Resistant Pest Management

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I R A C
INSECTICIDE RESISTANCE ACTION COMMITTEE

News and Review

Pesticide Resistance Management Activities by the U.S. Environmental Protection Agency

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Historically, the U.S. Environmental Protection Agency (EPA) has considered resistance and resistance management in its decisions to register and regulate pesticides. With a greater public focus on pollution prevention and pesticide reduction, the EPA believes that it is important to implement effective resistance management strategies. The Pesticide Resistance Management Workgroup of the Office of Pesticide Programs (PRMW)[1] was created, in part, to examine EPA's role in resistance pest management and to provide policy options to regulate pesticides to reduce selection for resistance.

In 1995, Lewis and Matten reviewed these activities for *Resistant Pest Management*. The EPA does not have an official policy on pesticide resistance management or a standard of data requirements for pesticide resistance management, although some regulatory decisions have included the effects of pesticide resistance. The EPA has addressed pesticide resistance issues under the following sections of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Section 18 (emergency exemption decisions), Section 6 (special review decisions based on unreasonable human health and/or environmental risks), and Section 3 (registration decisions).

This article updates the EPA's activities in pesticide resistance management. Three topics are discussed: (1) cooperation with Canada on a voluntary initiative on pesticide labeling for pest resistance management, (2) Section 18 policy revisions and resistance management, and (3) public hearings on resistance management considerations for plant-pesticides.

COOPERATION WITH CANADA ON A VOLUNTARY INITIATIVE ON PESTICIDE LABELING FOR PEST RESISTANCE MANAGEMENT

In December 1996, Canada's Pest Management Regulatory Agency (PMRA) proposed guidelines on pesticide labeling for pest resistance management. This was a voluntary initiative. Pest resistance management was based primarily on rotating pesticides with different modes of action.

The EPA provided comments to the PMRA on this voluntary initiative. The EPA believes that Canada's proposed guidelines on labeling pesticides for pest resistance management offer the U.S. an opportunity for a cooperative, international approach to pest resistance management. In principle, the EPA supports such an approach. Like Canada's PMRA, the EPA supports the development of sustainable pest management systems based on the incorporation of sound environmental strategies. Pest resistance management in conjunction with alternative pest management strategies and integrated pest management programs can make significant contributions to reducing pesticide risks to humans and the environment. The EPA will continue to work with the PMRA on this initiative to label pesticides for pest resistance management in Canada and the U.S.

SECTION 18 POLICY REVISIONS AND RESISTANCE MANAGEMENT

A second area where pest resistance management has become a key issue is emergency exemptions as described in Section 18 of FIFRA. These exemptions are issued on a state or regional basis. They allow a pesticide to be applied to a crop or in a situation not stated on the pesticide label, or to apply a pesticide not yet registered by the EPA. More than 30% of all emergency exemption requests in the last five years were associated with con-

1 Members of the PRMW are: Sharlene Matten (leader), Neil Anderson, Leonard Cole, Tobi Colvin-Snyder, Frank Ellis, Mary Beth Gleaves, Paul Lewis, Eric Maurer, Robert Rose, Douglas W.S. Sutherland, Dennis Szuhay, Steve Tomasino and Sandy Zavolta.

trol failures due to pest resistance to the alternative registered pesticides. Frequently, the EPA is urged to issue emergency exemptions for two or more pesticides with different modes of action in response to existing resistant pest problems or when a pest has a long history of resistance development. These exemptions seek to prevent pest resistance to pesticides before they are even registered. However under Section 18 guidelines, EPA can only grant an emergency exemption based on resistance when 1) pest resistance to the registered alternative(s) has already developed, 2) a pest control emergency exists, 3) the currently registered pesticides are ineffective, and 4) a significant economic loss is expected.

In November of 1996, a stakeholder meeting was held in Washington D.C. to consider revisions to the Section 18 regulations, including these emergency exemptions based on pest resistance management. At this meeting, the EPA decided to revise its Section 18 policy to allow emergency exemptions for two or more requested pesticides (with different modes of action) for resistance management based on strict criteria. These criteria must demonstrate a potential for pesticide failure and subsequent significant economic loss as a direct result of pest resistance. The EPA seeks to eliminate unfounded claims of resistance problems and limit emergency exemptions to the most serious situations associated with resistance.

Clarity in EPA's guidance and regulations for issuing emergency exemptions should reduce the number and frequency of Section 18 emergency exemptions considered and issued. Further reductions are anticipated as the number of pesticides with different modes of action become available. The EPA will continue to develop criteria for granting emergency exemptions based on pest resistance considerations.

PUBLIC HEARINGS ON RESISTANCE MANAGEMENT CONSIDERATIONS FOR PLANT-PESTICIDES

Recent attention has focused on the potential development of resistance to the δ-endotoxins of *Bacillus thuringiensis* (Bt) genetically-engineered into plants (Bt transgenic plants). The EPA calls these pesticides "plant-pesticides". There are other plant-pesticides that do not involve the insertion of Bt δ-endotoxins. However, pesticidal proteins in Bt plant-pesticides (like the CryI δ-endotoxins) are also widely used in a variety of Bt foliar sprays applied to many crops. Thus, pest resistance to Bt plant-pesticides could also affect the efficacy of the Bt foliar sprays. Therefore, industry, academia, government, user groups and environmental groups believe that the protection of Bt transgenic plants is important and that resistance management is critical.

Since May 1995, EPA has conditionally registered several Bt plant-pesticides including: (1) Bt-potato (Bt Cry IIIA δ-endotoxin) to control Colorado potato beetle, (2) Bt-corn (Bt CryIA(b) and CryIA(c) δ-endotoxins) to control European corn borer, and (3) Bt-cotton (Bt Cry IA(c) δ-endotoxin) to control pink bollworm, cotton bollworm and tobacco budworm. There are several different Bt-corn registrations held by different companies. For all of these Bt plant-pesticides, resistance management was a serious consideration to EPA. Resistance management plans were developed by the registrants and evaluated by the EPA. Long-term resistance management plans based on target pest biology and behavior, refugia, dose deployment adequacy, monitoring and reporting were conditional for Bt-corn and Bt-cotton registration.

A subpanel from the OPP Science Advisory Panel (SAP) reviewed the pesticide resistance management plan for Bt-potato in March 1995. No additional requirements concerning resistance management were necessary for the Bt-potato registration. SAP recommended that monitoring for resistance and dialogue with EPA continue and that the registrant update the resistance management plan as additional information became available. The SAP subpanel recommended that seven elements, identified by the EPA, be considered while updating the resistance management plan. These elements were: (1) knowledge of pest biology and ecology, (2) appropriate gene deployment strategy, (3) appropriate refugia (primarily for insecticides), (4) monitoring and reporting incidents of pesticide resistance development, (5) employment of IPM, (6) communication and educational strategies on product use and (7) development of alternative modes of action.

Today, questions continue to arise about the adequacy of these resistance management plans for Bt-potato, Bt-corn, and Bt-cotton. This past summer, Bt-cotton failures (Bollgard cotton) associated with cotton bollworm resistance were reported. Evidence collected by industry, academia, and government agencies (EPA and USDA) show that these reports were unsubstantiated. High bollworm infestations,

rather than resistance, led some growers to spray insecticides on their fields planted in Bollgard cotton. Nevertheless, EPA did hold two public hearings (Washington, D.C., March 1997 and College Station, TX, May 1997) to reevaluate the registration requirements for resistance management, particularly for Bt plant-pesticides. Four issues were discussed: (1) the requirement for resistance management plans, (2) scientific needs for resistance management plans, (3) the use of "public good" criteria for the requirement of resistance management plans, and (4) the performance of Bt-cotton. Full transcripts of both public hearings including comments from industry, academia, USDA researchers, users, and environmental groups are available to the public. EPA will evaluate these comments and decide on appropriate actions. If necessary, SAP members will meet in Fall of 1997 to evaluate specific data needs for updating the long-term resistance management strategies for Bt-corn and Bt-cotton.

GOALS

It is good public policy to manage pesticide use to minimize the development of pesticide resistance. Effective pesticide resistance management can reduce the total burden on the environment and reduce the overall human and ecological exposure to pesticides. Effective pesticide resistance management will prolong the availability and effectiveness of pesticides and provide growers access to a wider selection of pest control tools to manage pest populations. The EPA supports the efforts of registrants, academia, crop consultants, USDA researchers and extension agents, and pesticide users to promote pesticide resistance management through development of pesticide resistance management plans, appropriate pesticide labeling and education programs. The EPA will not allow this focus on pesticide resistance management to overly burden of the regulated community, jeopardize the registration of reduced risk pesticides, or exclude conventional pesticides that contribute to the overall concept of integrated pest management. The EPA continues to evaluate and refine the role that pest resistance management has in pesticide regulatory decisions.

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Resistance Around the Globe

Barnyardgrass (*Echinochloa crus-galli*) Resistance to Both Butachlor and Thiobencarb in China

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Grass species in the genus *Echinochloa* are serious pests in agriculture throughout the world and two species are considered the third and fourth most serious weeds in the world (Holm *et al.* 1977, Holm *et al.* 1979). They are especially competitive in rice where herbicide applications are economical even for growers among the developing countries (Ampong-Nyarko & De Datta 1991, Labrada *et al.* 1994). The following herbicides are frequently applied for the selective control of *Echinochloa* species in rice: 1) the photosystem II-inhibiting amide herbicide – propanil, 2) the chloroacetamide herbicide – butachlor, and 3) the thiocarbamate herbicide - thiobencarb. The sites of action for latter herbicides are unknown. Plants treated with thiobencarb display symptoms distinguishable from plants treated with butachlor, and thus different sites of action are proposed for these herbicides.

Despite the long history of herbicide use in rice, *Echinochloa* resistance to herbicides is a recent phenomenon (Gressel & Baltazar 1997). All three groups of herbicides used to control *Echinochloa* have been extensively applied to rice for over 20 years. These herbicides are not prone to rapid resistance evolution. However, *Echinochloa* has evolved resistance to atrazine at the target site, as well as resistance that is clearly not at the

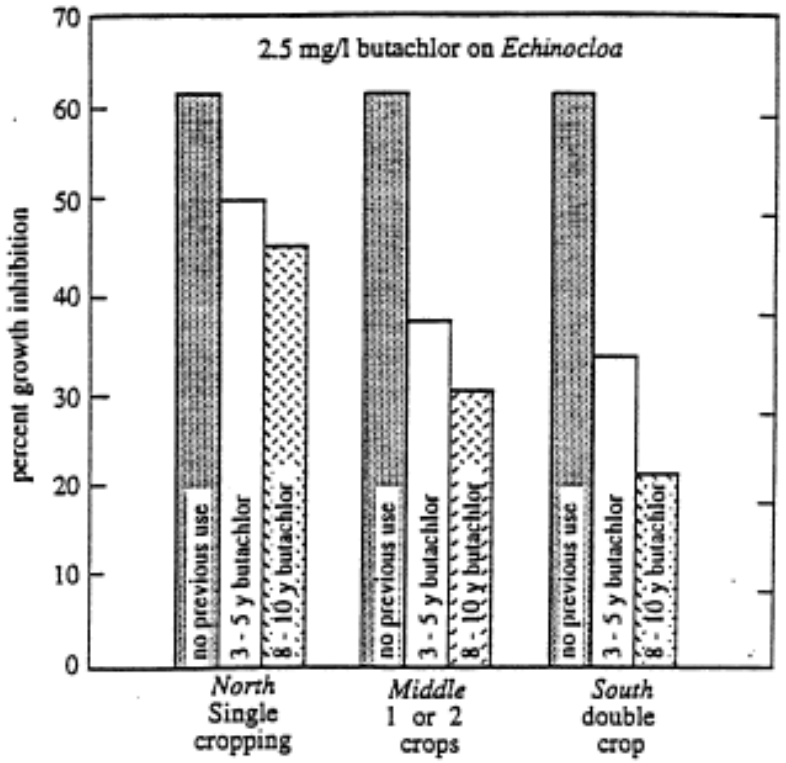

Figure 1. Appearance of *Echinochloa crus-galli* resistant to butachlor. The magnitude of resistance is a function of both the total number of treatments as well as the number of treatments per year (Huang & Lin 1993).

direct target (Gressel *et al.* 1982). Presumably propanil affects the same target site but selection pressure is not as strong as atrazine. Nevertheless, propanil resistance has evolved in *Echinochloa* spp. in Greece (Giannopolitis & Vassiliou 1989), Columbia (Fischer *et al.* 1993), U.S. (Baltazar & Smith 1994) and Costa Rica (Leah *et al.* 1994). This resistance is associated with elevated levels of herbicide-degrading acyl amidase in resistant weed biotypes and can be overcome by amidase inhibitors (Valverde 1996). Interestingly, these inhibitors do not prevent the herbicide degradation by the same enzymatic pathway in rice, so selectivity between rice and the weed remains (Valverde 1996).

In the mid 1970's, China began to rely on widespread applications of herbicides in rice. Each year, the area treated with herbicides increases by about 20%. About ten herbicides are marketed in China and two are commonly applied for weed control in rice. Butachlor and thiobencarb have been applied to rice grown in northeast China for over ten years, while butachlor has been applied in the central and southern China for over 15 years.

We documented *Echinochloa crus-galli* resistance to butachlor in 1993 (Figure 1). The magnitude of weed resistance in each population was a function of exposure to herbicide applications (Figure 2A). Such "creeping resistance" (Gressel 1995) is indicative of, but not proof for, a polygenic or other incremental genetic control mechanism (Gressel *et al.* 1996). Furthermore, these butachlor-resistant populations of *Echinochloa* were also resistant to thiobencarb (Figure 2B).

It is unclear whether thiobencarb resistance in Figire 2 is cross resistance due to a single mechanism, or multiple resistance due to sequen-

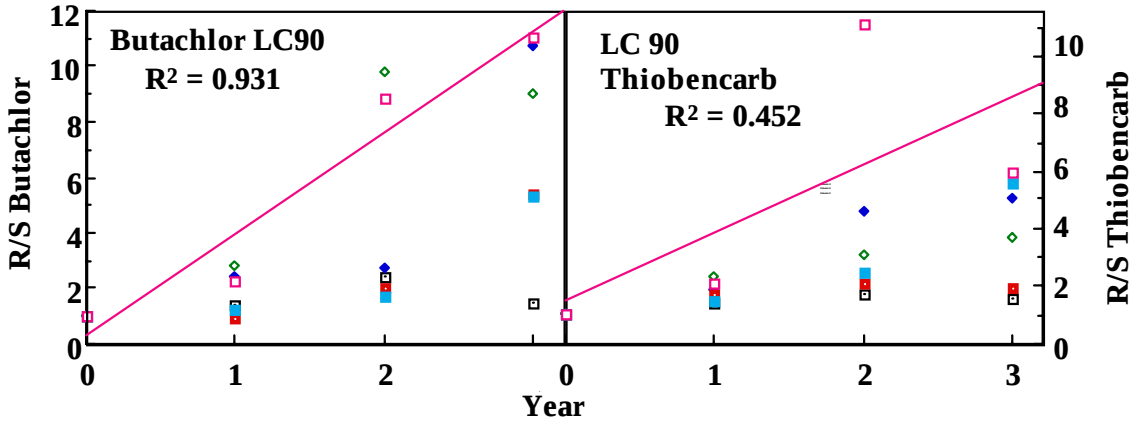

Figure 2. The effect of selection with butachlor on resistance of *Echinochloa* populations to (A) butachlor and (B) thiobencarb. Each symbol represents the same population over each year in both treatments. The populations were treated with thiobencarb or butachlor prior to the period monitored. The resistance factors (R/S) are based on the LC_{90} from probit analysis of dose-response curves. In year 0, the populations were not exposed to either herbicide. In years 1 to 3, the populations were exposed to butachlor only (Huang *et al.* 1995a, Huang *et al.* 1995b).

tial selections for different mechanisms. These farmers treated their fields with either thiobencarb or butachlor between 1980 and 1990, but only with butachlor in the three years illustrated in Figure 2. Still, most of the populations under recurrent butachlor selection were co-resistant to thiobencarb. Different biochemical mechanisms were associated with these resistances as indicated by the inhibitor studies. The resistance due to butachlor was overcome by a proprietary amide hydrolase inhibitor, whereas the resistance to thiobencarb was inhibited by diethylthiophosphophenol (SV-1) (Table 1). This suggests that selective synergists will suppress resistance in the weed without affecting rice (Gressel 1990). These synergists may allow farmers to continue controlling resistant *Echinochloa* with these two herbicides.

These herbicide-resistant *Echinochloa crus-galli* biotypes cover an estimated 2 million hectares of the rice growing area in China. This represents only 6% of the total area of rice planted in China, but the area affected is bound to increase as these herbicides continue to be applied year after year.

