1989 orchards. In 1988 this compound provided good control but in 1989 control was variable and in most orchards not acceptable. Resistance to fenvalerate did not change significantly between 1988 and 1989. At this time in

the Wenatchee area pyrethroids no longer provide acceptable control of over-wintered adult pear psylla even when combined with pbo.

Pyrethroid resistance survey results are presented in Table 2. In general, resistance to fenvalerate in pear psylla was higher in the northern end than in the southern end of the survey area, with the exception being lower in British Columbia than in central Washington and higher in Lake County, California than in Medford, Oregon. Some of the differences in resistance intensity can be explained by different amounts of pyrethroids used to control pear psylla during the ten years prior to 1988, but most of this difference can not be attributed to use history. Pear growers near Wenatchee, Washington, Hood River and Medford, Oregon, have used about equal amounts of pyrethroid per hectare for psyllid control, yet in these areas psyllids averaged 76.9, 12.8 and 3-fold resistant respectively to fenvalerate. Another possible reason for different levels of pyrethroid resistance in different growing areas is the difference in intensity of pear culture between areas and the nature of surrounding vegetation. In the arid areas such as Wenatchee and Yakima there is little growth of abandoned pear trees and thus they support very low psyllid populations. In wetter growing areas abandoned trees support larger populations of non-selected psyllids that could decrease frequency of resistance genes in the area's population.

Table 2. Area mean levels of fenvalerate resistance in *C. Pyricola* from the major pear growing areas of western North America, 1988. (Croft et al. 1989).

Region	Area	Fold resistance <sup>1</sup>		
		Maximum	Minimum	Mean
B. C.	Okanagan	43.5	8.1	21.1
Canada				
Wash.	N. Wash.	31.9	11.7	21.6
Wash.	Wenatchee	136.2	23.9	76.9
Wash.	Yakima	152.2	21.0	55.9
Oregon	Hood River	31.2	4.8	12.8
Oregon	Willamette	20.0	1.0	8.4
Oregon	Medford	5.0	<1.8	3.0
Calif.	Lake Co.	9.6	8.4	9.2
Calif.	Placerville	1.8	<1.8	<1.8

<sup>1</sup> Based on susceptible population from Oregon State University, Entomology Farm.

Pyrethroids were used for post-bloom control of pear psylla in two pear growing areas of western North America, Lake county, California and central British Columbia, Canada, in other areas these compounds were restricted to prebloom use. A comparison of pyrethroid resistance in Lake county with that in Medford, Oregon, indicates that summer use of pyrethroids promotes resistance faster than prebloom use since the average level of resistance in the former area is significantly greater than that of the latter even though less total compound was used. Resistance in British Columbia has likely been influenced by summer use of pyrethroids but in contrast to other areas of production discussed here, the most commonly used pyrethroid in British Columbia has been permethrin, with recent substitution of deltamethrin and cypermethrin in some orchards: but fenvalerate has not been used. It appears that selection with permethrin has conferred a moderate level of resistance to fenvalerate.

The Willamette Valley is an area of diversified agriculture and native forest. Pear orchards are small and widely separated from each other. Abandoned or weed trees are vigorous to produce moderate populations of psyllids. In this area there is not the strong regional resistance found in areas of concentrated pear production. Instead, moderate levels of resistance have developed in individual orchards. Although there probably is dilution of resistance by mixing of populations during winter dispersal, susceptible individuals are removed from the population by the initial pyrethroid application each year.

Studies on the biochemistry of resistance indicate that reduced penetration and increased detoxification of insecticides are important mechanisms conferring resistance in pear psylla. Esterases are of major importance in the detoxification of a variety of pesticides in this insect (van de Baan 1988) but pyrethroid toxicity to resistant psyllids provided by synergism with pbo supports the importance of mixed-function oxidase activity as a mechanism of resistance.

What have we learned from studies of resistance in pear psylla? First, it seems obvious that resistance management with this insect must be a pre-planned program, not a reactionary one. We need to manage susceptibility. Managing susceptibility in individual orchards is feasible in some areas (the Willamette Valley and Placerville), but in areas of concentrated pear production (Wenatchee, Yakima) areawide action will be necessary in order to delay development of resistance. The spread of resistance in psyllid populations is so fast once it develops that there are few measures short of changing pesticides that can be taken to preserve effectiveness of affected pesticides. Better understanding of dispersal of pear psylla, especially of winter adults, would aid in predicting spread of resistance within and between areas of production. There is real need with pear psylla for a more diversified control, including not only several effective pesticides from different chemical classes but also the use of cultural practices that make trees less susceptible to attack and damage and the augmentation of biological

control. This means that we need soft or selective programs that allow survival of biocontrol agents and reduce dependence on chemical control. With pear psylla there does not seem to be much reversion of populations back to a susceptible state after discontinuing the use of a compound; the only exception may be with Thiodan, which has not been used on pear for several years in Washington due to its loss of effectiveness. In the spring of 1989 it was quite effective against winter adults.

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## Behavioral Response of *Plutella xylostella* (Lepidoptera: Plutellidae) Populations to Permethrin Deposits

An assay was designed to quantitatively measure behavioral response of diamondback moth populations, collected as part of a nationwide survey, to insecticide deposits. Cabbage leaf disks were treated with droplets of permethrin and arranged to form a graded series of droplet densities. Larvae were exposed to the gradient for 24 hr periods, after which their positions along the gradient and amount of feeding on each disk were recorded. Populations showed varied behavioral response to the gradient as measured by average position. Feeding data supported the results of the larval position data. When our measure of behavioral response is compared to a measure of physiological response, the  $LC_{50}$  in a leaf-dip bioassay, a negative correlation between the two is evident for most populations. These data suggest that irritation and intoxication are responsible for behavioral and physiological responses, respectively, and they arise from the same physiological process. Populations that did not fit this pattern were present also, however, indicating that behavioral and physiological responses could also arise from different physiological mechanisms.

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## Evaluating resistance to permethrin in *Plutella xylostella* (Lepidoptera: Plutellidae) populations using uniformly sized droplets.

A series of assays were designed to expose 3rd instar *Plutella xylostella* larvae to discrete deposits of uniformly sized spray droplets containing permethrin. Larvae from 5 US populations were evaluated for resistance to this pyrethroid. The  $LC_{50}$  for dip and spray droplet (100 - 120um diameter droplets) tests differed by 10 - 1000 fold, for the same population. Inter-population differences in resistance also spanned 3 orders of magnitude in both assays. The spray assay  $LC_{50}$  of larvae from 3 of the populations exceeded 40g/l (equivalent to approx. 100 times the concentration recommended for conventional spray application). Applying different droplet densities to the upper surface of leaf discs had no significant effect upon subsequent mortality in 4 of the populations, emphasizing the importance of obtaining spray deposition on leaf undersides.

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## Fungal Resistance to Sterol Demethylation Inhibitors Molecular Mechanism and baseline Sensitivities

*Ustilago avenae* -strains with differential levels of resistance to sterol demethylation inhibitors (DMIs) comprise the model system for studies on the molecular mechanisms of resistance. A sensitive wild-type and DMI-resistant strain with the highest resistance level were investigated with regard to their initial and long-term responses to the treatment with the DMI triadimenol. At a discriminatory dose of 2 mg/L, reproduction of the sensitive strain was inhibited subsequent to a log-phase of 6 h. The few cells still emerging were morphologically altered and remained in cell aggregates. The initial response pattern was not different for the DMI-resistant mutant. Reproduction was severely blocked, and new cells also remained in aggregates. However, the inhibitory effect on both sporidia reproduction and segregation of daughter cells was only transient, and full growth resumed 12 h after inhibitor treatment. Concomitant to the different patterns of reproductive responses, substantial differences were also apparent with respect to the sterol contents and metabolism. During the initial inhibitory phase, sterol precursors strongly accumulated in both strains, indicating that the target site of the resistant mutant was saturated to a degree not different from the sensitive strain. In contrast to the lasting accumulation of sterol precursors observed for the sensitive strain, this effect was only transient for the resistant mutant. The precursor content declined, and desmethyl sterols, comprising the pool of authentic membrane sterols, increased proportionally.

The 'stop-and-go' mechanism observed for the DMI-resistant mutant of *U. avenae* comprises the first report of an induced expression of resistance of fungi to an agricultural fungicide. So far, mechanisms of resistance have been described as constitutive systems, such as the mutational change of the target site leading to the decreased binding of the inhibitor. The nature of the induced system accounting for the expression of resistance could be explained by the desactivation of the inhibitor, the synthesis of excess target enzyme compensating for higher quantities of inhibitor, or the oxidative degradation of accumulating sterol precursors. These possibilities are currently under investigation. The induced expression of resistance might also explain, why the sensitivity distribution of pathogen populations to DMI fungicides is continuous in character, and why separate sub-populations with high levels of

resistance have not yet been observed. An extremely high level of resistance might be counteracted by the induced metabolic effort necessary to express resistance while maintaining viability.

The continuous sensitivity distribution of wild-type populations of *Venturia inaequalis*, the casual agent of apple scab, was characterized for the DMI flusilazole. ED<sub>50</sub>-values were determined for 300 single-conidia isolates. The sensitivities ranged from 0.6 to 170 ppb, with a mean value at 8 ppb. The distribution was lognormal in character. These baseline data will be mandatory for the monitoring of populations towards resistance to DMI fungicides currently introduced for the control of apple scab, and for the development of simplified monitoring methods.

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## Bioassay for Resistance in pear psylla i

Work on pesticide resistance a Wenatchee involved a continuing survey of pyrethroid resistance and development of base line toxicity data for avermectin B<sub>1</sub> in pear psylla, *Psylla pyricola*. The pyrethroid resistance survey done in cooperation with entomologists in British Columbia, Canada, Oregon and California documented that resistance is spreading and intensifying throughout the pear growing areas of the Pacific Northwest. Resistance is strongest in central Washington in areas of intense pear culture and weakest in northern California and Medford, Oregon. The foothills growing area of northern California is one of low intensity pear culture with a well-managed IPM program so one can understand the low level of resistance there but in the Medford, Oregon, area pears are intensively grown and about the same amount of pyrethroids have been applied there as in central Washington. This relationship is worthy of further study.