Table 1. Suppression of toxicity (butachlor and thiobencarb) by amide hydrolase and monooxygenase inhibitors. (Huang *et al.* 1994)

Herbicide	Herbicide inhibitor ratio	Amide hydrolase LC_{50} (mg/l)		Monooxygenase LC_{50} (mg/l)	
		S	R	S	R
Butachlor	alone	2.32	3.59	NA	NA
	10:1	1.86	2.85	NA	NA
	1:1	1.31	1.78	NA	NA
Thiobencarb	alone	3.22	5.44	1.62	2.14
	10:1	3.34	5.31	NA	NA
	1:3	NA	NA	2.05	1.60
	1:5	NA	NA	1.39	1.71

The amide hydrolase inhibitor is proprietary and the monooxygenase inhibitor was diethylthiophosphophenol.
NA = Not available.

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Biological Significance, Genetic Mechanism, and Potential of Esterase-Mediated Insecticide Resistance in the Greenbug

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The greenbug, *Schizaphis graminum* (Rondani)(Homoptera: Aphididae), has long been a pest of small grains. The greenbug population in the United States is a composite of many genotypes. Some individuals are capable of thriving on many crop cultivars, including those once resistant to this pest, and some individuals are resistant to insecticides once applied to control them. Management practices utilize natural enemies of the greenbug, as well as greenbug-resistant crop cultivars, and chemical insecticides. Thus, a basic understanding of greenbug biology will improve management strategies for this pest species.

Recent studies have elucidated the mechanism of organophosphate (OP) resistance in greenbugs. This involves both detoxification of the insecticide by esterases and target site insensitivity (Seigfried & Ono 1993). Two elevated esterase patterns (R1 and R2) from individual OP-resistant greenbugs have been identified on electrophoretic gels and each resistant insect had one elevated esterase, but not both (Wilde *et al.* 1994). These esterase polymorphisms have been characterized by Shufran *et al.* (1996) and associated with certain levels of insecticide resistance when compared to susceptible (S) clones (R2 > R1 > S).

Much attention has been given to insecticide resistance in the greenbug, biotype formation and, more recently, inheritance of specific characters for virulence to wheat and sorghum. With the laboratory breeding protocol developed by Puterka & Slosser (1983, 1986), it is possible to screen for and identify virulent genotypes not yet known to exist in the field (Ullah 1993). This protocol also allows researchers to create and test new genotypes for insecticide resistance before these genotypes appear in the field.

Drawing on the resistance mechanisms observed in both *Culex pipiens* (Diptera) and *Myzus persicae* (Sulzer) (Homoptera), we suspected that gene amplification may underlie esterase-mediated insecticide resistance (Field *et al.* 1988, Mouches *et al*. 1990) in greenbug. Typically, the amplicon is inherited as a single dominant gene (Takada 1978, Blackman *et al.* 1996, Ferrari & Georghiou 1991). In the peach-potato aphid *(M. persicae*), the expression of these esterase genes is due to DNA methylation (Field & Devonshire 1992).

To further our understanding of insecticide resistance in the greenbug, several studies were undertaken to determine the biological significance, the potential development of new resistant genotypes, and the underlying genetic mechanism of insecticide resistance in this species. Insecticide resistant and susceptible greenbugs were examined for their ability to enter the sexual phase, their fecundity through the sexual phase, the inheritance of insecticide resistance, and the genetic mechanism of insecticide resistance at the molecular level.

MATERIALS & METHODS

Sexual morph induction, crosses, and handling of eggs and hatched nymphs were performed as described by Puterka & Peters (1995). Individual crosses were made with a single male and five oviparae (egg-laying females). Esterase determination of field-collected greenbug clones, the sexual morphs derived from those clones, and any subsequent progeny resulting from crosses with those clones was conducted with native polyacrylamide gel electrophoresis as described by Shufran *et al.* (1996). Genomic DNA was isolated from greenbugs as described by Jowett (1986), then used for Southern blots (Southern 1975) and dot blots. Blots were probed with an E4 esterase gene fragment from the aphid *Myzus persicae* (Sulzer) (Field & Devonshire 1992). Insecticide resistance assays were conducted on greenbug clones with a surface-residue vial bioassay as described by Shufran *et al.* (1996).

RESULTS & DISCUSSION

Fecundity and sexual morph production

The reproductive potential for the two phenotypes (R1, R2) of insecticide-resistant greenbugs was compared to susceptible (S) greenbugs. Several clones (collected from Colorado, Kansas, and Texas) were induced into the sexual phase (when possible) and inbred or outcrossed to S clones. Most R2 clones could not be induced into the sexual cycle. Only oviparae produced from these clones entered the sexual phase. Seven of ten S clones, five of ten R1 clones, and six of thirty R2 clones entered the sexual cycle. Greenbug clones from the S, R1, and R2, were induced into the sexual cycle and inbred or crossbred

to determine their reproductive capacity. Crosses with susceptible females averaged the highest number of eggs per cross, followed by R1 females, and then by R2 females. This study substantiates that fecundity in the greenbug is variable and suggests that insecticide resistance may have an effect on the holocycle that results in reduced capacity for sexual reproduction.

Inheritance and molecular genetic mechanism

The genetics of organophosphate resistance including the underlying genetic mechanism and the mode of inheritance was investigated in the greenbug. Resistant greenbugs with the R1 and R2 esterase pattern and S greenbugs were induced into the sexual cycle, sib-mated and reciprocally crossed to determine the pattern of inheritance of esterase-mediated insecticide resistance. Examination of the esterase profiles of the sexual morphs produced showed no differences between the sexual morphs and the parthenogenic clones from which they originated. R1 males, in particular, did not segregate into susceptible and resistant esterase types as would be expected for a sex-linked trait, thus the R1 resistance gene lies on an autosome. Both resistances associated esterases in the greenbug are inherited in a simple Mendelian fashion as single dominant genes. Double heterozygotes, produced in the F1 generation in crosses between R2 and R1 insects were designated pattern 3 (R3). DNA from S, R1, R2, and R3 clones of the greenbug was used for Southern and dot blot hybridizations. Blots were hybridized with a probe containing a portion of an esterase gene from the peach-potato aphid, *Myzus persicae*. DNA from R1 and R3 aphids displayed a restriction fragment length polymorphism (RFLP) and contained a two-fold amplification in an esterase that was associated with resistance in *M. persicae*. Meanwhile, the R2 aphids showed no RFLP or evidence that an amplified esterase gene similar to that in *M. persicae* was present. Thus, it appears that the R1 and R2 elevated esterases are the result of unrelated genes, and/or mechanisms. The R1 related gene sequences are methylated differently in the resistant and susceptible clones. The exact role of DNA methylation in the greenbug is currently unknown, but it is likely that DNA methylation promotes gene expression.

Resistance in the R3 genotype

The R3 greenbug clone, created through laboratory breeding experiments between R1 and R2 insecticide-resistant clones, has not been discovered in the field. The response of the R3 clone to parathion and carbofuran was compared to S, R1, and R2 greenbug clones with an insecticide residue vial bioassay. All three resistant clones (R1, R2, and R3) were significantly more resistant to parathion than the S clone. The R2 and R3 clones were significantly more resistant to parathion than the R1 clone, but no significant differences occurred between the R2 and R3 clones. No significant differences were found among the clones when exposed to carbofuran, indicating little or no cross resistance. Therefore, control measures effective against R2 insects should be effective against R3 insects, if they are discovered in the field.

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Comparison of the Cotton Aphid Resistance Level between Xinjiang and Shandong Populations

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In 1997, over 2 million hectares of cotton were planted in Xinjiang, the largest cotton production area in China. A number of pests species are associated with cotton in this area including the cotton aphid (*Aphid gossipii*), cotton spider mite (*Tetranychus cinnabarinus*), cotton bollworm (*Helicoverpa armigera*), and another species, *Acyrthosiphon gossippii*. The cotton aphid is the most serious pest. In recent years, many pesticide companies and factories in China and abroad have gathered in Xinjiang, an important pesticide market.

During the 1980's, acreage was first planted in Shandong, an area now dominated by cotton aphid and bollworm populations with extremely high levels of pesticide resistance – the highest in China. We wish to avoid a repeat of the Shandong situation by learning from the experience and properly managing the use of pesticides to control pests and avoid resistance development in Xinjiang. This could prove important to the future of cotton production in China.

From 1995 to 1996, we monitored aphid resistance and field efficacy of twenty pesticides. The results were compared between Xinjiang and Shandong. Preliminary studies found cotton aphids from Xinjiang to be resistant to some pesticides.

In this report, we will examine the response of cotton aphid from Xinjiang and Shandong to eight major insecticides.

Table 1. Comparison the cotton aphid resistance level between Xinjiang and Shandong strains.

	LD50 (µg/head)					+/- Ratios*			
Pesticide Treatment	China Base line	Xinjiang (1996)	Shandong Base line (1980)	Shandong Highest	Shandong (1996)	Xinjiang 1996	Shandong Base line	Shandong Highest	Shandong (1996)
Deltamethrin	0.000025	0.01917	0.000011	0.41230 (1988)	0.045889	766.8	0.4	16,492.0	1,835.6
Fenvalerate	0.000383	2.510013	0.000251	0.728449 (1988)	0.057890	6553.5	0.7	1,901.2	151.1
Monocrotophos	0.0017	0.2428	0.0017	3.5158 (1988)	0.3216	142.8	1.0	2,068.1	189.2
Parathion	0.0211	0.8261	0.0211	6.2791 (1986)	0.6073	39.1	14.0	297.6	28.8
Omethoate	0.0042	0.0426	0.0042	5.5020 (1988)	0.3189	10.1	1.0	1,310.0	75.9
Methomyl	0.00469	0.017926	0.00469	0.1375 (1991)	0.0178	3.8	1.0	29.3	3.4
Aldicarb	0.0100	0.0573	0.0105	0.0394 (1984)	0.0220	5.7	1.0	3.9	2.2
Imidacloprid	NA	0.077929	NA	NA	0.056421	NA	NA	NA	1.4

*Ratio = LD_{50} sample population/ LD_{50} China Base line
NA = Not available

MATERIALS & METHODS

The resistance level (LD_{50}) in each aphid population was measured with the topical application method assay recommended by FAO (1980). The field efficacy of these eight insecticides were observed over one week at a field site in Shihenzhi, Xinjiang, 1996.

The response of cotton aphid to eight pesticides was bioassayed. These pesticides were deltamethrin, fenvalerate, monocrotophos, parathion, omethoate, methomyl, aldicarb and imidacloprid. Most products were technical grade material. In the field, all products were commercial formulations. We formulated two pesticide mixtures (Fupei I and Fupei II) and applied them in the field efficacy trials.

All bioassays were performed on an aphid population collected at the Shihezhi Plant Protection Station, Xinjiang. The field efficacy trials were carried out at the same farm.

RESULTS & DISCUSSION

High levels of deltamethrin resistance was detected in cotton aphids from Xinjiang (766-fold) and Shandong (1,835-fold), the highest level was detected in Shandong in 1988 (16,492-fold) (Table 1). Cotton aphids from Xinjiang showed more resistance to fenvalerate than any other pesticide tested (6,554-fold). Aphids from Shandong also showed resistance levels at 151-fold in 1996 and 1,901-fold in 1988. Pesticide resistance to monocrotophos, parathion, omethoate, and methomyl was detected in aphids from Shandong.

Table 2 shows that imidacloprid, omethoate, monocrotophos and the two Fupei formulations provided good control of a cotton aphid population in the resistant-plagued Xinjiang area. Deltamethrin, fenvalerate and parathion were not as effective.

The results indicated that the Shandong aphid populations were not only resistant to most pesticides but that resistance levels sometimes exceeded that of Xinjiang, an area of extremely high resistance levels. We face a serious problem in preventing further resistance development. We must work with the government, universities, pesticide companies and producers to prevent an outbreak of resistant cotton aphids in the Shandong area. In 1982 and 1985, several resistant aphid outbreaks in the Xinjiang area reduced output by 30%. Pyrethroids were once effective pesticides for cotton pests but now resistance in cotton aphid and bollworm has rendered pyrethroids nearly worthless.

The causes of resistance have been explored for many years. The results are as follows:

Table 2. Field effect observations of cotton aphid to 8 pesticides in Shihenzhi, Xinjiang, 1996.

Pesticide Treatment	Concentration (ppm)	Application Rate (g/ha)	Initial Density	1st day		3rd day		7th day		Average Effect (%)
				Density	Effect (%)	Density	Effect (%)	Density	Effect (%)	
Fupei I EC (25%)	166.7	125.0	504	2	99.7	0	100.0	21	99.1	99.6
Fupei II EC (25%)	166.7	125.0	510	4	99.4	6	99.6	66	97.1	98.7
Imidacloprid WP (10%)	66.7	50.0	483	74	88.3	7	99.5	18	99.2	95.7
Omethoate EC (40%)	266.6	200.0	464	16	97.4	17	98.7	66	96.8	97.6
Monocrotophos EC (40%)	266.6	200.0	482	33	94.8	55	95.9	252	88.4	93.0
Deltamethrin EC (2.5%)	16.7	12.5	508	12	98.2	176	87.5	828	63.9	83.2
Parathion EC (50%)	333.3	250.0	499	262	59.9	193	86.1	913	59.4	68.5
Fenvalerate EC (20%)	133.3	100.0	514	486	27.7	1008	29.5	2432	-4.9	17.4
Check	Water	0	516	675	0	1435	0	2328	0	0

-If we continuously apply one type of pesticide at high concentrations, high frequency and over a large area for many years, widespread pest resistance will develop quickly.

-At present, cotton-aphid cross resistance has occurred to all pyrethroid pesticides. However, both cotton aphid and bollworm are most resistant to fenvalerate, a leading cotton pesticide in China.

-Our results indicate that cotton aphid and bollworm resistant to fenvalerate are cross resistant to parathion. Parathion, an organophosphate, has been used to control cotton pests for 41 years. These fenvalerate-resistant populations were initially exposed to parathion. It is likely that resistance to parathion quickly led to cross resistance to fenvalerate and other pyrethroids.

-We examined the cross resistance in the cotton aphid to three major organophosphorus pesticides - parathion, monocrotophos and omethoate.

Bioassays indicated that parathion resistant aphids showed cross resistance to fenvalerate, but did not show resistance to monocrotophos or omethoate. Cross resistance was indicated between monocrotophos and aldicarb but monocrotophos did not show cross resistance to pyrethroid. We observed that aphids with cross resistance to monocrotophos and pyrethroids were most susceptible to omethoate. Due to this negative cross resistance relationship, resistance to omethoate is less than other pesticides in Xinjiang now. However, the increase in omethoate applied in cotton is bound to be followed by the decreasing efficacy of omethoate, but perhaps increase the susceptibility of the cotton aphid to pyrethroids. Exploitation of negative cross resistance may be a way to manage aphid resistance, but it must be done on a wide-spread area for many years

-We also examined the cross-resistance of cotton aphid to two carbamate pesticides (methomyl and aldicarb) and imidacloprid.

The methomyl resistant aphid showed negative cross resistance to monocrotophos and pyrethroids, but its efficacy has been closely related to resistant degrees of other pesticides over the past eleven years. Nearly 3 million tons of technical methomyl was used in Shandong to control cotton pests with serious resistance problem. Due to the aphid cross resistance between aldicarb and monocrotophos, the efficacy of aldicarb was quickly reduced in Xinjiang. In Shandong, aldicarb was applied to control the cotton aphid for only three years, from 1982 to 1984.

Imidacloprid was first used to control cotton pests after 1990. Base line susceptibility of the cotton aphid has not been established yet. The field efficacy is better than other pesticides, but use of imidacloprid is limited by product costs. Nevertheless, we plan to examine the cross resistance of imidacloprid with other pesticides.

We are convinced from our research that the best approach to control resistant cotton aphids is to alternate pesticide mixtures. Based on cross resistant cotton aphids, we designed two pesticide mixtures (Fupei I and II) to control cotton aphids in the field. In 1996, they faired better than imidacloprid (Table 2), the most effective pesticide in Xinjiang.

Fitness of an Insecticide-Resistant Parasitic Wasp

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Malathion has been used extensively as a chemical protectant on stored grain since the late 1950's. Widespread use of this phosphorodithioate insecticide has lead to significant levels of malathion resistance in beneficial insects as well as insect pests in stored grains. Insecticide resistance in a pest species is the direct cause of many control failures. In contrast, insecticide resistance in beneficial species is a desirable trait that can allow beneficials to be integrated with chemical control technologies in pest management programs.

The amount of natural control that beneficial species exert on insect pests in stored grain is difficult to measure and likely to be sporadic. As a result, augmentative releases of parasitic wasps in the stored-grain ecosystem is necessary. When parasitoids carrying a desirable gene for insecticide resistance are used in augmentative release programs, we want to know the stability of that resistance gene when the released and resident parasitoids interbreed. Resistance stability in the wasp population is critical if resistance confers fitness disadvantages in the absence of pesticide selection pressure.

We measured several fitness parameters in two strains of *Anisopteromalus calandrae*

(Howard), a pteromalid wasp that parasitize rice weevils, *Sitophilus oryzae* (L.), in wheat. The resistant (R) strain of *A. calandrae* is more than 2,500-fold resistant to malathion compared with the susceptible (S) strain.

Development time, Fecundity, and Progeny sex ratio

The fitness parameters for S and R strains of *A. calandrae* were compared in five separate studies. In the first study, male progeny from both strains had a significantly shorter development time (ca. one day) compared to female progeny. GLM analysis showed no significant difference in mean development time (data weighted by number of progeny produced) at 25°C and 75% RH between the two strains ($F = 0.56$; $df = 1,57$; $p = 0.46$) for either sex ($F = 68.9$; $df = 1,57$; $p < 0.01$) or strain × sex interaction ($F = 0.93$; $df = 1,57$; $p = 0.34$). Also, there were no significant differences in number of progeny produced between strains (Box-Cox transformed data) ($F = 0.41$; $df = 1,56$; $p = 0.53$), sex ($F = 204.4$; $df = 1,56$; $p < 0.01$) or strain × sex interaction ($F = 3.26$; $df = 1,56$; $p = 0.08$), progeny sex ratio (proportion females arcsin transformed and weighted by number of progeny produced) ($F = 4.03$; $df = 1,27$; $p = 0.055$), or percentage parasitization (arcsin transformed) of host larvae ($F = 0.14$; $df = 1,28$; $p = 0.71$) between the two strains.

In the second study, 4- to 5-day-old females from both strains of *A. calandrae* produced the most progeny. Average daily numbers of female progeny produced were 10.6 ± 2.2 at day 4 and 8.1 ± 3.7 at day 5 in the S and R strains, respectively. There was a significant effect between parent female age and numbers of progeny produced ($F = 8.54$; $df = 9,210$; $p < 0.01$). However, the GLM analysis revealed a significant strain effect ($F = 2.03$; $df = 1,210$; $p = 0.16$) and strain × parent female age interaction ($F = 1.01$; $df = 9,210$; $p = 0.43$). GLM analysis of arcsin-transformed proportion females weighted by number of progeny produced showed that sex ratio of progeny did not change as a function of parent female age ($F = 0.48$; $df = 9, 164$; $p = 0.89$) or strain ($F = 3.82$; $df = 1,21$; $p = 0.06$) or strain × parent female age interaction ($F = 0.45$; $df = 9,164$; $p = 0.91$).

We also examined the effect of host density on parasitoid success. The mean number of progeny produced by individual parent females in both strains of *A. calandrae* was a function of host density ($F = 87.0$; $df = 2,53$; $p < 0.01$). However, there was no significant difference between progeny production of the S and R strains at any density ($F = 1.45$; $df = 1,53$; $p = 0.23$) or host density × strain interaction ($F = 0.82$; $df = 2,53$; $p = 0.45$). GLM analysis of arcsin-transformed percentage parasitization weighted by number of weevils + number of parasitoids emerged showed that percentage parasitization did not vary with either host density ($F = 0.10$; $df = 2,53$; $p = 0.90$) or strain ($F = 0.99$; $df = 1,53$; $p = 0.33$) or host density × strain interaction ($F = 0.40$; $df = 2,53$; $p = 0.67$). GLM analysis of arcsin-transformed proportion females weighted by number of progeny produced showed that sex ratio did vary with density ($F = 9.9$; $df = 2,53$; $p < 0.01$). There was not a significant strain effect on sex ratio ($F = 1.20$; $df = 1,53$; $p = 0.28$) but there was a significant strain × density interaction ($F = 5.15$; $df = 2,53$; $p < 0.01$). The S strain produced a higher proportion of female progeny at the highest density.

Changes in the frequency of the R allele were followed with Hardy-Weinberg equations for six generations in the absence of selection. Based on the initial ratio of female and male genotypes, and assuming random mating among genotypes as well as a stable allele frequency, the expected mortality of F_1 females and males was 25% and 50%, respectively. In experiment 1, the initial mortality for both female and male progeny significantly exceeded these values, but stabilized after 2 to 3 generations. In this experiment, mortality of female progeny increased from 50% to 65% and remained stable at about 65%. Mortality of male progeny was approximately 75% to 80% throughout the study. In experiment 2, mortality of F_1 female progeny (28.7%) was not significantly different from expected. However, mortality of female progeny increased to approximately 50% in the F_2, then remained stable for the duration of the study. Mortality of male progeny fluctuated throughout the 2nd experiment, but generally was similar to that of male progeny in the 1st experiment.

In a final study on the stability of the R allele, virgin hybrid SR females were mated to S males to initiate a parasitoid population. Mortality among female and male progeny was not significantly different from expected over two generations based on Chi-square analysis.

In summary, there were no significant differences between strains in development time, progeny production, progeny sex ratio (except in the density study), or parasitization effectiveness. In R/S allele competition tests, we detected no significant fitness advantage of one allele over another. Shifts in allele frequencies confined to a single generation were attributed to genetic drift, not fitness selection.

It is apparent that even in the absence of a selecting insecticide, the R strain of *A. calandrae* can compete successfully in the stored grain ecosystem with parasitoids lacking the R allele. Even if this were not the case, multiple releases of parasitoids with a desirable trait may be able to overcome any minor fitness disadvantage that the released parasitoid might have relative to the resident parasitoid population.

From Monitoring to Implementation: A Stepwise Approach to Resistance Management with Imidacloprid

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INTRODUCTION

Imidacloprid's distinguishing feature is a distinct mode of action compared to most conventional insecticides. Therefore, it provides excellent control of contemporary multi-resistant pests such as whiteflies, aphids, leafhoppers and planthoppers, and Colorado potato beetles. Cahill *et al.* (1996b) recently published a survey of resistance in *Bemisia tabaci*. The survey did not detect any signs of reduced susceptibility to imidacloprid in *B. tabaci* collected from different countries of the world, except Spain. This paper reports on baseline and cross resistance studies for *B. tabaci*, field monitoring for *B. tabaci* susceptibility to imidacloprid in Almeria, Spain, and proposes general directives for resistance management.

METHODS

Insects

After investigating several established and new bioassays, a systemic bioassay where *Bemisia tabaci* is continuously exposed to imidacloprid was selected for resistance monitoring. Fully expanded cotton leaves were cut from the plant and the petiole immediately immersed into solutions of formulated imidacloprid over a range of concentrations. The leaves were kept immersed for 15 to 18 h. Then, discs were cut from treated leaves, placed onto agar in petri dishes and 20-30 *B. tabaci* females added. Mortality was scored at 24 and 48 h (Elbert *et al.* 1996).

A number of populations were tested including a susceptible laboratory strain from Sudan (SUD-S) and strains recently collected from Pakistan (PAK), the Netherlands (NED-3), USA (CAL-1) and from the Almerian region of southern Spain (ALM-2 and LMPA-2) (Table 1). Polyacrylamide gel electrophoresis (PAGE) identified the CAL-1 and NED-3 strains as B-types. However, the PAGE pattern of the Spanish strains were unique to that area, as were the PAGE pattern of the Pakistani strains. Both strains may represent distinct biotypes (Elbert *et al.* 1996).

Table 1. Origin and pesticide resistance history of six *Bemisia tabaci* strains.

Strain	Country of Origin	Original Host	Collected	OP Resistance	PYR Resistance	Endosulfan Resistance
SUD-S	Sudan	cotton	1978	no	no	no
PAK	Pakistan	cotton	1992	high	high	moderate
NED-3	Netherlands	gerbera	1992	high	high	moderate
ALM-2	Spain	tomato	1994	high	high	high
LMPA-2	Spain	melon	1995	high	moderate	not tested
CAL-1	USA	cotton	1995	high	moderate	high

RESULTS

Baseline/Cross resistance

Bioassays with imidacloprid compared the response of a susceptible strain of *B. tabaci* (SUD-S) to PAK and NED populations that were multi-resistant to organophosphates, carbamates, pyrethroids and endosulfan. Results from single bioassays were consistent and pooled on the basis of strain or geographical origin to yield composite LC_{50}s of 1.4 ppm imidacloprid for SUD-S and 1.8 ppm for PAK (Table 2). To compare resistance factors for each field population, a composite log-dose-response curve was estimated from the appropriate baseline data. This response curve has a LC_{50} of 1.7 ppm with a slope of 1.4.

These bioassays show that the resistance mechanisms among the field strains did not confer cross resistance to imidacloprid. Thus, imidacloprid can serve as an excellent tool for management of resistant pest populations.

Resistance Monitoring

Special focus was given to southern Spain, where imidacloprid has been used commercially since 1992. Intensive vegetable cropping areas were selected for resistance monitoring (in Almeria and Murcia). In Almeria, 19,000 ha of vegetables are grown under plastic in two crop cycles per year. *B. tabaci* resistance to numerous insecticides has developed and is widespread in this region.

Figure 1 shows efficacy results with imidacloprid, expressed as LC_{50} values. The bioassay revealed ALM-2 as the least susceptible strain to imidacloprid. However, the resulting resistance ratios were low and less than 5.0. This included a typical B-strain population from California, CAL-1, and serves as the susceptible strain with a response similar to SUD-S (Elbert & Nauen 1996). The results for the Spanish strains contrasted with those published by Cahill *et al*. (1996a), where high LC_{50}s ranged between 20 and 25. This discrepancy is attributed to different responses in the reference strain, SUD-S, between the two laboratories. Both Bayer and IACR Rothamsted are collaborating closely to explain these observed differences.

Field performance in southern Spain

To investigate the reported imidacloprid resistance situation in Spain, efficacy trials of imidacloprid on *B. tabaci* was conducted between 1988 and 1996 in the field. The results are summarized in Figure 2. Imidacloprid (Confidor 200 SL) was applied to field plots at a rate of 0.01 % a.i. Whitefly infestation pressure was high at the first application and remained high in untreated plots throughout the trial. Excellent control of *B. tabaci* was demonstrated 8 to 17 days after the second application in each trial perfomed between 1988 and 1996. Efficacy ranged between 90 and 100 %. Even after consecutive applications in commercial field since 1992, a high level of efficacy (93 to 99 %) was observed between 1994 and 1996. No loss in efficacy of imidacloprid against the whitefly *B. tabaci* was detected under typical conditions. Nevertheless, a two-year project is underway with IACR Rothamsted to design a resistance management strategy for imidacloprid against *B. tabaci* in southern Spain.

Table 2. Relative efficacy of imidacloprid against *Bemisia tabaci* strains SUD-S, PAK and NED-3.

Strain	LC_{50} (ppm)	95% conf. limits	Slope	s.e.	no. of tests
SUD-S	1.4	(1.0-1.9)	1.2	0.07	4
PAK	1.8	(1.4-2.3)	1.5	0.07	6
NED-3	3.0	(0.87-4.8)	1.7	0.37	1
All strains	1.7	(1.5-2.1)	1.4	0.05	11

Guidelines for resistance management with chloronicotinyls

Based on the described results and considering the IRAC guidelines, priniciples for resistance management of chloronicotinyls can be proposed. These guidelines are designed for insects with a high potential for resistance development, *i.e.* aphids, whiteflies, hoppers and Colorado potato beetles.

1. Long-term rotation acts against rapid selection of resistant populations.
2. Use effective doses (full recommended rates) of each pesticide when applying tank mixtures.
3. Season-long control should not be based on products representing one pesticide class with the same mode of action.
4. Pesticide mixtures and rotations should include imidacloprid.

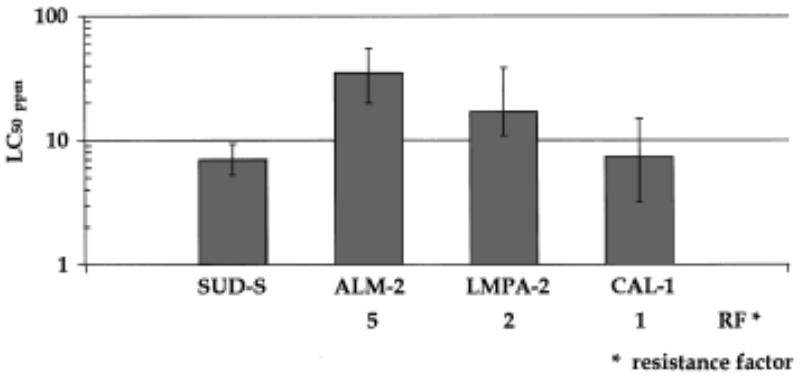

Figure 1. Imidacloprid efficacy against *Bemisia tabaci* strains SUD-S, ALM-2, LMPA-2 and CAL-1.

A list of effective combinations is in preparation.

5. Use non-specific products to prevent resistance development.

6. All possible cultivation techniques should be used, as well as physical and biological pest control methods.

7. Crop protection products should be used to reduce the risk to beneficial organisms.

8. All applications should be at the doses and spray intervals recommended by the label.

9. Ensure uniform spray coverage.

10. When resistance reduces effectiveness, do not carry out a follow-up treatment with a pesticide with the same mode of action.

11. Monitor the pest responses to imidacloprid whenever possible to detect resistance before it becomes widespread or creates control problems.

Control practices for these key insect pests differ considerably between countries, crops, plant varieties and climate. Therefore, any general strategy must be flexible. Effective implementation and adaptation of resistance management strategies to local conditions must be achieved by regional specialists.

CONCLUSIONS

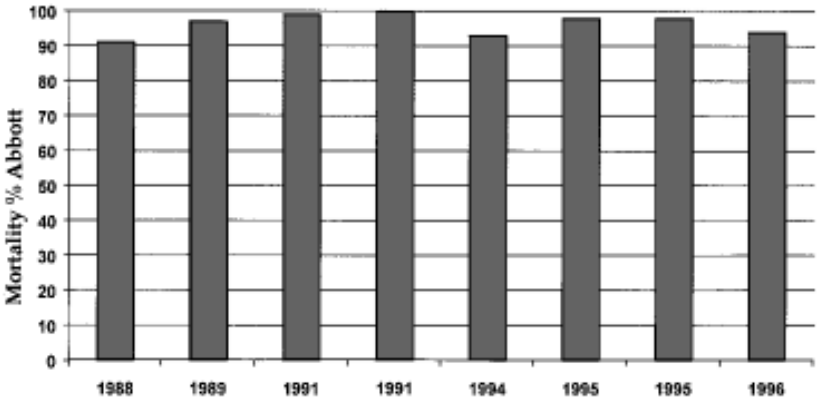

Figure 2. Field perfomance of imidacloprid against *Bemisia tabaci* in southern Spain 1988-1996.

Imidacloprid offers a new and valuable tool to manage resistant pests. As demonstrated with whiteflies, multi-resistance insect pests do not show cross resistance to imidacloprid. Nevertheless, suitable monitoring methods for whiteflies were developed, validated and baseline data established. A monitoring program is underway to survey any shifts of the susceptibility of whiteflies against imidacloprid. General guidelines for a resistance management program are proposed. Special guidelines for the management of *B. tabaci* in Spain will be developed in collaboration with M. Cahill, IACR Rothamsted.

This paper is the summary of a presentation given at the ESA Annual Meeting in Louisville (Kentucky, USA) on December 10, 1996.

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The *in vivo* Mechanism of Housefly Resistance to Permethrin

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INTRODUCTION

The objective of this study was to identify the resistance mechanisms in houseflies to pyrethriods collected at Akagi, Gunma prefecture (Akagi County, Japan). Electrophysiological studies were conducted to investigate the effect of permethrin on the nervous system of the housefly. The *in vivo* metabolism of permethrin was studied with ^{14}C labeled compound and with synergist tests. We also compared the locus of the recessive resistance gene on the third chromosome with that of the *kdr* gene. We found that reduced nerve sensitivity and increased metabolism by microsomal cytochrome P-450 dependent monooxygenase system were the

major mechanisms of housefly resistance to pyrethroids in the Akagi strain.

MATERIALS & METHODS

Houseflies

The initial colony was started from individuals collected in Akagi, Gunma prefecture in 1984. Akagi PP5 and Akagi PP15 strains were selected with permethrin for 5 and 15 generations, respectively. The 228e2b strain was established by Dr. J. Keidling in 1976 from Danish flies. CSMA and Osaka-S are wild susceptible strains. The susceptible Bx^2 strain has one dominant marker on the third chromosome. The susceptible *bgp* strain has three recessive markers on the third chromosome.