Avermectin B<sub>1</sub> bioassay techniques for adults and nymphs have been developed and base line toxicity data developed so that susceptibility of pear psylla to this compound can be monitored in the future. For nymphs a floating leaf-disk technique is suitable. Shoot growth from untreated pear trees is sprayed in the lab with 5 serial dilutions of avermectin and allowed to dry. 2.2cm circular leaf disks are punched from treated foliage and floated on moist filter paper in 14 cm Petri dishes. Ten 1st - 3rd instars from the test population are transferred to each disk. Mortality is determined after 3 days by examining nymphs under magnification. Five to 10 disks of 10 nymphs each seem to produce reliable data. I prefer running serial dilutions to using single diagnostic dosages because serial dilutions give an indication of changes in slope.

Adult psylla are bioassayed from the same treated shoot sample as used for nymphs by confining them on leaves in modified Munger cells (H. Tashiro. 1067. J. Econ. Entomol. 60: 354-6). Cells made from 3/16 in plastic sheet are 1 in. in diameter. Ten adults are placed in each cage and mortality is determined after 4 days. Cells are placed about 1/2 in above water in shallow pans and paper towel wicks are used to keep leaves turgid.

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## Geographical and Seasonal Variation in Pesticide Resistance in the Cotton Aphid, *Aphis gossypii*, in California Cotton

The cotton-melon aphid, *Aphis gossypii* Glover, is an occasional pest of cotton in San Joaquin Valley cotton. However, it is a serious pest if the sticky honeydew it produces is deposited on open cotton bolls. Sticky cotton breaks during the cotton spinning process leading to rejection of California cotton by spinners. Growers depend on pesticides to obtain rapid control of aphids, especially in the latter half of the growing season. During the 1986-88 field seasons, *A. gossypii*, appeared in higher than normal densities and the broad spectrum pesticides used to control it gave highly variable results. It was the purpose of my project to determine if pesticide resistance played a significant role in the observed pesticide efficacy problems and, if so, to begin to develop a resistance management program for *A. gossypii*.

Alate nymphs, alate adults and apterous adults were screened for their response to three organophosphates (oxydemeton-methyl, chlorpyrifos and dicotophos), a chlorinated hydrocarbon (endosulfan) and a pyrethroid (biphenate). While the three OPs and endosulfan had been used in cotton for aphid control, the pyrethroid had not. A 24 hour leaf dip bioassay was used to assess the response of the various stages of aphids. Apterous adults were the least tolerant stage for all pesticides tested. Therefore, lower discriminating concentrations were chosen to detect resistance in this stage for use in the geographical survey.

Thirteen populations of *A. gossypii* were collected from throughout the San Joaquin Valley and assayed for their response to the five pesticides. Resistances to oxydemeton-methyl, chlorpyrifos, dicotophos, and endosulfan were found in 4 to 5 populations. The majority of the resistant populations were found on the east side of the Valley where aphid populations develop first and where early season aphid pesticide applications are more common. At the end of the season, aphid from these sites were recollected and bioassays indicated that only 2 to 3 sites had highly resistant aphids. The wide geographical and seasonal variability in pesticide resistance in *A. gossypii* coupled with

the relatively low selection pressure for aphids in the San Joaquin Valley (0-2 applications/season) suggests that resistance should be manageable. I am continuing efforts to survey for resistance and develop rapid resistance detection methods.

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## Parasitode Resistance in California

*T. pallidus* was selected for resistance to azinphosmethyl in the laboratory. The resistant strain was mass reared and approximately 75,000 parasites were released into five commercial walnut orchards in California during 1988. The resistant strain established in four of the five sites, survived field rates of azinphosmethyl or methidathion, persisted throughout the growing season, had measurable impacts on walnut aphid populations, and dispersed to nearby nonrelease sites. These sites were monitored during the spring 1989 to determine whether the resistant strain had successfully overwintered. In all four sites, the resistant strain was found to have overwintered, although the resistance levels were variable. We also found, using clip cages and foliage collected from treated orchards, that the azinphosmethyl-resistant strain of *T. pallidus* is cross resistant to chlorpyrifos, endosulfan, methidathion, or phosalone. A mode of inheritance test was conducted and the data are currently being analyzed.

During the 1989 growing season, aphid and mummy counts in each orchard are being monitored to determine how well the overwintered parasites are able to control aphids. In addition, additional parasites were mass reared and released into a commercial walnut orchard. We hope to establish the resistant strain in the San Joaquin Valley of California; since the wild population of *T. pallidus* is abundant and disperses readily, we are interested in learning how to optimize methods for enhancing establishment of the resistant strain.

The carbaryl-resistant strain of *A. melinus* was released into two commercial citrus orchards in the San Joaquin Valley during the summer of 1989. We are monitoring establishment and cross resistances to pesticides used in citrus IPM. In addition, we are attempting to develop a mass selection method so that commercial producers could maintain this strain for augmentative releases.