Toxicity/ Synergist bioassays

The relative toxicity of insecticides towards these housefly strains was determined with a topical application method. The role of detoxification in resistance was investigated with two synergists, piperonyl butoxide (PB) and S,S,S-tributyl phosphorotrithioate (DEF). In the synergist bioassay, insecticide and synergist were applied simultaneously to the individuals.

Penetration study

^{14}C labeled permethrin was applied topically to the thorax of the Akagi PP15 and the CSMA individuals at the rate equivalent to the LD_{10} of the CSMA strain. At set intervals after application, the surface of each housefly was rinsed with hexane into a liquid scintillation vial. The hexane was allowed to evaporate and scintillation cocktail added to the vials. Then, the radioactivities on the flies and in the vials were determined by liquid scintillation.

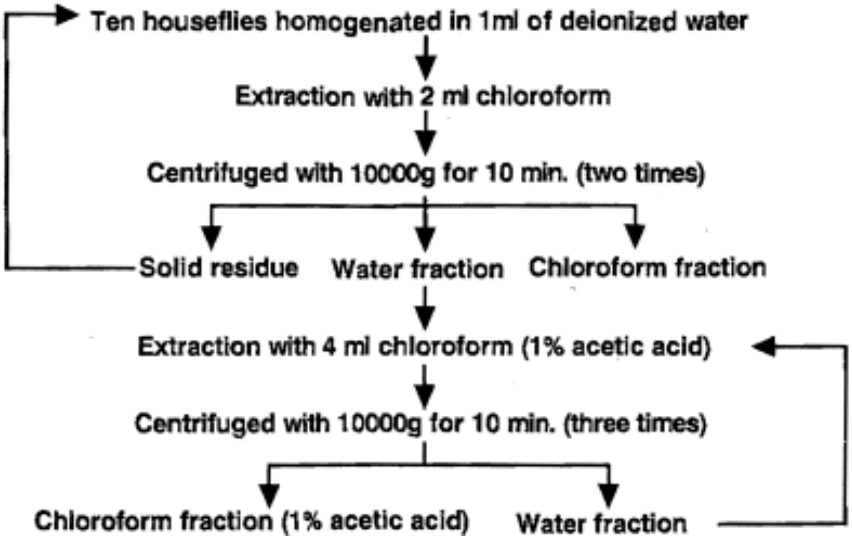


Figure 1. Extraction procedure for permethrin (parent compound) and metabolites.

Metabolic study

After the ^{14}C labeled compound was topically applied, permethrin and metabolites were extracted from treated houseflies with the procedure depicted in Figure 1.

Electrophysiological study

The electrical activity induced by permethrin topically applied to houseflied was monitored as shown in Figure 2.

Analysis of recessive resistant factor on the third chromosome

This analysis was done by crossing and backcrossing the resistant F_1 adults with a Bx^2 strain possessing a dominant marker on the third chromosome (Figure 3).

Mapping of the third chromosomal recessive resistance gene

Resistant houseflies were crossed

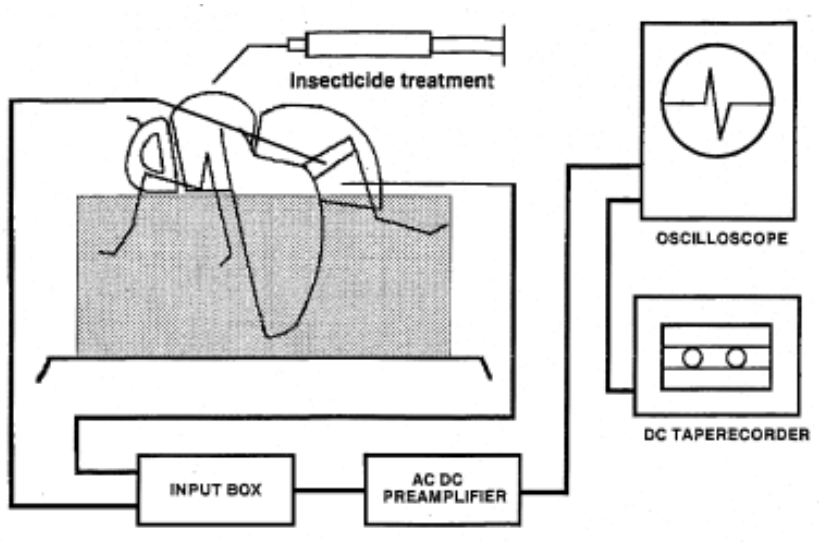


Figure 2. Schematic drawing of the method used to record nervous system activity.

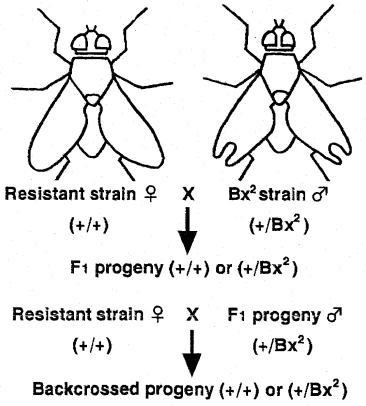


Figure 3. Housefly crosses to isolate recessive resistance factor on the third chromosome.

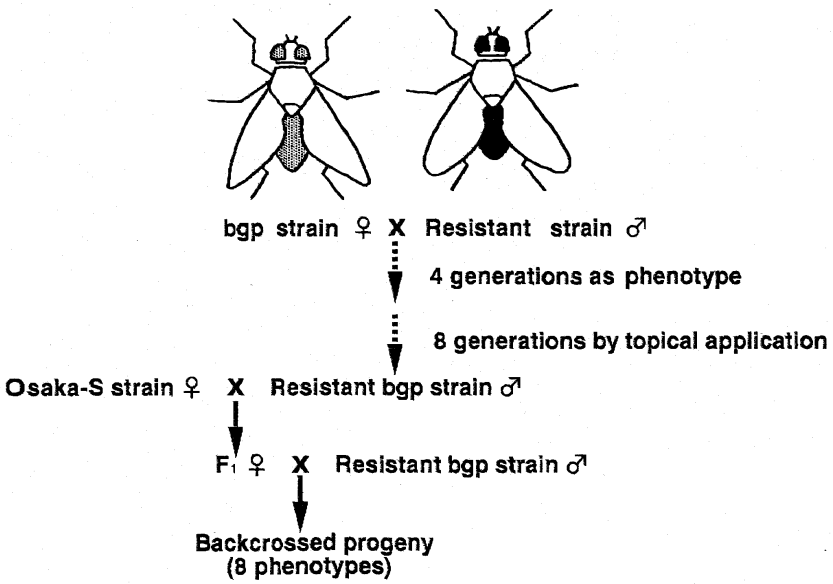


Figure 4. Housefly crosses to determine recombination values among visible marker genes and the recessive resistance gene on the third chromosome.

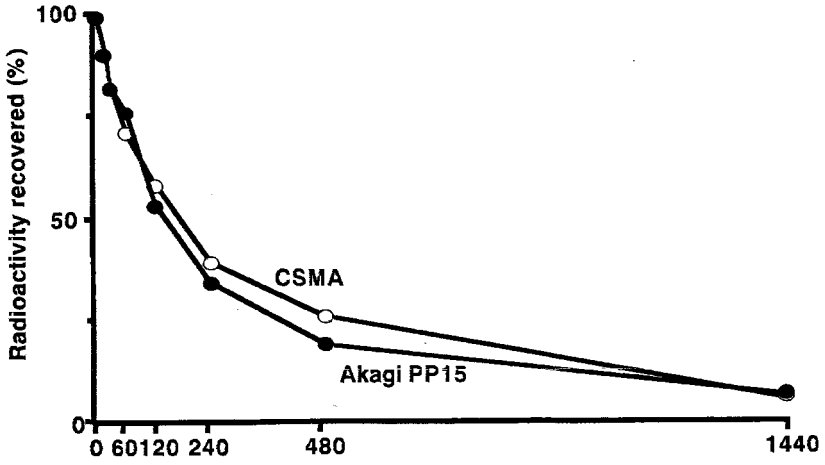


Figure 5. Percent ^{14}C recovered from hexane rinses at various time after treatment with ^{14}C permethrin (= LD_{10} for CSMA strain).

Table 1. Susceptibility of Akagi, Akagi PP15, 228e2b and CSMA housefly strains to permethrin, cypermethrin and fenitrothion.

Insecticides	LD_{50} (µg/ female fly)			
	CSMA	Akagi	Akagi PP15	228e2b
permethrin	0.021	7.1 (340)[a]	73 (3500)	4.4 (210)
cypermethrin	0.010	5.8 (580)	51 (5100)	2.7 (270)
fenitrothion	0.064	2.3 (36)	2.2 (34)	1.1 (17)

[a]Resistance Ratio = LD_{50} value resistant fly/ LD_{50} value of CSMA strain.

with the *bgp* strain to establish resistant strains with markers (Figure 4). Resistant strains were segregated by eight morphological phenotypes. A dose of 0.1 mg permethrin was applied to each backcrossed progeny segregated by eight morphological phenotypes. Another dose of 1.0 mg was applied to each backcrossed progeny from the crossing experiment with the resistant Akagi *bgp* strain (Figure 4). The recombination values were calculated by the formula proposed by Tsukamoto (1965).

RESULTS & DISCUSSION

LD_{50} values for the three resistant housefly strains, as well as the susceptible CSMA strain, to insecticides are given in Table 1. The Akagi colony shows high resistance to both pyrethroids and moderate resistance to fenitrothion. The Akagi PP15 strain was almost ten times more resistant to permethrin and cypermethrin than the original Akagi colony. A synergistic effect was recognized with PB on the Akagi colony and the Akagi PP15,

Table 2. Synergistic effects of piperonyl butoxide (PB) and S,S,S-tributyl phosphorotrithioate (DEF) with permethrin for the Akagi, Akagi PP15, 228e2b and CSMA housefly strains.

Insecticides	LD_{50} (µg/female fly)			
	CSMA	Akagi	Akagi PP15	228e2b
permethrin alone	0.021	7.1	73	4.4
with PB	0.0012 (18)[a]	0.19 (37)	0.45 (160)	0.20 (22)
with DEF	0.0063 (3.3)	4.7 (1.5)	19 (3.8)	2.6 (1.7)

[a]Synergist Ratio = LD_{50} value without synergist/ LD_{50} value with synergist.

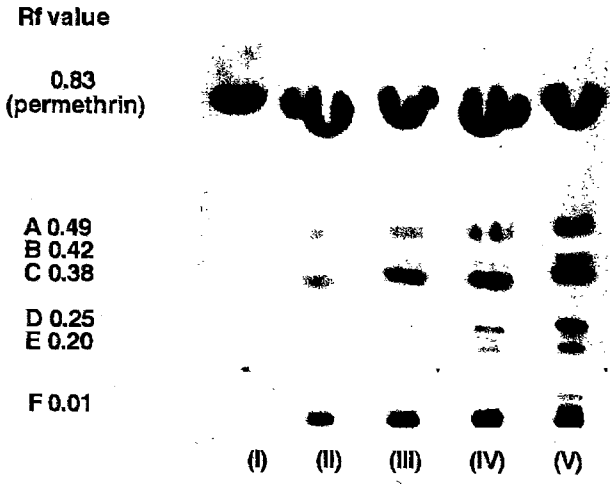


Figure 6. Radioautograph of ^{14}C permethrin (I), chloroform and chloroform (1% acetic acid) soluble metabolites of ^{14}C permethrin in the CSMA (II, IV) and Akagi PP15 (III, V) housefly strains after 30 and 120 minutes of treatment, respectively.

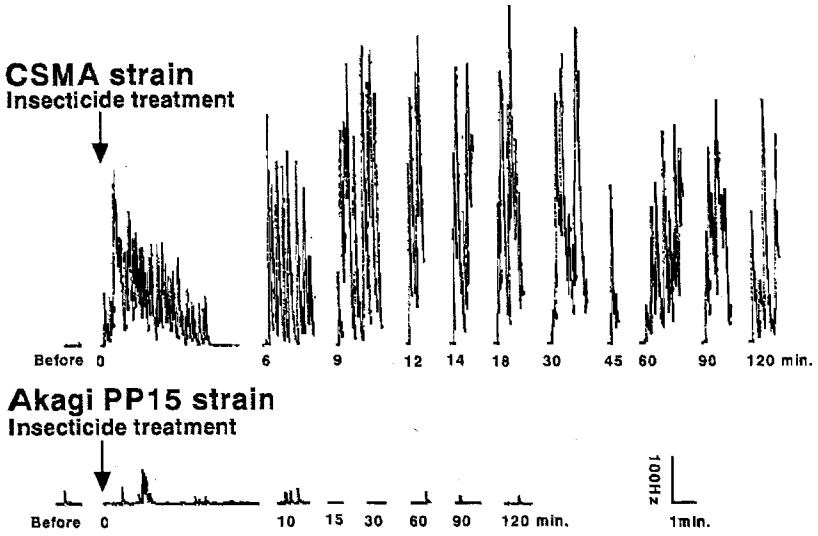


Figure 7. Changes in firing frequency in the femur nerve after topically applying 1 mg permethrin to individuals from the CSMA strain and the Akagi PP15 strain.

Table 3. Percent ^{14}C permethrin in the CSMA houseflies and Akagi PP15 houseflies after 30 and 120 minutes after treatment.

Time (min)	Percent of applied dose	
	CSMA	Akagi PP15
30	5.6	5.2
120	19	17

Table 4. Susceptiblity of backcrossed progeny (females of resistant strain x males of F1 progeny +/Bx^2) to insecticides.

Insecticide	Chromosome III genotype	LD_{50} (µg/female fly)		
		Akagi	Akagi PP15	228e2b
permethrin	+/+	7.3	37	2.5
	+/Bx^2	0.11	0.19	0.14
fenitrothion	+/+	3.0	1.3	0.51
	+/Bx^2	3.0	0.81	0.59

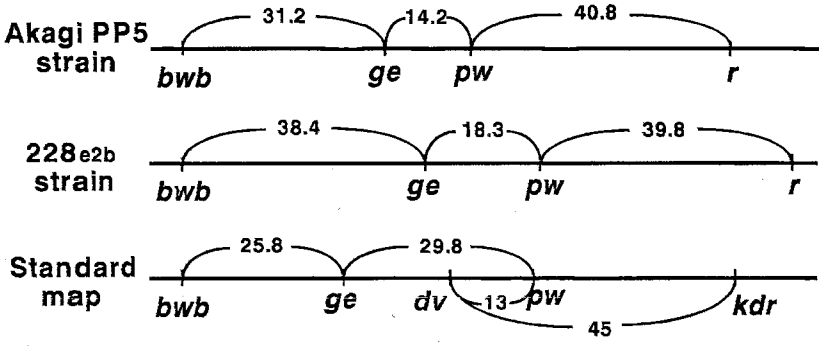
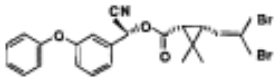
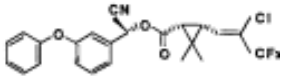
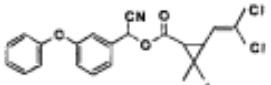
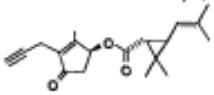


Figure 8. Linkage maps for the third chromosome in Akagi PP5 and 228e2b housefly strains compared to the standard map reported by Hiroyoshi (1977).

Table 5. Susceptibility of backcrossed housefly progeny (females of Akagi PP15 strain x males of F1 progeny +/Bx^2) to pyrethroids.

Compound	Structure	LD_{50} (µg/female fly)		C*kdr*[a]
		+/+	+/Bx^2	
deltmethrin		>40	0.032	>1300
λ-cyhalothrin		>40	0.068	>590
cypermethrin		>40	0.096	>420
prallethrin		18	0.80	23

[a]C*kdr* = LD_{50} backcrossed progeny (+/+)/ LD_{50} of backcrossed progeny (+/Bx^2)

but not with DEF (Table 2). This indicates that the cytochrome-P450-dependent monooxygenase system plays a role in housefly resistance to permethrin. LD_{50} values of Akagi colony and Akagi PP15 strain to permethrin and PB were still larger than that of CSMA strain to permethrin alone, so we suspect another mechanism must exist. Reduced penetration was not observed in Akagi PP15 strain in the study with ^{14}C labeled permethrin gene. Furthermore, when we compared the contribution of *kdr* factor to the resistance of some pyrethroids in Akagi PP15 strain by crossing it with Bx^2 strain (Table 5) a remarkable difference in the LD_{50} values to the pyrethroids with 3-phenoxybenzyl alcohol moiety was recognized between backcrossed progeny from the two genotypes (+/+ and +/Bx^2). The alcohol moiety appeared associated with *kdr* factor (Table 5). On the other hand, the contribution of *kdr* factor to prallethrin with cyclopentenolone alcohol was less than that of the pyrethroids described above.