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## Tobacco Budworm Resistance Update

A genetic analysis of pyrethroid resistance in tobacco budworm (*Heliothis virescens*, Lepidoptera: Noctuidae) was initiated. Populations from both the Mississippi delta and the Rio Grande Valley are being compared to a susceptible laboratory strain. These results will be interpreted in light of genetic analysis for the PEG-87 strain of *H. virescens*. PEG-87 is completely resistant to cypermethrin at field strengths (larval LD<sub>50</sub> = 325 ug, analyzed by M. J. Firko, June, 1989)

LD<sub>50</sub>'s of cypermethrin by different tobacco budworm populations in the lower Rio Grande Valley ranged from 0.0625 to 0.125 ug/larva (15 to 25 mg) in both 1983 and 1989; thus only variation in susceptibility was exhibited among the different populations within the valley.

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## GENETICS OF INSECTICIDE RESISTANCE IN *HELIOTHIS VIRESCENS* FROM COTTON AND TOBACCO

**O**bjective: Develop management strategies for this major pest of cotton throughout the Americas through understanding the dynamics of resistance genes in populations.

**A**pproach: Co-investigator David Heckel (Biological Sciences, Clemson University) and I share a Competitive Grant from USDA to construct a genetic linkage map of this insect and to map resistance genes. The following physiological mechanisms of resistance are under investigation:

### Progress:

- Genetic segregation of acetylcholinesterase insensitivity was observed.
- SS,RS,RR genotypes were discriminated using several inhibitors, some inhibiting RR.
- With David Heckel, this gene was found to be linked to one of his marker enzyme loci.
- A rapid microtiter plate assay was adapted for application in the field.
- Possible organophosphorus resistance-breaking compounds have been discovered.
- Segregation of one factor for permethrin resistance was observed in another strain.

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## CLONING OF THE $\beta$ -TUBULIN GENE FROM BENOMYL-SENSITIVE AND BENOMYL-RESISTANT FIELD STRAINS OF *VENTURIA INAEQUALIS*.

Widely differing levels of benomyl-resistance in *Venturia inaequalis* has been attributed to allelic mutations in the  $\beta$ -tubulin gene. To study this phenomenon at the molecular level, genomic DNA was isolated from 6-week old broth cultures of a benomyl-sensitive (WC-S) and a benomyl-resistant (KV3C) field isolate of *V. inaequalis* and partially digested fractionated DNA (16-20 kb) and BamHI/EcoRI digested lambda EMBL3 DNA with ligated DNA ligase and packaged to prepare a library. The library was screened for clones with a heterologous *Erysiphe graminis*  $\beta$ -tubulin probe. DNA sequence analysis of the clones showed extensive sequence similarities with the probe thereby confirming that the  $\beta$ -tubulin gene has been cloned.

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## THE EXPRESSION OF RESISTANCE OF *USTILAGO AVENAE* TO TRIADIMENOL IS AN INDUCED RESPONSE.

A strain of *U. avenae* sensitive to triadimenol (sen) and a resistant laboratory mutant (rl) were treated with triadimenol (2mg/L) after 15 hr of growth in liquid culture. Initially, reproduction of both strains was almost completely blocked; however, the inhibitory phase was transient for rl, and full growth resumed after 10hr. This pattern of initial growth inhibition and subsequent recovery was correlated with a decline of sterol precursors, as analyzed by GC-MS. Although precursors (pre-dominantly 24-methylenedihydrolanosterol) accumulated during the phase of growth inhibition, and also were still prominent at the onset of renewed growth, they were absent after 24 hr of treatment with triadimenol. Pulse-labeling of sterols at

various time intervals after treatment with the inhibitor revealed that the continuous disappearance of precursor sterols is not explained by dilution of the inhibitor from the target site.

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## BASELINE-SENSITIVITY OF THREE POPULATIONS OF *VENTURIA INAEQUALIS* TO FLUSILAZOLE.

One hundred monoconidial isolates of *V. inaequalis* were collected from each of two abandoned orchards (orchards 1 and 2), where no sterol demethylation inhibitors (DMI) had been used, and from a research orchard where DMI fungicides had been used for 12 years (orchard 3). The mean ED<sub>50</sub> values based on colony diameter were 0.0083  $\mu$ g flusilazole/ml, 0.0072  $\mu$ g/ml, and 0.0105  $\mu$ g/ml for orchards 1, 2, and 3, respectively. ED<sub>50</sub> values for individual isolates ranged from 0.002 to 0.064  $\mu$ g/ml, 0.0001 to 0.0469  $\mu$ g/ml, and 0.0011 to 0.1108  $\mu$ g/ml, in orchards 1, 2, and 3, respectively. There was no significant difference between the mean of the log<sub>10</sub> transformed ED<sub>50</sub> values of any orchard. Our results indicate that the three populations examined had similar mean ED<sub>50</sub> values observed in our study also indicates that small sample sizes are unlikely to represent accurately the sensitivity of populations of *V. inaequalis* to DMI fungicides.