ACKNOWLEDGMENTS

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Monitoring Strategies for Early Detection of Lepidoptera Resistance to *Bacillus thuringiensis* Insecticidal Proteins

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INTRODUCTION

The insect control effectiveness of transgenic plants expressing *Bacillus thuringiensis* (Bt) proteins is seriously threatened by the potential development of resistance (Tabashnik 1994). An important component of preemptive Bt resistance management strategies is an efficient resistance monitoring program. Monitoring data helps evaluate the effectiveness of resistance management strategies and permits early detection of resistant phenotypes. Under favorable circumstances, this could allow remedial resistance management measures to be implemented prior to control failure (ffrench-Constant & Roush 1990).

Insecticide resistance is usually monitored by determining the log-dose probit mortality responses of insect strains and statistically comparing the LD_{50}s and slopes of the probit regression lines (Robertson & Preisler 1992). However, LC_{50}s and slope estimates are not adequately sensitive for detecting resistance when the incidence is low, e.g. < 10^{-3} (Roush & Miller 1986). Diagnostic doses that unambiguously discriminate between resistant and susceptible phenotypes are a more efficient means of finding resistant phenotypes because each individual tested provides useful data.

Heliothis virescens (F.) and *Helicoverpa zea* (Boddie) are major pests targeted for control by commercial transgenic cotton lines that produce the CryIA(c) insecticidal protein. In this study, we compared the relative suitability of larval mortality and larval growth inhibition assays for monitoring the sensitivity of *H. virescens* and *H. zea* to the CryIA(c) protein. Diagnostic doses of CryIA(c) protein for both mortality and growth inhibition assays were calculated. The effectiveness of the growth inhibition assay, in combination with a diagnostic dose of CryIA(c) protein, was tested against a CryIA(c)-resistant strain of *H. virescens*.

MATERIALS & METHODS

CryIA(c) protein dose-mortality response and larval growth inhibition bioassays were previously described (Sims *et al*. 1996). Bt protein was mixed into a standard Lepidoptera diet and poured into individual wells of multiple well assay trays. One 1st instar *H. virescens* or *H. zea* larva was added to each well and confined by ventilated Mylar plastic. Assays (starting n = 24 - 48 larvae) were incubated at 28°C and evaluated at 7 days by scoring the number of survivors per concentration or by weighing larvae in groups of 10 - 48 and calculating the mean larval weight. The dose-response function of treatments was fit with either probit analysis (mortality data) or non-linear regression analysis for larval weight data (Sims *et al*. 1996).

We re-evaluated the combined dose mortality responses of 12

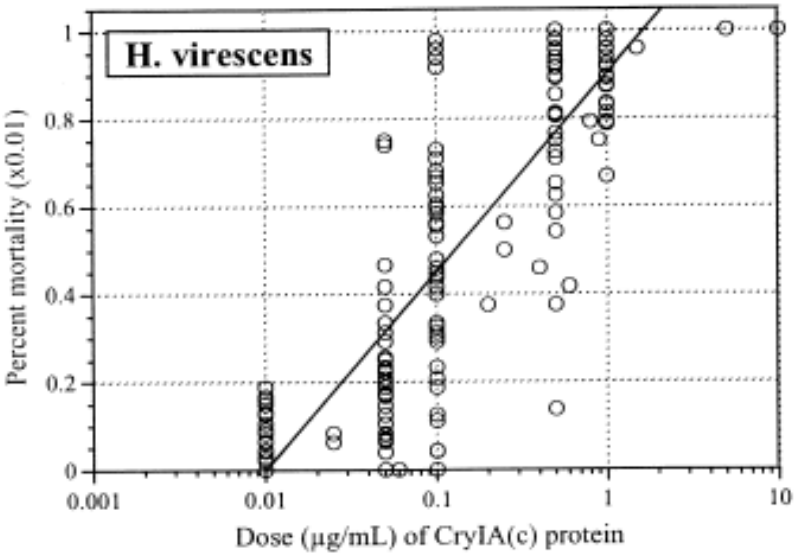

Figure 1. Mortality response of *Helicoverpa virescens* larvae to purified 63-kD CryIA(c) protein.

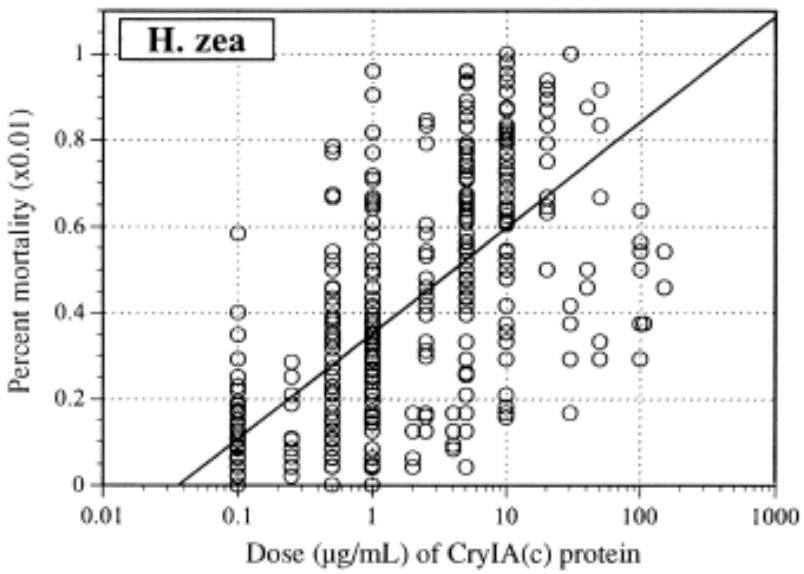

Figure 2. Mortality response of *Helicoverpa zea* larvae to purified 63-kD CryIA(c) protein.

strains of *H. virescens* and 15 strains of *H. zea* to purified 63 kDa (trypsin-activated) CryIA(c) protein (Stone & Sims 1993). The strains represented a significant proportion of each species' distribution. All data, within each species, were combined and probit analysis used to estimate LC_{99} values and 95% confidence limits. The LC_{99} values were selected as diagnostic doses for each species. The total numbers of assays contributing to the analyses for *H. virescens* and *H. zea* were 234 and 456, respectively.

Larval growth inhibition, in response to purified full-length CryIA(c) protein, was studied for *H. virescens* and *H. zea* laboratory colonies from the USDA, Stoneville, MS and two *H. zea* colonies initiated from individuals collected in Brooksville, MS. The data set for each species was fit by nonlinear regression to estimate EC_{99} values, *i.e.* the concentrations required to reduce larval weight to 1% of the mean control weight, and 95% confidence intervals. The total number of assays contributing to the analyses for *H. virescens* and *H. zea* were 178 and 173, respectively.

We evaluated potential diagnostic doses for the growth inhibition assay on larvae from a North Carolina strain of *H. virescens* (YHD2) selected for over 1000-fold resistance to CryIA(c) protein (Gould *et al.* 1995) and on F_1 hybrids derived from crosses to a non-selected susceptible *H. virescens* strain (YDK). The CryIA(c) protein, within a lyophilized transgenic cotton leaf tissue matrix, was incorporated into insect diet at 4, 20, 60, and 80 mg/mL diet resulting in 0.24, 1.20, 3.6 and 4.8 ug/mL concentrations of active CryIA(c) protein, respectively. Diets containing appropriate concentrations of leaf tissue from non-transgenic C312 cotton served as controls for weight comparisons.

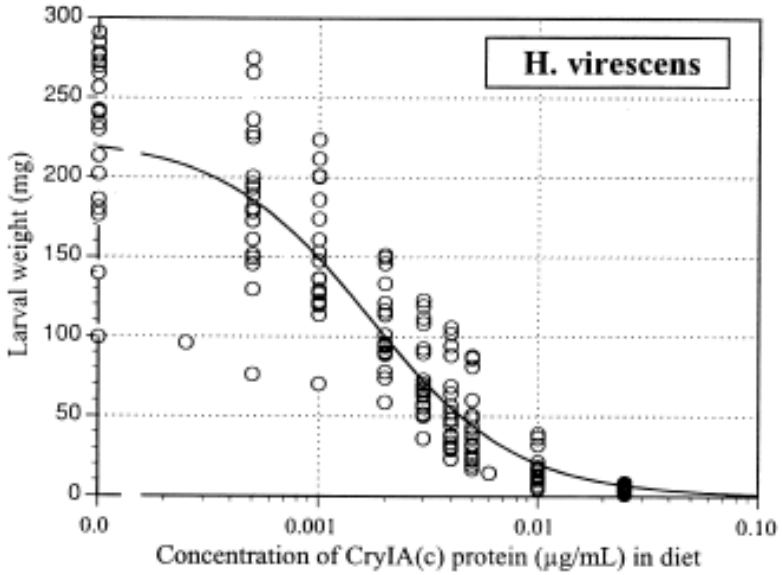

Figure 3. Growth inhibition of *Heliothis virescens* larvae in response to purified 130-kD CryIA(c) protein.

RESULTS & DISCUSSION

The dose-mortality reponse results are presented in Figures 1 and 2. LC_{99} estimates for the 63-kDa protein were 3.3 mg/mL (95% CI = 2.3 - 5.3) for *H. virescens* and 6,661 mg/mL (95% CI = 1,003 - 2.12 x 10^5) for *H. zea*. Because transgenic cotton produces the non-activated, full-length CryIA(c) protein (~ 130-kD) approximately twice the molecular weight of the trypsin-resistant core, the LC_{99} estimates for the full-length CryIA(c) protein was 6.6

mg/mL for *H. virescens* and 13,322 mg/mL for *H. zea.*

Larval growth inhibition results are shown in Figures 3 and 4. EC_{99} values were 0.058 mg/mL (0.030 to 0.086) for *H. virescens* and 28.8 mg/mL (-7.4 to 65.1) for *H. zea*. These estimates were considerably lower (114-fold less for *H. virescens*, 463-fold less for *H. zea*) than the corresponding LC_{99} estimates for the full-length CryIA(c) protein.

Resistant YHD2 larvae developed significantly faster on all CryIA(c) concentrations compared to larvae from the susceptible strain (YDK) (Fig. 5). The mean weight of presumptive F_1 heterozygotes for the resistance trait (*i.e.* YHD2 x YDK and YDK x YHD2) can be distinguished from the mean weight of YDK larvae reared on diet treated with 4 mg/mL of transgenic leaf powder (Fig. 6).

A larval growth inhibition assay based on sublethal doses of CryIA(c) protein was considerably more sensitive than dose-response mortality assays and is likely to be superior for detecting incipient susceptibility changes in *H. virescens* and *H. zea* to the Bt CryIA(c) protein. Since both growth and mortality assays require ingestion of the insecticidal protein mixed into a diet matrix, little additional effort is required to set-up and visually score growth inhibition tests compared to the mortality assays.

Since *H. virescens* and *H. zea* are not equally susceptible to the CryIA(c) protein, different diagnostic doses and accurate identification were necessary prior to the assays. The CryIA(c) EC_{99} doses calculated for *H. virescens* (0.058 mg/mL) and *H. zea* (28.8 mg/mL) were reasonable diagnostic dose estimates. However, the EC_{98} (6.6 mg/mL, 0.1 to 13.0) was more practical for *H. zea*, because it provided adequate discrimination (stunting) of susceptible larvae at a much lower concentration. These diagnostic doses either kill susceptible larvae or prevent them from reaching 3rd instar. Size differences between 1st and 2nd instar versus 3rd instar are obvious but errors (false positives) can be further minimized by concurrently testing larvae on control diet to provide a direct size comparison. Essentially all healthy larvae of both species tested on control diet were ≥ 3rd instar (usually ≥ 100 mg) at 7 days.

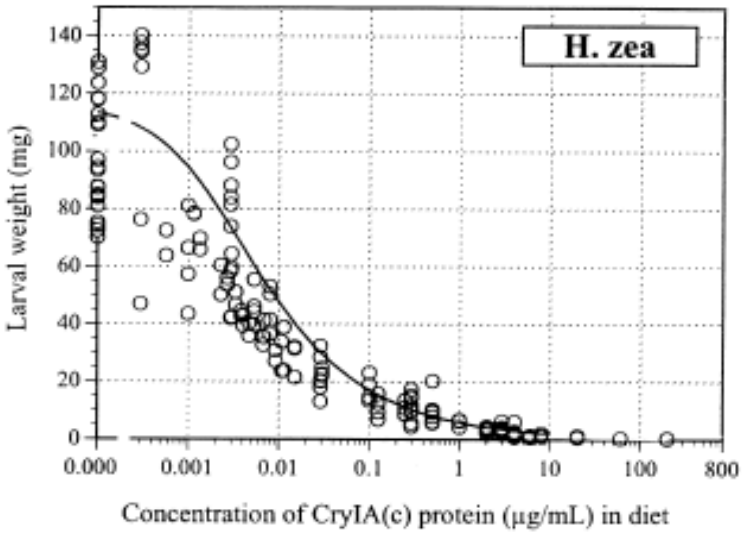

Figure 4. Growth inhibition of *Helicoverpa zea* larvae in response to purified 130-kD CryIA(c) protein.

The larval growth assay, combined with a diagnostic dose, unambiguously separated resistant homozygotes of the CryIA(c) resistant strain of *H. virescens* from susceptible insects. However, the diagnostic dose was only partially successful at detecting resistant F_1 heterozygotes. A detailed analysis of individual growth rates indicated that a significant proportion of presumptive F_1 heterozygotes grew at the same rate as susceptible YDK larvae (Gould *et al.* 1995). Neverthe-

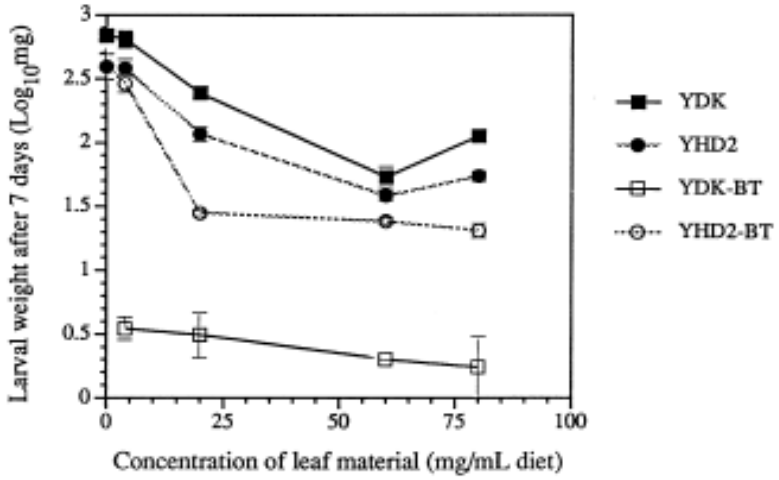

Figure 5. Effect of CryIA(c) protein, in transgenic cotton leaf tissue, on weight gain (± 1 SEM) of susceptible (YDK) and resistant (YHD2) *H. virescens* larvae.

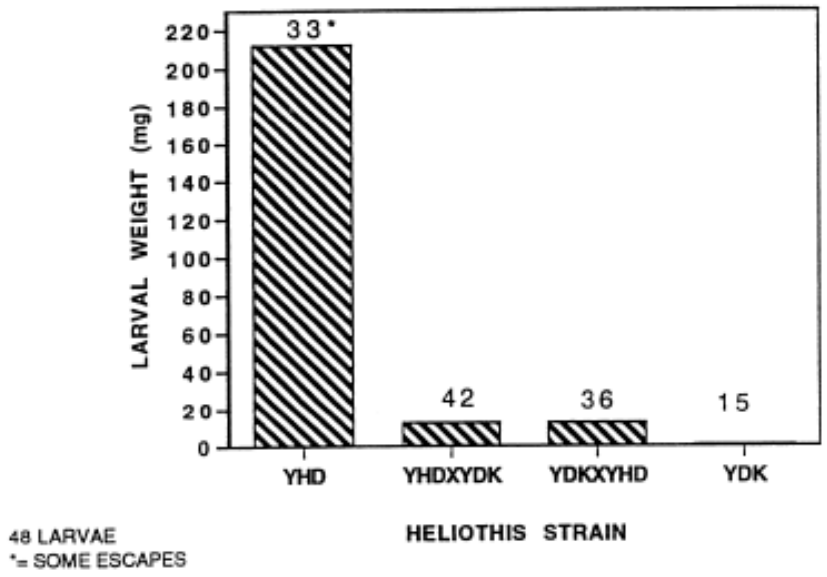

Figure 6. Weight of 11-day-old larvae from the CryIA(c) resistant, susceptible and reciprocal F_1 hybrids to a diet-incorporated, discriminating dose of CryIA(c) protein in transgenic cotton leaf tissue.

less, the sensitivity of the growth assay and the potential for detecting any resistant heterozygotes significantly increases the probability of detecting resistance while it is still rare (Roush & Miller 1986).