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## HOMOLOGY BETWEEN THE COPPER RESISTANCE OPERON OF *PSEUDOMONAS SYRINGAE* PV *TOMATO* AND PLASMIDS IN COPPER-RESISTANT STRAINS OF *XANTHOMONAS CAMPESTRIS* PV *VESICATORIA* AND *ERWINIA HERBICOLA*.

Copper-resistant strains of *Xanthomonas campestris* pv *vesicatoria* and *Erwinia herbicola* were isolated from a tomato leaf sample with bacterial spot disease. The *X. c. vesicatoria* strain grew on media supplemented with up to 1.5 mM cupric sulfate, and the *E. herbicola* isolate grew on

media with 2.6 mM cupric sulfate. Southern blot experiments showed homology between the copper resistance operon of *Pseudomonas syringae* pv. tomato and a 100 kilobase plasmid in the *X. c. vesicatoria* strain. A larger plasmid of about 200 kilobases in the *E. herbicola* strain hybridized with the *P. s.* tomato copper resistance operon. No homology was detected between the *P. s.* tomato copper resistance operon and DNA of copper sensitive strains of either *X. c. vesicatoris* or *E. herbicola*.

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### VARIATIONS IN TOLERANCE TO BENOMYL AMONG COLLETOTRICHUM CLOESPORIOIDES ISOLATES FROM MANGO.

In 1987 and 1988 a mango grove located in Dade County Florida USA lost over 50% of the crop to mango anthracnose caused by *C. gloeosporioides*. The grove was sprayed with 1 1/2 lbs of benomyl weekly in flower and every 3 to 4 weeks after fruit set by the owner in 1987 and by a professional grove management company in 1988, with no noticeable control of anthracnose in both years. In the summer of 1988, 100 infected fruits were harvested randomly from the grove from which 84 single spore colonies were isolated. The isolates were screened at 0, 1, 10, and 100 ppm of benomyl. Out of 84 single spore colonies, nearly 40% were tolerant to 10-100 ppm of benomyl, while 60% were sensitive, showing little or no radial growth. These results may explain the lack of anthracnose control by benomyl in the grove.

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### SMALL, CONJUGATABLE PLASMID IN COPPER-RESISTANT STRAINS OF XANTHOMONAS CAMPESTRIS PV VESICATORIA. V. DITTAPONGPITCH.

Thirty-two strains of *Xanthomonas campestris* pv. *vesicatoria* isolated from pepper and tomato were tested for sensitivity to 200 g/ml copper sulfate in sucrose peptone agar. Sixty percent were copper resistant. Plasmid profiles indicated the presence of at least two plasmids in all strains.

All copper-resistant strains contained an approximately 3 kbp plasmid. This plasmid was transferred via conjugation of copper-sensitive strains. Transconjugates contained the 3 kbp plasmid and were copper resistant. Preliminary analysis indicated the plasmid was digested by restriction enzymes PstI, Sau3A, AluI, and TaqI, but not by EcoRI, BamHI, HindIII, or XhoI.

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### FUNGICIDE RESISTANCE IN BOTRYTIS CINEREA ISOLATES FROM PENNSYLVANIA GREENHOUSES

Twenty *Botrytis cinerea* isolated from infected greenhouse floricultural crops in Pennsylvania were grown on a range of concentrations of benomyl, chlorothalonil, cupric hydroxide, mancozeb, thiophanate methyl + mancozeb, vinclozolin, and zineb *in vitro*. Five isolates were resistant to only benomyl and 14 were resistant to both benomyl and vinclozolin. Isolates with fungicide resistance infected and sporulated on excised geranium (*Pelargonium*) leaf disks that had been treated with the label rate of the fungicide to which they were resistant. Linear growth rates and sclerotium formation *in vitro* and sporulation *in vivo*, used as saprophytic and parasitic fitness parameters, were compared among isolates.

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### DIAGNOSTIC MEDIA FOR THE DETECTION OF FUNGI (*BOTRYTIS CINEREA*) RESISTANT TO VINCLOZOLIN AND BENOMYL.

A diagnostic medium was developed for the detection of *Botrytis cinerea* strains resistant to vinclozolin and benomyl. The medium contains 0.04% (w/v) brom cresol purple, 10% 0.1N NaOH, and 2% agar. After autoclaving, filter-sterilized dextrose is added to 4% then 40 ppm vinclozolin or 10 ppm benomyl and 50 ppm streptomycin sulfate are added. Germination and growth of resistant spores causes a color change from red to yellow in 18-48 hours after inoculation. Laboratory and field tests demonstrated selectivity against fungal contaminants, making the medium useful for field monitoring of resistance. Comparisons between this method and other techniques such as agar diffusion tests and spore

germination on fungicide amended media (PDA, MA or WA) showed excellent correlations. This medium has also been used for the detection of resistant strains of *Monilinia fructicola* to benomyl.

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## WRCC-60 RESEARCH PROGRESS REPORT--HAWAII

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### Insecticide Resistance in Diamondback Moth (*Plutella xylostella*)

We are monitoring levels of susceptibility to pyrethroids in field populations. We are also quantifying variation among field populations in susceptibility to *Bacillus thuringiensis* (Bt). We are initiating a project to determine the genetic basis of variation in susceptibility to Bt.