A future application of growth assays could be the analysis of allelic frequencies of resistance prior to field release of transgenic plants. This could be done by screening populations for individuals surviving to 3rd instar with a diagnostic (EC_{99}) dose. Following transfer to fresh diet, completion of development and adult mating, resulting progeny would be tested against an appropriate diagnostic dose for the presence of genetic factors having major effects on susceptibility. This approach, based on larval growth, might also be a more useful method for obtaining resistant insect strains compared to recurrent selection based on larval survival.

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Organophosphate Resistance and its Biochemical Mechanisms in Brazilian and U.S. Populations of the Lesser Grain Borer, *Rhyzopertha dominica*

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Organophosphate resistance was detected in lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), collected from eight sites in the states of Minas Gerais and São Paulo in Brazil and from seven sites in northeast Kansas in the United States. Each field-collected population of lesser grain borer showed resistance to the organophosphates -- malathion, pirimiphos-methy and chlorpyrifos-methyl (Guedes *et al.* 1996). Malathion resistance ranged from 2.1- to 12.2-fold at LC_{50}; pirimiphos-methyl resistance ratios ranged from 2.4- to 9.2-fold; and chlorpyrifos-methyl resistance ratios ranged from 5.6- to 167.9-fold. To our knowledge, this is the first report of pirimiphos-methyl resistance in *R. dominica* populations

from the United States, and resistance development is probably in the initial stage. Furthermore, moderate resistance to chlorpyrifos-methyl was detected in Brazilian and U.S. populations of lesser grain borer for the first time. Since chlorpyrifos-methyl is not used against stored grain insects in Brazil, this resistance must have evolved through selection by other organophosphates.

Possible biochemical mechanisms for organophosphate resistance were examined in the populations of lesser grain borer. Low synergism, non-synergism and even antagonism of pesticide toxicity by triphenyl phosphate, diethyl maleate, and piperonyl butoxide in all 15 resistant populations suggested that carboxylesterases, glutathione *S*-transferases, and cytochrome P450 monooxygenases do not play a major role in organophosphate resistance in lesser grain borer (Guedes *et al.* 1997a). This hypothesis was strongly supported by *in vitro* colorimetrical studies. In contrast, all resistant populations of *R. dominica* showed higher acetylcholinesterase activity than the susceptible population and were less sensitive to inhibition by malaoxon. Quantitative and qualitative changes in acetylcholinesterase activity may contribute to organophosphate resistance in these populations of lesser grain borer.

Phosphotriesterase activity towards paraoxon among 15 organophosphate-resistant populations of lesser grain borer from Brazil and the United States was surveyed. These resistant populations had significantly higher phosphotriesterase activity than the susceptible population (Guedes *et al.* 1997b). The kinetic parameters, K_m and V_{max}, were 5.2×10^{-3} M and 80.2 nmol/h/mg protein, respectively, for the susceptible population and 5.8×10^{-3} M and 189.5 nmol/h/mg protein, respectively, for the resistant population from Uberlândia, Brazil. Enhanced phosphotriesterase activity may be another major organophosphate resistance mechanism in *R. dominica*. The substrate, paraoxon, is an organophosphate insecticide, thus we suspect that the higher enzymatic activity in resistant populations will allow those populations to hydrolyze other organophosphates, especially those with similar chemical structure to paraoxon.

Further studies with purified acetylcholinesterase isolated from resistant lesser grain borer indicate that this enzyme is a true acetylcholinesterase (unpublished data). Kinetic studies show that the affinity of the acetylcholinesterase to the substrates acetylthiocholine, acetyl-(b-methyl) thiocholine, and propionylthiocholine was similar between the susceptible and resistant (Uberlândia, Brazil) populations. However, the affinity to *S*-butyrylthiocholine was significantly lower in the resistant population than in the susceptible population (unpublished data). For each substrate investigated, the hydrolyzing efficiency of purified acetylcholinesterase from the resistant population was about 2-fold higher than the susceptible population. Acetylcholinesterase from the resistant population was 13- and 32-fold less sensitive to inhibition by paraoxon and malaoxon, respectively, than acetylcholinesterase from the susceptible population. However, acetylcholinesterase from the resistant population was about 3-fold more sensitive to carbaryl and chlorpyrifos-methyl oxon and 205-fold more sensitive to carbofuran than the acetylcholinesterase from the susceptible population. These results support our contention that an altered acetylcholinesterase contributes to target site insensitivity leading to malathion resistance in lesser grain borer. Nonetheless, this mechanism does not contribute to but actually counteracts chlorpyrifos-methyl resistance in this species.

In summary, resistance to chlorpyrifos-methyl is probably due to increased phosphotriesterase activity in this population, whereas resistance to malathion was due to increased phosphotriesterase activity and reduced sensitivity of acetylcholinesterase. Phosphotriesterases might be more efficient in hydrolyzing phenyl and heterocyclic organophosphates, such as parathion and chlorpyrifos-methyl than aliphatic organophosphates like malathion. This would explain the higher resistance levels to chlorpyrifos-methyl than to malathion in the resistant population of lesser grain borer.

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Pyrethroid-Resistant *Helicoverpa zea* in Cotton in South Carolina

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INTRODUCTION

New strategies are needed to manage insecticide resistance in transgenic crops with pesticidal activity. In the case of transgenic cotton expressing the *Bacillus thuringiensis* toxin (Bt-cotton), an important factor is the relative tolerance of several cotton pests to the toxin. Among the two major lepidopterous pests of cotton in the southeastern USA, tobacco budworm, *Heliothis virsecens* (F.), is very susceptible to *Bacillus thuringiensis* toxin, while corn earworm, *Helicoverpa zea* (Boddie), exhibits a wide range of responses to the toxin (Sims *et al.* 1996). The practical consequence is that Bt-cotton must be sprayed once or twice to control large populations of *H. zea.* The most effective and economical insecticides for this application has been the synthetic pyrethroids.

In 1996, conventional cotton growers in Estill, SC experienced failure controlling *H. zea* larvae with pyrethroid applications. Investigation and modification of various factors related to application techniques did not correct the problem. Cyhalothrin was applied 5 or 6 times with additional applications of deltamethrin and cypermethrin.

MATERIALS & METHODS

The Estill96 laboratory colony was started from larvae collected from the problem fields on September 13 and 17, 1996. F_1 and F_2 adults were bioassayed in the laboratory. The response of P, F_1, F_2 adults and F_1 larvae to pyrethroids was compared to a susceptible laboratory strain of *H. zea.* This susceptible strain (*H. zea*-S) was provided by Mr. F. Stell, Clemson University, and thought to have originated at the USDA research laboratory in Stoneville, MS.

Susceptibility tests of virgin adults fed for one day were performed in insecticide-coated glass scintillation vials (Campanhola & Plapp 1989, Pimprale *et al.* 1996). One moth of either sex was held in each vial at 21-23°C and mortality was assessed at 24 h. Individuals were considered dead when they were unable to cling to the side of the vial when disturbed. Susceptibility tests of larvae were performed by topical application of technical grade insecticides in acetone (Brown *et al.* 1996).

RESULTS & DISCUSSION

Field generation adults were resistant to cypermethrin. When exposed to 2.5µg per vial of cypermethrin, the mortality for Estill96 adults was 17.6% (n = 34) compared to 92% mortality for *H. zea*-S (n = 25). There was no control mortality for *H. zea*-S (n = 22).

There was also resistance to cyhalothrin in Estill96 adults (Table 1). Preliminary probit analysis indicated approximately 28-fold resistance to cyhalothrin. A descriminatory dose of 2.5µg cyhalothrin per vial killed all *H. zea*-S adults while most Estill96 adults survived. Analysis of hybrid progeny produced by mating Estill96 to *H. zea*-S indicated that this resistance is expressed as an incompletely dominant trait.

We also observed 5-fold resistance to permethrin in Estill96 F_1 larvae when compared to our baseline data (unpublished results) obtained in 1982 from a collection made in Elliott, SC. The median

Table 1. Adult susceptibility to cyhalothrin for three strains of *Helicoverpa zea:* a laboratory colony (*H. zea*-S); F_1 and F_2 adults of a collection from Estill, SC (Estill96); and the hybrid progeny of those strains (Hybrid).

Dose (µg/vial)	% Mortality at 24 h (n)		
	H. zea-S	Hybrid	Estill96
0	0 (22)	-	0 (12)
0.125	50 (10)	-	-
0.625	53.3 (15)	0 (20)	-
1.25	94.4 (36)	45 (20)	12.5 (8)
2.5	100 (15)	50 (20)	6.6 (106)
5.0	-	89.5 (19)	-
10	-	100 (20)	80 (10)
30	-	-	100 (4)

lethal dose of permethrin for Estill96 larvae was 3.06 µg/g (C.L. = 2.48 - 3.85, slope = 2.1) while the median lethal dose for Elliott82 was 0.62 µg/g (C.L. = 0.42 - 0.79, slope = 2.5). Resistance to permethrin in larvae was similar to that reported previously for corn earworm larvae collected in 1991 from sweet corn in Illinois and from cotton in Missouri (Abd-Elghafar *et al.* 1993).

Resistance to cypermethrin and to cyhalothrin in adults appears to be high compared to previous records. The average mortality for *H. zea* collected from cotton in September in Louisiana from 1988 - 1993 to cypermethrin (2 µg/vial) was 74% and the lowest value was 52% mortality in 1993 (Bagwell *et al.* 1997). We observed only 17.6% mortality at 2.5 µg/vial of cypermethrin and only 6.6% mortality at 2.5 µg/vial cyhalothrin, a dose that killed all of our susceptible laboratory strain.

This resistance case seems to be unusual in that there have been few problems controlling corn earworm with pyrethroids in South Carolina in the past and there were no similar reports of failures in other areas of South Carolina in 1996. It is possible that this is an isolated case where resistance developed due to intensive use of pyrethroids. Similar hot spots have been observed in Louisiana (J. B. Graves, personal communication). Monitoring in 1997 will include both Estill and a control area around Florence.

A management plan has been established with Bt-cotton introduced to help control the pyrethroid-resistant population. If monitoring indicates continued resistance, then alternative insecticides will be recommended to reduce pyrethroid selection pressure. Unfortunately, this will greatly increase costs to the growers who have already invested in Bt-cotton.

The establishment and spread of pyrethroid-resistant *H. zea* would have serious implications for the economically successful use of Bt-cotton. This situation must be closely monitored and economically feasible alternative strategies must be developed quickly. Of course, there is also the question of resistance developing to Bt-cotton, so introducing this plant/pesticide to control pyrethroid-resistant insects also comes with a risk to the overall management plan.

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Reduced Acetylcholinesterase Sensitivity in a Diamondback Moth Population from South China

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INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.), is an important pest of cruciferous vegetables in South China. In different regions, field populations of this moth have different levels of resistance to insecticides. In recent years, farmers have expanded the cultivated area of vegetables; consequently, there is a great need for simple and rapid, yet sensitive methods to detect and monitor the resistance levels of DBM to conventional insecticides. Reduced acetylcholinesterase (AchE) sensitivity to inhibition is an important mechanism of resistance to organophosphate (OP) and carbamate insecticides in many insect species (Hama 1983, Dary *et al.* 1990) including the DBM (Sun *et al.* 1986, Tung *et al.* 1992). Some

Table 1. Insecticide toxicity among three strains of diamonback moth.

Strain	Insecticide	LD_{50} µg/insect	Y=a+bx	R/S
JS	Dichlorovos	0.27	7.86+2.39x	1.0
	Malathion	0.78	6.71+1.17x	1.0
	Methomyl	0.25	6.31+1.17x	1.0
GBR	Dichlorovos	3.67	5.10+2.06x	13.8
	Malathion	7.86	4.76+0.86x	10.1
	Methomyl	1.35	5.42+0.86x	5.4

Table 2. Percent inhibition of AchE activity in DBM by propoxur and paraxon.

Strain	Percent inhibition	
	propoxur	paraxon
S-strain	52.94	84.66
R-strain	50.97	43.76
DG population	43.96	35.87

biochemical methods for characterizing reduced AchE sensitivity to insecticides among resistant insects (Moores 1998, Ferrari 1993) are widely recognized and utilized in practice. A dot-blot method on nitrocellulose membrane for identification of insensitive AchE in resistant DBM individuals has created an opportunity for early detection and subsequent monitoring of resistance among field populations.

METHODS

The resistant strain (GBR) was started from individuals collected on a vegetable farm in Guanzhou province in 1986 and reared in the laboratory under the selection pressure with OPs. The susceptible strain (JS) was obtained from Japanese Sumitomo Chemical Co. Ltd.

Topical applications of insecticides were used to bioassay DBM. A dot-blot assay on nitrocellulose membrane was done as described by Dary *et al.* (1991) with some modifications. To obtain measurable residual AchE activity without insecticide exposure, we performed all assays on homogenate from DBM heads. One hundred heads from three-day-old adult female DBM were homogenized in 5 ml of ice-cold 100 mM sodium phosphate buffer (pH 7.5) in wells of porcelain plates. After homogenization, an additional 45 ml buffer was added to each well. A piece of tissue paper (1 by 1 cm) was placed on top of the homogenate in each well to exclude the head fragments. Five ml homogenate was aspirated through the piece of tissue paper and spotted onto nitrocellulose membrane (5 by 3 cm). The membranes were treated with appropriate concentrations of propoxur and paraoxon, as well as ethanol, for inhibiting the susceptible AchE. The blots were rinsed and then immersed in the developing solution to color-tag AchE activity. After 3.5 h incubation at room temperature, blots were rinsed in distilled water and dried. The staining intensity of each dot-blot was determined with RCP densitometer (Tobias Associated INC.).

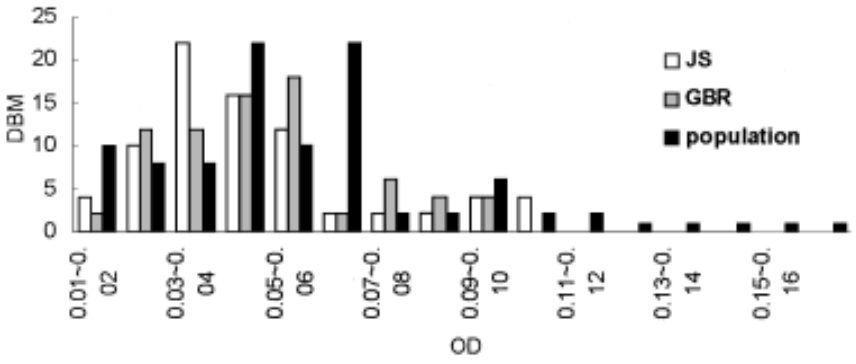

Figure 1. Distribution frequency of residual AChE activities of (Propoxur inhibitor) in the susceptible JS strain, the resistant GBR strain and a field population.

RESULTS

The GBR strain was significantly less susceptible to both dichlorovos and malathion as well as methomyl than JS strain (Table 1). At the LD_{50}, the resistance ratio of the GBR strain relative to JS was 13.8 and 10 for dichlorovos and malathion, respectively, and 5.4 for methomyl.

The percentage of inhibition of AchE activity in GBR strain and field population (DG) was 50.97% and 43.96%, respectively, in the presence of propoxur, 43.76 and 35.87%, respectively, in the presence of paraoxon (Table 2). The results showed that GBR strain and DG field population have higher degrees of AchE insensitivity to OP and carbamate than the susceptible strain. The frequency distribution of AchE activity in the presence of propoxur (Figure 1) and paraoxon

(Figure 2) revealed that there was also a wide range of inhibition and that some individuals were heterozygous for the AchE insensitivity gene.

Our main goal was to determine whether AchE insensitivity was present in the field populations of resistant DBM. If AchE insensitivity is involved in resistance then this biochemical technique can be valuable for defecting and monitoring changes in the frequency of resistant individuals within resistant DBM populations.

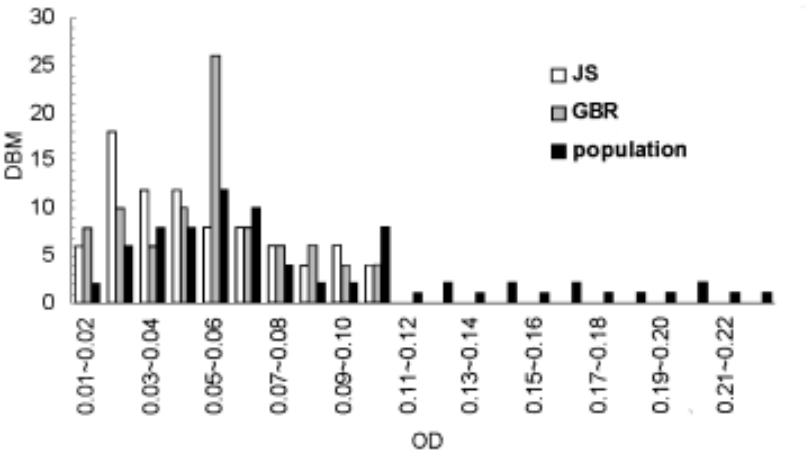

Figure 2. Distribution frequency of residual AChE activities of (Paraoxon inhibitor) in the susceptible JS strain, the resistant GBR strain and a field population.