### Insecticide Resistance in *Liriomyza* Leafminers and Their Parasitoids

We are determining susceptibilities of *L. sativae* and *L. trifolii* to permethrin and fenvalerate at 10 sites throughout Hawaii using diagnostic assays.

We are determining susceptibility to insecticides in *Diglyphus begini*, the most abundant parasitoid of *Liriomyza* leafminers in Hawaii. *D. begini* were more tolerant to oxamyl and fenvalerate than permethrin and methomyl. The fenvalerate LD<sub>50</sub> was 14-fold greater for females from a heavily treated bean field compared with females from an untreated population. The fenvalerate LD<sub>50</sub> of the heavily treated population was 20 times more than the recommended field rate. Susceptibility in the parasitoids *Ganaspidium utilis* and *Chrysocharis oscinidis* is also being measured.

### Resistance Management Theory & Practice

We are analyzing an extensive database and conducting simulations to clarify the influences of generation turnover, introduced vs. native status, taxonomic order, and pest severity on evolution of resistance.

Sequences, mixtures, rotations, and mosaics are potential strategies for resistance management. Review of findings from theoretical models suggests that, under certain conditions, mixtures might be especially effective for resistance management. The assumptions of such models, however, are probably not widely applicable. Potential disadvantages associated with mixtures that are usually not considered in modeling studies include disruption of biological control, promotion of resistance in secondary pests, and intense selection for cross-resistance. Results from limited experimental work suggest that pesticide combinations do not consistently suppress resistance

development. More thorough evaluation of tactics that seek to optimize benefits of more than one insecticide will require rigorous experiments with specific pesticide-pest combinations. Because of the difficulty in generalizing results across systems and the potential negative impacts of multiple insecticide use, emphasis on minimizing insecticide use is recommended.

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## Resistance to Soil Insecticides Widespread in New York Populations of the Colorado Potato Beetle

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### Summary

Resistance is widespread throughout New York State to insecticides commonly applied in granular formulations to the soil at planting for control of Colorado potato beetle. Resistance appears to be severe enough that very little mortality is caused by Furadan, the only soil insecticide still registered for control of Colorado potato beetle, at least in most areas of the state. Similarly severe resistance also occurs to Thimet, which is no longer labeled for Colorado potato beetle control. Di-Syston has not been considered effective against Colorado potato beetle in New York State for over ten years. Growers, Cooperative Extension personnel, consultants and other professionals can now test for resistance to Furadan and Thimet through simple dip techniques. Soil applications of Furadan and Thimet appear to select more strongly for insecticide resistance than foliar applications of related insecticides. This probably occurs because soil-applied insecticides provide a long-lasting concentration of insecticide in the plant. If this effect also occurs in other pests, an important tactic for resistance management may be to avoid the use of soil insecticides.

### Current Status of Resistance

To examine the effectiveness of soil insecticides against the current background of insecticide resistance, we obtained collections of overwintered adult beetles from six counties. These adults were held in the laboratory for several days and fed high quality potato foliage to encourage egg production. Potatoes (cv. Katahdin) were planted on June 5, 1989 and treated with maximum labeled rates of Thimet 20G, Furadan 15G, Di-Syston 15G or without insecticides (an untreated check) at the Cornell University Vegetable Research Farm in Freeville, NY. About 50% of the plants had emerged from the soil by June 22, 1989. Egg masses produced by the adults collected in each county were placed in cages on the soil

insecticide-treated and untreated potato plants at Freeville beginning June 27. We also monitored egg laying and larval survival of native beetles at Freeville and the survival of larvae from a population originally collected in North Carolina where the Colorado potato beetle is highly susceptible to insecticides.

The native population of Colorado potato beetles at Freeville is moderately susceptible compared to others in the state, but none of the soil insecticide treatments provided more than 20% mortality of the larvae. More than 80% mortality of the susceptible North Carolina larvae was achieved among larvae placed in the field as eggs June 27-30 on plants treated with Furadan and Thimet. No significant mortality was observed in the offspring of adults collected from any of the New York counties placed in the field after July 5. However, these insecticides provided 30 - 40% mortality of the highly susceptible larvae from North Carolina in the period following July 5. At no time did Di-Syston provide more than 20% mortality, even among the susceptible larvae from North Carolina.

We have also developed three different laboratory methods for determining resistance of larvae and adults to Thimet and Furadan. Each of these procedures results in 95-100% mortality of the susceptible North Carolina beetles, but less than 10% mortality of beetles obtained from most locations in New York.

We recognize that soil insecticides may still be useful for control of other pests, such as the potato leafhopper. In addition, we observed in our small plot trials that the native Freeville adults laid fewer egg masses on soil-insecticide treated plants. However, this could have resulted from an ovipositional preference phenomenon since our treated and control plots were at their greatest and least separation from each other, within 18 and 3 feet of each other, respectively. In the absence of such choices in large treated plantings, the adults may simply deposit their eggs unaffected by the presence of soil insecticide. We will be seeking cooperating farms next year to examine this hypothesis in large scale plantings and to examine the cost-effectiveness of alternatives to soil applications of insecticides that can control potato leafhopper while not selecting strongly for resistance in the Colorado potato beetle. Where soil insecticides are necessary for control of potato leafhoppers, Di-Syston may provide an alternative that does select strongly for resistance in Colorado potato beetle.