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Selection for Resistance in the Cotton Bollworm to Insecticide Mixtures

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We have controlled the cotton bollworm (*Heliothis armigera* Hubner) (CBM) with nearly sixty insecticides for over forty years in cotton areas of northern China. This intense pesticide reliance has lead us to classify CBM in these areas as low, moderate, high or extremely resistant to pesticides. In these areas with extremely high levels of CBM resistance, we support and implement integrated pest management (IPM) as the best approach to control CBM today.

Presently, cotton growers accounting for 70% of total cotton acreage rely on a single insecticide to control CBM. In China, we recommend that growers routinely mix insecticides for CBM control, but we are concerned that we may select for multiresistant CBM. Nevertheless, we must recommend mixtures as emergency measures to control CBM. This report compares CBM resistance development in response to single, 2-component and 3-component insecticide mixtures after 20 generations of selection. This study was carried out in the laboratory on CBM collected in the cotton area of Liaocheng, Shandong province in China. This area has a CBM population with extremely high levels of pesticide resistance.

MATERIALS & METHODS

The highly resistant CBM were collected from the Liaocheng cotton area of Shandong Province in China and continuously reared on artificial diet for two years (1993-1995) during this study. All CBM adults bioassayed emerged from pupa that weighed more than 380 mg.

We selected CBM with two series of three insecticides singularly and in all possible combinations. One series included cyhalothrin (CYH), phoxim (PHO) and parathion-methyl (PAM). Insecticides were selected to avoid cross resistance and represented two pesticide groups, three chemical types and

Table 1. Change in resistance of CBM to pesticide mixtures after 20 generations of continuous selection (1993-1995).

Pesticide	Component Proportion	LD_{50} (µg/g) (Resistance Ratio)				
		Base-line	F_5	F_{10}	F_{15}	F_{20}
CYH + PHO + PAM	0.5:9.5:20	0.1539 (1.0)	0.1846 (1.2)	0.2462 (1.5)	0.7233 (4.7)	0.8618 (5.6)
CYH + PHO	0.5:25.5	0.0458 (1.0)	0.0687 (1.5)	0.1053 (2.3)	0.3664 (8.0)	0.4625 (10.1)
PHO + PAM	25:25	0.2049 (1.0)	0.3688 (1.8)	0.635 (3.1)	1.8031 (8.8)	3.0120 (14.7)
CYH + PAM	0.5:25.5	0.2570 (1.0)	0.4112 (1.6)	0.6939 (2.7)	2.1588 (8.4)	2.9041 (11.3)
CYH	-	0.0387 (1.0)	0.2360 (6.1)	0.4721 (12.2)	0.7778 (20.1)	1.0255 (26.5)
PHO	-	0.1504 (1.0)	1.2633 (8.4)	2.2259 (14.8)	3.3998 (24.6)	4.6473 (30.9)
PAM	-	0.9217 (1.0)	7.0971 (7.7)	12.816 (13.9)	20.441 (22.2)	26.452 (28.1)

two modes of actions. However, they have been used to control CBM in large areas for many years. Thus, high resistance and multiple resistance are already present in this CBM population. The other series included cyfluthrin (CYF), endosulfan (END) and quinalphos (QUI). Again, to avoid cross resistance, these insecticides represented three pesticide groups, three chemical types and three modes of action. Most growers have not applied these insecticides to control CBM in the field. However, bioassays and laboratory efficacy trials indicate CBM resistance has already been expressed for each insecticide. In each series, the effect of 3-component insecticide mixtures is compared to 2-component mixtures and single insecticide treatments. This gave us a total of 14 pesticide treatments. All insecticides used were technical products.

Fourteen populations of laboratory-reared CBM were selected for 20 generations with one of the pesticide treatments. Baseline susceptibility of each CBM population was determined prior to selection. Third instars were selected with the topical application assay recommended by FAO (1980). In each treatment, LD_{50} was used to select fourteen CBM populations for resistance. These values were assessed every five generations and updated LD_{50}s were used to select the next five generations. The change in response for each population to their respective insecticide treatment was bioassayed by immersing 4th to 5th instar CBM in insecticide mixtures. Mortality was assessed 24 hours after exposure.

Table 2. Reduction in efficacy of pesticide mixtures towards CBM after 20 years of continuous selection (1993-1995).

Pesticides	Number tested	Percent Mortality (24 h)				
		F_1	F_5	F_{10}	F_{15}	F_{20}
30% CYH + PHO + PAM	100	96	96	96	92	89
26% CYH + PHO	100	82	80	77	72	65
50% PHO + PAM	100	56	56	54	50	41
26% CYH + PAM	100	74	71	62	53	47
2.5% CYH	100	21	20	18	10	6
50% PHO	100	34	29	25	19	16
50% PAM	100	37	32	26	20	15

Table 3. Change in resistance of CBM to pesticide mixtures after 20 generations of continuous selection (1993-1995).

Pesticide	Component Proportion	LD_{50} (µg/g) (Resitance Ratio)				
		base-line	F_5	F_{10}	F_{15}	F_{20}
CYF + END + QUI	1:14:10	0.0632 (1.0)	0.0695 (1.1)	0.0821 (1.3)	0.1137 (1.8)	0.1390 (2.2)
CYF + END	1:28	0.0460 (1.0)	0.0390 (1.5)	0.0966 (2.1)	0.2484 (5.4)	0.3359 (7.3)
END + QUI	17:13	0.0079 (1.0)	0.1186 (1.6)	0.3165 (2.6)	0.6838 (5.8)	0.8960 (7.6)
CYF + QUI	1:20	0.0384 (1.0)	0.0652 (1.7)	0.0921 (2.4)	0.1920 (5.0)	0.2726 (7.1)
CYF	-	0.0401 (1.0)	0.2646 (6.6)	0.4211 (10.5)	0.6416 (16.0)	0.9022 (22.5)
END	-	0.2160 (1.0)	1.1232 (5.2)	1.7938 (8.3)	2.3976 (11.1)	3.1536 (14.6)
QUI	-	0.1569 (1.0)	0.8624 (5.5)	1.1446 (7.3)	1.6150 (10.3)	2.5715 (16.4)

RESULTS & DISCUSSION

Tables 1 and 3 show a gradual increase in the LD_{50} of CBM populations selected by each insecticide treatment over 20 generations in both pesticide series. Table 5 shows that the rate of increase for the 3-component mixtures was 5.6-fold for the CYH + PHO + PAM series and 2.2-fold for the CYH + END + QUI series. Mean increase was 3.9-fold for both series. The resistance ratio for 2-component mixtures increased much faster than 3-component mixtures, and after 20 generations ranged from 7.1 to 14.7-fold resistance. Meanwhile, the resistance levels for single component applications ranged from 14.6 to 30.9-fold after 20 generations. Resistance ratios among the 2-component mixtures were 2.5 times higher than the 3-component mixtures and the single component applications were 5.9-fold higher.

Table 2 and 4 show that mixture efficacy was reduced for all treatments after 20 generations of selection in both pesticide series. The efficacy of the 3-component mixtures was reduced by 7.3% and 3.1% for CYH + PHO + PAM and CYF + END + QUI, respectively (Table 5). Mean reduction of efficacy for 3-component mixtures was 5.2%. Efficacy reduction among 2-component mixtures was greater than 3-component and ranged from 13 to 36% with a mean of 21.8%. Efficacy reduction among single component mixtures ranged from 25.6 to 71.4% and averaged 50.6%. Overall, efficacy reduction in 2-component mixtures was 4.2% higher than 3-component mixtures.

Table 4. Reduction in efficacy of pesticide mixtures towards CBM after 20 years of continuous selection (1993-1995).

Pesticides	Number tested	Percent Mortality (24 h)				
		F_1	F_5	F_{10}	F_{15}	F_{20}
25% CYF + END + QUI	100	98	98	97	95	95
29% CYF + END	100	92	90	86	81	74
30% END + QUI	100	90	88	86	82	77
21% CYF + QUI	100	92	89	86	83	80
5.7% CYF	100	42	35	30	27	14
35% END	100	78	73	68	63	58
25% QUI	100	83	79	74	68	60

Table 5. Comparison of CBM resistance levels and mixture efficacy of three-component, two-component and single component mixtures after 20 generations of selection pressure (1993-1995).

	Resistance Ratio			Pesticide Efficacy		
Pesticides	Resistane ratio[1]	Average increase	Relative component effect	Ratio[2]	Average decrease	Relative component effect
CYH + PHO + PAM	5.6			7.3		
CYH + END + QUI	2.2			3.1		
Three-component		3.9	1.0		5.2	1.0
CYH + PHO	10.1			20.7		
PHO + PAM	14.7			26.8		
CYH + PAM	11.3			36.5		
CYF + PAM	7.3			19.6		
END + QUI	7.6			14.4		
CYF + QUI	7.1			13.0		
Two-component		9.7	2.5		21.8	4.2
CYH	26.5			71.4		
PHO	30.9			52.9		
PAM	28.1			59.5		
CYF	22.5			66.7		
END	14.5			25.6		
QUI	15.4			27.2		
Single component		23.0	5.9		50.6	10.0

[1]LD_{50} (µg/g) F_{20}/ LD_{50} (µg/g) F_1
[2](1.0 - Efficacy in F_{20}/Efficacy in F_1) x 100

Efficacy reduction in single component mixtures were 10% higher than 3-component mixtures. This data indicates that a 3-component mixture is significantly better than 2-component mixture and far better than single insecticides in reducing resistance development. Twenty generations of selection for these laboratory strains of CBM is equivalent to five years of selection for field populations.

In conclusion, a 3-component mixture of insecticides made up of single components with minimal cross-resistance can delay resistance and improve control of CBM significantly better than 2-component mixtures and single component insecticide sprays.

Selection of Colorado Potato Beetle Resistant to CryIIIA on Transgenic Potato Plants

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Developments in insect-resistant plants should provide more effective, less costly, and more environmentally attractive pest control than pesticide applications. Field expression of the *Bacillus thuringiensis* δ-endotoxin genes in transgenic plants has been an effective mean to suppress insect pest populations (Adang *et al.* 1987, Fischhoff *et al.* 1987, Vaeck *et al.* 1987, Delannay *et al.* 1989). However, the recent discovery that several important species of insect pests, including Colorado potato beetle, have the capacity to evolve resistance to *B. thuringiensis* δ-endotoxins raises questions regarding the long-term durability of this bio-pesticide insecticide in pest control (McGaughey & Whalon 1992). Therefore, insect resistance will be a critical consideration as *B. thuringiensis* d-endotoxin applications (transgenic plant releases or conventional d-endotoxin sprays) increase. Without caution and a wise resistance management program, the effectiveness of *B. thuringiensis* δ-endotoxin will be lost in only a few years.

A recent study (Wierenga 1997) determined the stage-specific mortality of Colorado potato beetle exposed to transgenic potato plants containing the *B. thuringiensis* CryIIIA gene. When the larvae were fed on transgenic plant for 96 h, less than 2% of first and second susceptible instar survived. However, resistant second instar larvae experienced less than 50 % mortality. A comparison of mortality for adults fed for two weeks on transgenic plants indicated that susceptible adults could not survive, while the resistant beetles experienced only 25% mortality.

This study was followed by another that selected Colorado potato beetle larvae and adults with commercially available transgenic potato plants containing CryIIIA δ-endotoxin. The Colorado potato beetle strain used for the experiment was 700-fold resistant to CryIIIA δ-endotoxin and was maintained in our laboratory at 25 ± 2°C and 16:8 h (L:D) photoperiod. The origin and maintenance of both susceptible and resistant strains were described earlier (Whalon *et al.* 1993, Rahardja & Whalon 1995, Trisyono & Whalon 1997).

Transgenic Russet Burbank potato petioles (5 leaflets) were inserted into 2-ml vials filled with water, and transferred to individual petri-dishes (15 cm diameter). Twenty larvae were placed on each leaf and held at 25 ± 2°C and 16:8 h (L:D) photoperiod in a growth chamber. Foliage was checked daily and water replenished as needed. Larvae were placed on the foliage and allowed to move and feed freely. After 96 h exposure to transgenic foliage, surviving larvae were transferred to regular potato foliage in rearing cages until they pupated. The emerged adults (T1 generation) were maintained and allowed to move, feed and lay eggs on caged transgenic potato plants. The progeny from T1 (T2 generation) were treated similarly on transgenic plants.

The larval selection experiment was initiated by exposing larvae (480 first instar, 179 second instar and 60 third instar) to transgenic potato foliage. No neonates survived the selection (Table 1) while successive larval stages did survive at progressively higher percentages. The number of beetles that emerged as adults from selection of second and third instars was 1 (< 1%) and 7 (~10%), respectively. The average number of egg masses produced by each surviving female was 28±16 which is in agreement with the fecundity of the resistant strain reported by Trisyono & Whalon (1997). Egg viability (65%) was lower than that observed in conventionally selected Colorado potato beetles (98%, Trisyono & Whalon 1997). From 1,313 larvae fed on transgenic plants, only 12 adults survived. Of these adults, nine were

Table 1. Number or individual Colorado potato beetle stages (neonate, second and third instar) selected with commercial transgenic potato plants.

Stage	# selected	# 2nd instar survived	# 3rd instar survived	# 4th instar survived	# Adult emerged
Neonate	480	0			
Second Instar	179	127	124	28	1
Third Instar	60		42	18	7

females and three were males and they produced 18 egg masses. None of the eggs produced by these adults were viable.

This experiment demonstrated that laboratory selected, highly resistant beetles could survive on B.t transgenic plants for a short period of time. If alternative host plants (horsenettle or non-transgenic potato) were encountered after selection, beetles could survive. Conversely, successive generational exposure or continuous exposure to transgenic plants resulted in 100% mortality of this resistant strain within three generations. The adults from the third (last) generation fed on transgenic plant did not produce any viable eggs. We do not know whether mating did not occur, sperm were infertile or eggs were non-viable.

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Susceptibility of the Cocoa Mirid, *Helopeltis theivora* Waterhouse, to γ-HCH, Cypermethrin and Deltamethrin

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Abstract. The susceptibilities of the cocoa mirid, *Helopeltis theivora*, from Serdang, Selangor, Malaysia to g-HCH, cypermethrin and deltamethrin were evaluated in the laboratory by time-response and topical application techniques. Cypermethrin and deltamethrin were more toxic to the mirid than γ-HCH. An increase in the LT_{50} values over ten years indicates impending resistance in this mirid. Time-response bioassays with synergists suggest that 1) penetration may play a role in the susceptibility of the mirid to γ-HCH, 2) microsomal monooxygenases play a role in the metabolism or detoxification of the pyrethroids, and 3) that conjugation is not the major route for metabolism of these insecticides.

INTRODUCTION

The mirid, *Helopeltis theivora* Waterhouse is one of the key pests of cocoa in Malaysia. This mirid feeds predominantly on cocoa cherelles and pods, and causes serious crop damage or loss during cherelle stage (Muhamad & Way 1995). Cocoa is grown widely in this country and insect pest management in cocoa relies on insecticides as one of the main features (Chung & Wood 1989, Ho 1994). Widespread use of insecticides has lead to resistance development in many insects. Prudent insecticide use in cocoa must be taken seriously as resistance problems in mirids may arise (Dzolkhifli *et al.* 1986). Liew *et al.* (1992) and Ho (1994) have shown degrees of tolerance between populations of the cocoa mirid and possible development of resistance to γ-HCH. These mirid populations show no resistance to the synthetic pyrethroids. This paper describes 1) the susceptibility of *H. theivora* to γ-HCH, cypermethrin and deltamethrin; and 2) the effect of synergist piperonyl butoxide and maleic acid diethyl ether on the toxicity of those insecticides.

MATERIAL & METHODS

Insect Colony

The cocoa mirids were collected from Serdang, Selangor, Malaysia and cultured with the technique described by Muhamad & Khoo (1983). Fourth and fifth instars from the F_1 generation were used in all experiments.

Chemicals

Technical grades of γ-HCH (99% a.i.), deltamethrin (99.5% a.i.) and cypermethrin (50% a.i.) were used to prepare stock solutions. The synergists piperonyl butoxide (PB) and maleic acid diethyl ether (MADE) were obtained from Aldrich and Sigma, respectively. The stock solutions for time-response bioassay were prepared in olive oil. In the topical application bioassay, solutions of the insecticides and synergists were prepared in acetone.