Soil applications of Furadan and Thimet appear to select more strongly for insecticide resistance than foliar applications of similar insecticides. In field trials in nearby plots, the effects of foliar insecticides were also investigated by placing early instar larvae in cages on treated plants. Heterozygous (F<sub>1</sub>) larvae resistant to organophosphorous and carbamate insecticides survived both soil and foliar applications throughout the trials. However, whereas soil insecticides killed significant numbers of susceptible North Carolina larvae for at least three weeks after emergence of the plants, foliar applications of organophosphorous and carbamate insecticides, including Furadan, did not kill larvae longer than one week.

It has long been recommended in resistance management to avoid persistent pesticides and formulations. If the example of the Colorado potato beetle is typical, a direct and easily affordable aid to the management of resistance may be to avoid the use of soil insecticides.

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## Farmer Practicable Procedure for Detection of Soil Insecticide resistance in Colorado Potato Beetle

### Equipment Needed:

Plastic teaspoons, gallon milk jugs or similar gallon containers, Disposable cups with lids, permanent marker, tea strainer, rubber gloves, taper towels, newspaper, respirator, liquid chlorine laundry bleach, commercial formulations of insecticides (Thimet 20G, Furadan 4F)

### Procedures:

**Beetle Collection.** Collect 20-40 adult Colorado potato beetles for each soil insecticide to be tested. Collect the adults from as many plants as possible and in several places in the field to minimize the possibility that they are closely related. The larger the sample size the better, but there is probably little to be gained by sample sizes of much greater than 50 per insecticide. Hold the adults in cups out of direct sunlight

### Preparation of Insecticide Solutions.

Mark all containers and pipettes (or teaspoons) with the name of the insecticide to be tested and a skull and crossbones (poison symbol), and signal word "Poison!" Handle all insecticides with caution; wear rubber gloves when handling either insecticide or insecticide-treated beetles. Label at least two cups for each of the insecticides to be tested, one for dipping and the others to hold the insects after they have been dipped. Similarly label another set of cups with the word "water".

- To prepare Thimet 20G at the proper concentration (18 gm of granules per liter water), add one teaspoon of Thimet granules to a cup ( 8 fluid oz) of water. Allow to stand for about ten minutes to partially dissolve the granules, then stir thoroughly.
- To prepare Furadan 4F at the proper dilution (1 part to 250), add 3 teaspoons of Furadan to 1/2 gallon of water. Rinse the teaspoon in the gallon container. Mix or shake thoroughly. Add water to bring the total volume to one gallon. Mix and shake again. Pour the solution into one of the disposable cups.

- In another clean disposable cup, add only water.

### Dipping.

Follow this sequence. Place up to 10 adult beetles in the tea strainer and dip into the cup containing only water, swirling gently for 5 seconds. Remove the strainer, blot the excess fluid on newspaper or a paper towel, transfer the beetles into a labelled clean disposable cup, add insecticide-fed potato foliage, and place a lid on the cup. Repeat this process with each of the insecticide solutions. Place the treated beetles and their cups out of direct sunlight but where temperatures are close to those in the field (e.g., shade of a barn). *Don't forget to dip beetles in a water check; this is absolutely necessary to ensure that the beetles didn't die for some reason other than pesticide exposure!* After use, triple rinse all containers (disposable cups and lids, tea strainer, gallon jug, plastic teaspoons) with water and then soak in a 10% solution of chlorine laundry bleach overnight. Save used Furadan solutions and rinses in a labeled container and store in your pesticide storage facility for later field application. Bury the Thimet solution in the crop field. Destroy (break, cut, or crush) all contaminated teaspoons, gallon jugs, disposable cups and lids and discard in the trash.

Score mortality 24 hours later by transferring the beetles onto a paper towel or newspaper. Score as dead any adult that cannot right itself or crawl away after 5 minutes. Caution: some adults will "play possum" before crawling away so don't score them immediately after removing from the cup! Alternatively, place an incandescent light bulb of at least 150 watts several inches above the adults. The light and heat will rapidly stimulate activity in healthy adults. Record your results. Note: Less than 50% mortality probably indicates the soil insecticides will not kill a significant number of larvae under field conditions.

Finally, triple rinse the disposable cups and lids with water and then soak them and the newspaper or paper towels used for blotting in a 10% solution of chlorine laundry bleach overnight. Save rinses from the cups containing Furadan-treated beetles and store in your pesticide storage facility for later field application. Bury the rinses from the cups containing Thimet-treated beetles in the crop field. Destroy (break, cut, or crush) disposable cups and lids and discard in the trash along with the blotting newspaper.

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## Susceptibility of *Heliothis* spp. to Pyrethroids in Missouri during 1988 and 1989.

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In response to the development of resistance in tobacco budworm (TBW), *Heliothis virescens* (F.), to pyrethroids, a resistance monitoring program was initiated in Missouri. A pheromone trap system was set up throughout the state, with the majority of the traps located in the cotton producing region of southeast Missouri. Adult male *Heliothis* were collected from pheromone traps and tested for susceptibility to pyrethroids using the adult vial bioassay.

Populations of corn earworm (CEW), *H. zea* (Boddie), were tested using 10, 5, 1, 0.5 and 0.1 g of cypermethrin in per vial. Twenty moths were used for each dose. Based on the low LD<sub>50</sub>'s of CEW populations in southeast Missouri in 1988, pyrethroid resistance was not present. However, statistically higher responses were documented in migratory populations in central Missouri, an area with little to no insecticidal selection pressure. This may indicate the possibility of influxes of tolerant moths into the area. Too few TBW were collected to perform any bioassays. Although, analysis of 1989 CEW bioassays has not been completed, preliminary results appear similar to those in 1988.

Based on two seasons of monitoring, we conclude that CEW is by far the main *Heliothis* sp. in southeast Missouri and that populations of this insect are susceptible to pyrethroids.

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## Insecticide Resistance in Western Flower Thrips in Missouri

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Control failures of organophosphate, pyrethroid and carbamate insecticides used against the western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), have been reported in several locations throughout the state of Missouri by greenhouse operators. Control failures were occurring despite the use of frequent and heavy applications of insecticides. The glass vial bioassay used to document resistance in *Heliothis* spp. was slightly modified for these studies. Vials were coated with doses of diazinon (100, 50, 10, 5, 1, 0.5 and 0.1 g/vial) or cypermethrin (10, 5, 1, 0.5 and 0.1 g/vial). Food consisting of one square cm of flower leaves was added six hours after placing the thrips in the vials. Thrips were checked for mortality at 24 hours.

Adult female WFT were collected from a colony that was obtained from a producer in Kansas City MO. This

producer had experienced control failures of WFT by organophosphate, carbamate and pyrethroid insecticides. Identical tests were performed on adult female WFT collected from a greenhouse in Columbia that had no control problems with any of the three classes of insecticides.

With diazinon the  $LC_{50}$ 's of the "Kansas City" and "Columbia" strains were 49.3 and 4.6 g per vial, respectively. With cypermethrin the  $LC_{50}$  of the "Columbia" strain was 3.7 g per vial, no mortality was observed with the "Kansas City" strain even at concentration of 10 g per vial. Thus, resistance is present in WFT to organophosphate (diazinon) and pyrethroid (cypermethrin) insecticides. Since the "Kansas City" strain had no history of exposure to either of these specific compounds, it appears that there is cross resistance within classes as well as between classes of insecticides.

Future research will focus on determining the susceptibility profile between and within different classes of insecticides. Mechanistic studies also will be conducted.

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## RECENT DEVELOPMENTS IN RESISTANCE DETECTION IN ANOPHELINE VECTORS OF MALARIA

In Guatemala, changes in cross-resistance relationships and the relative significance of resistance mechanisms have been documented using a microplate resistance monitoring scheme. The frequency and level of elevated esterase resistance to fenitrothion has increased in *Anopheles albimanus* throughout the Pacific Coast. Cross-resistance to deltamethrin has been shown to be due to the elevated esterase at higher levels. The highest levels of esterase are now producing cross-resistance to malathion. The frequency of the insensitive acetylcholinesterase mechanism has declined precipitately, reflecting a country-wide shift in agriculture and public health from fenitrothion, which selects for insensitive acetylcholinesterase as well as the elevated esterase, to deltamethrin, which selects for the esterase only.

We have concentrated on integrating kinetic (time-mortality) bioassays (conducted using simpler, less expensive materials) with microplate-based assays to produce a comprehensive system for detecting resistance and mechanism. A means has been devised to express results of both types of assays in a similar fashion on the same chart. Resistances to organophosphates, carbamates, organochlorines, and pyrethroids have now been detected and mechanisms identified in the field (in Ecuador) using this approach. Resistance mechanism frequencies and resistance levels for the known resistance mechanisms in all

currently-used insecticide classes may now be conveniently derived from the same mosquitoes.

A microplate-based method for detecting glutathione s-transferase DDT resistance in *Anopheles albimanus* and *Anopheles arabiensis* has been developed. This technique uses only a small fraction of a mosquito homogenate, but requires UV detection.

The *kdr* resistance mechanism has been selected from Guatemalan *Anopheles albimanus*. The mechanism gives high-level resistance to DDT, but lower levels for the pyrethroids permethrin and deltamethrin. The resistance is temperature-sensitive and crosses to methoxychlor. An experimental protocol has been developed and tested which allows the glutathione s-transferase and *kdr* mechanisms to be conveniently distinguished in the field.

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## Recent Publications on Resistance

- A new book, -Pesticide Resistance in Arthropods- edited by R. Roush and B. Tabashnik will be published by Chapman and Hall early in 1990. Contributors include J. Bloomquist, B. Croft, J. Daly, R. French-Constant, G. Georgioui, M. Hoy, F. Plapp, Jr., D. Prec, J. Scott, and D. Soderlund.
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