Bioassay technique

We used the technique recommended by the FAO for detecting cocoa mirid resistance to insecticides. Insects were exposed to a single insecticide concentration (Busvine 1980). Earlier studies (Muhamad & Dzolkhifli 1996) showed that this technique is rapid, simple and requires few insects. Ten mirid nymphs were placed in plastic cylinders lined with treated filter paper (12 x 15 cm) and knockdown was recorded at 20 minutes intervals. Knockdown occurred when nymphs failed to cling to the filter paper. In the controls, mirids were exposed to the filter papers treated with the carrier solvent (Dzolkhifli *et al.* 1986). There were five replicates per treatment.

Mirid susceptibility to the insecticides was further tested by topical application. The insecticide (0.6 ml/nymph), with or without PB, was applied with an Arnold microapplicator (Burkard Manufacturing Co. Ltd.). We used five nymphs per replicate and a minimum of 3 replicates per treatment. The mirids were placed in a container with cocoa pod slices. Nymph mortality was recorded 24 h later. With a similar procedure, the insecticides with and without MADE were applied to nymphs. Mortality was recorded 72 h later.

Data were subjected to probit analysis (Finney 1971) with a probit program (S103, Statistical Research Service, Canada Dept. of Agriculture, 1986, unpublished). The median lethal exposure time (LT_{50} = time required to knockdown 50% mirids tested) and median lethal dose (LD_{50}) were obtained for the mirids.

Table 1. A reduction in susceptibility of field-collected *Helopeltis theivora* to γ-HCH, 1982 to 1992.

Year tested	Slope +/- S.E.	LT_{50} (min)	Fudicial limit (min) lower-upper
1982	4.82 +/- *na	63.0	*na
1985	5.32 +/- 0.64	72.3	64.2 - 80.0
1992	4.41 +/- 0.45	94.2	86.9 - 101.9

*na = not available

RESULTS & DISCUSSION

With the time-response technique, the values for γ-HCH determined for 1982, 1985 and 1992 were 62 min, 72 min and 94 min, respectively (Table 1). Even though the increase in the LT_{50} values was not much, it showed increasing mirid tolerance to γ-HCH and impending resistance in the cocoa mirids to γ-HCH. The LT_{50} values of cypermethrin and deltamethrin for 1982 and 1985 were not determined. These pyrethroids became

Table 2. Toxicity of γ–HCH, cypermethrin and deltamethrin and synergism by piperonyl butoxide (PB) on the mirid *H. theivora*. The insecticides with or without PB were tested with topical application bioassay.

Treatment	LD_{50} (ng/nmph)	Fudicial limit lower - upper	RP*	SR**
γ-HCH alone	26.7	18.4 - 62.9	-	
+ PB	7.44	2.83 - 40.19		3.6
cypermethrin alone	2.52	1.26 - 5.29	10.6	
+ PB	0.33	0.15 - 0.16		7.6
deltamethrin alone	2.58	1.39 - 4.03	10.3	
+ PB	0.32	0.16 - 0.54		8.1

*RP: Relative potency = (LD_{50} of γ-HCH)/ (LD_{50} or pyrethroids)
**SR: Synergism ratio = (LD_{50} of insecticide alone)/ (LD_{50} of insecticide + PB)

Table 4. Effect of synergist piperonyl butoxide applied topically on the LT_{50} of γ-HCH, cypermethrin and deltamethrin of *H. theivora*.

Treatment	LT_{50} (min)	Fudicial limit (min) lower - upper	*SR
γ-HCH alone	94.2	86.9 - 101.9	
+ PB	50.3	46.9 - 53.8	1.9
cypermethrin alone	584.1	544.5 - 631.7	
+ PB	81.8	75.3 - 88.2	7.1
deltamethrin alone	142.2	132 - 153.9	
+ PB	67.1	63.9 - 70.4	2.1

*SR: Synergism ration = (LD_{50} of insecticide alone)/ (LD_{50} of insecticide + PB)

a widely used alternative to γ-HCH in the mid-eighties.

Through topical application, the relative potency (RP) of cypermethrin and deltamethrin to γ-HCH was determined. Both cypermethrin and deltamethrin are more toxic (about 10 times) than γ-HCH (Table 2). Based on the LD_{50} values after 72 h, cypermethrin was more potent (RP = 52) compared to deltamethrin (RP = 24) to γ-HCH (Table 3). Currently, cypermethrin and deltamethrin are the recommended insecticides for the mirid control (Ho 1994).

Table 4 shows the effect of synergist PB applied topically followed by time-response bioassay on the LT_{50} values of γ-HCH, cypermethrin and deltamethrin. The synergistic ratio observed for γ-HCH was 1.9. This low value indicated that microsomal monooxygenase may not play an important role in the metabolism of γ-HCH. However, we argue that the uptake of g-HCH through insect cuticle was not very efficient. Thus, only a minor proportion of γ-HCH was able to enter through the insect cuticle and subjected to the microsomal monooxygenases. Further evaluation by topical application of γ-HCH, with and without PB, (Table 2) gave a synergistic ratio of 3.6. The increase indicated that penetration may play some role on the susceptibility of the mirid to γ-HCH. The increase also showed that microsomal monooxygenases may be important in the metabolism of γ-HCH. Muhamad and Dzolkhifli (1996) showed that the topical application bioassay was more sensitive in detecting resistance in mirids than the time-response technique.

More than 7-fold synergism of PB on cypermethrin and deltamethrin was observed in both bioassay techniques with the exception of deltamethrin in the time-response bioassay (Tables 2, 4 and 5). These results indicate that microsomal monooxygeneses play an important role in the metabolism or detoxification of cypermethrin and deltamethrin.

When the mirids were treated with synergist MADE, the synergistic ratios for all the toxicants tested were found to be very low for each insecticide tested (Table 3). Cypermethrin and deltamethrin show synergistic values of less than 1.0 indicating an antagonistic effect. These results indicate that conjugation is not the major route for the detoxification of the toxicants tested.

Cypermethrin and deltamethrin are more toxic to the mirid than γ-HCH. The metabolism of these in-

Table 3. Toxicity of γ–HCH, cypermethrin and deltamethrin and synergisms by maleic acid diethyl ether (MADE) to the mirid *H. theivora*. The insecticides with or without MADE were tested with a topical application bioassay.

Treatment	72 h LD_{50} (ng/ nymph)	RP*	SR**
γ-HCH alone	6.14	-	
+ MADE	4.55		1.3
cypermethrin alone	0.12	51.2	
+ MADE	0.17		0.7
deltamethrin alone	0.26	23.6	
	0.46		0.56

*RP: Relative potency = (LD_{50} of γ-HCH)/ (LD_{50} or pyrethroids)

**SR: Synergism ratio = (LD_{50} of insecticide alone)/ (LD_{50} of insecticide + MADE)

secticides by microsomal monooxygenases appears to be more important than conjugation. The increase in tolerance of the mirid to γ-HCH over time coupled with higher synergism of PB with all three insecticides indicates the impending resistance in the mirids. Thus, there is a need to devise chemical strategies now and to integrate these strategies for management of the cocoa mirid as soon as possible.

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Synergistic Suppression of Fenvalerate Resistance in Field Populations of *Helicoverpa armigera* in Tamil Nadu

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INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* Hubner, is a serious polyphagous noctuid that damages a number of crops in Tamil Nadu, India. In cotton, there has been an increase in synthetic pyrethroids applied to control *H. armigera* since it displaced *Spodoptera* and *Earias* spp. as the key pest species. Resistance to synthetic pyrethroids in field populations of *H. armigera* was reported from several locations in India (Pasupathy & Regupathy 1994, Armes *et al.* 1992). Physiological and biochemical mechanisms responsible for pyrethroid resistance are: 1) slower rate of pesticide penetration through cuticle; 2) an enhanced rate in pesticide metabolism through mixed function oxidases (MFOs), esterases, or both enzyme systems. Selection pressure through unrestricted and inappropriate applications of insecticides may have resulted in the development of insecticide resistance through multiple resistance mechanisms in *H. armigera* populations from Tamil Nadu (Armes *et al.* 1994). As part of a regular program to monitor resistance in field populations of *H. armigera*, the detoxification mechanisms were examined with pesticide synergists.

MATERIAL &METHODS

H. armigera larvae, collected from the field, were reared on semi-synthetic diet in 12-well tissue cul-

Table 1. Synergist suppression of fenvalerate resistance in *H. armigera*. Fenvalerate topically applied at rate of 0.2 µg/larva.

	Synergist rate	No. of larvae dosed	Percent survival
Fenvalerate	-	4545	76.6
FEN + PBO	50 µg	5193	22.7
FEN + PP	25 µg	4891	30.4
FEN + PRF	0.1 µg	4603	66.8

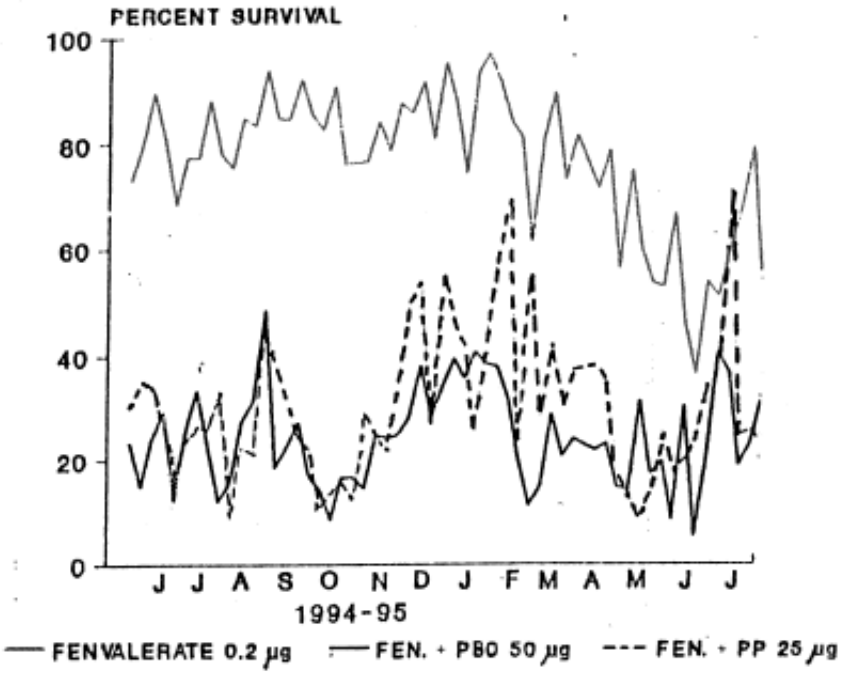

Figure 1. Synergistic suppression of fenvalerate resistance in *H. armigera* by PBO and PP.

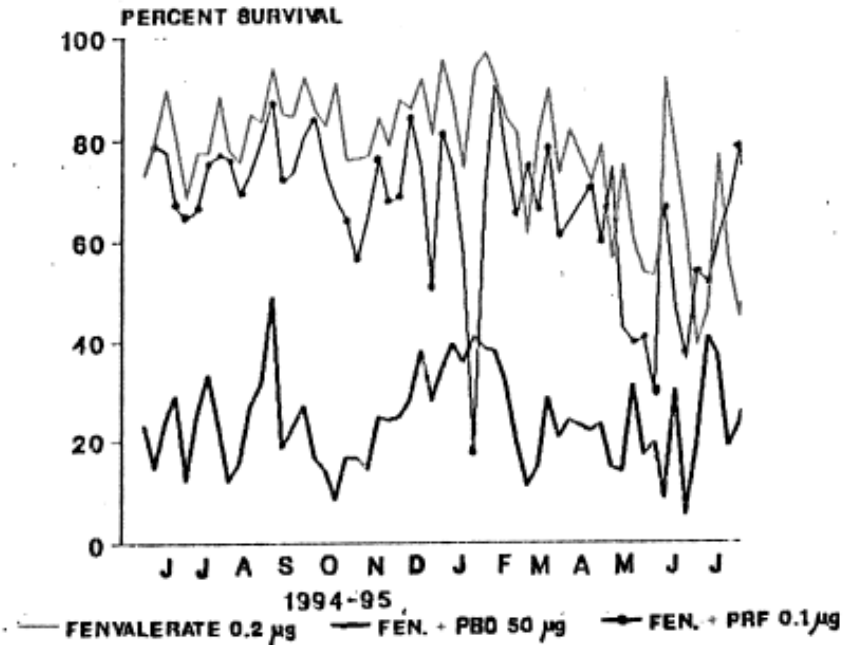

Figure 2. Synergistic suppression of fenvalerate resistance in *H. armigera* by PRF.

ture plates. Trays with eggs and larvae were kept at 25 ± 2°C. The larvae were sorted and 30-40 mg larvae were placed in separate tissue culture plates for bioassays. Discriminating doses of fenvalerate were topically applied with a Hamilton microsyringe to the thoracic dorsum of larvae reared on synthetic diet. Each larvae was observed for mortality every 24 h up to 144 h. Percent survivorship (number of dead larvae/ number dosed* 100) was calculated and expressed with a standard error ($P * 100\text{-}P/ n\text{-}1)^{-1/2}$ where P = % larvae surviving a discriminating dose and n = total number of larvae tested. The following discriminating doses in 1.0 ml solution were applied per larva.

1. Fenvalerate 0.2 mg/ml
2. Fenvalerate 0.2 mg + Piperonyl butoxide (PBO) 50 mg/ml
3. Fenvalerate 0.2 mg + Propargyloxphthalimide (PP) 25 mg/ml
4. Fenvalerate 0.2 mg + Profenofos (PRF) 0.1 mg/ml

RESULTS & DISCUSSION

The resistance to fenvalerate was prevalent throughout the observation period (June 1994 to July 1995) with survival as high as 90%. There was some seasonal fluctuations in resistance with the lowest level observed during April and May, 1995 (56.4 and 53.1%, respectively) (Fig. 1).

PBO suppressed fenvalerate resistance by 48 to 91% with a mean of 70.3%. The suppression of fenvalerate resistance by propargyloxphthalimide (PP) was similar to PBO. This suggests that the synergists affect a similar resistance mechanism (Table 1). Synergism between piperonyl butoxide and fenvalerate present in this study is similar to that in the diamondback

moth, *Plutella xylostella* L. (Viropong *et al*. 1988). The synergistic activity of PBO and PP may be attributed to inhibition of microsomal oxidation by cytochrome P-450.

The suppression of fenvalerate resistance by PRF was 6.4 to 40.4% with a mean reduction of only 14.5%. The highest suppression level of fenvalerate resistance by profenofos was observed during November 1994 and May 1995 (Figure 2). These synergist bioassays suggest that the predominant mechanism involved is mixed function oxidases (MFOs) not esterases. McCaffery *et al.* (1989) found levels of fenvalerate resistance in *H. armigera* also. The synergistic activity of PBO with synthetic pyrethroids was demonstrated with *Tribolium cataneum* (Herbst) (Ishaaya *et al*. 1983), houseflies (Saito *et al.* 1992), horn flies (Sparks & Byford 1988), and Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Silcox *et al.* 1985).

The toxicity of several insecticides is limited by detoxifying oxidases, esterases and other enzyme systems in resistant insects. Enzyme inhibitors may enhance the potency of insecticides in these insects.

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Tobacco Budworm Response to Pyrethroid Insecticides in the Winter Garden Area and in the Lower Rio Grande Valley

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INTRODUCTION

For the past two decades, pyrethroid insecticides especially cypermethrin, have been used widely to control the tobacco budworm, *Heliothis virescens* (F.), on cotton in the Winter Garden Area, south Texas and in the Lower Rio Grande Valley that runs from south Texas, USA to Tamaulipas, Mexico. In the Lower Rio Grande Valley, budworm toxicity to permethrin and fenvalerate was first determined by Davis *et al.* (1975) and Harding *et al*. (1977). The toxicity of cypermethrin and cyfluthrin to this insect was determined by Wolfenbarger *et al.* (1982).

MATERIALS & METHODS

The initial toxicity of cypermethrin and five other pyrethroids against field-collected budworms was conducted to determine variability in larval resistance or susceptibility. Toxicity of nine pyrethroids was also determined for a susceptible laboratory strain. This laboratory strain was maintained in isolation for two decades. Eggs or larvae collected from each field location were separately reared to adults, allowed to mate and produce progeny. LD_{50} values were determined by topically applying insecticides to larvae (15 to 28 mg) (Davis *et al.* 1975, Wolfenbarger *et al.* 1989). Mortalities were determined 48 or 72 h post-treatment. Differences in LD_{50}s were indicated when 95% confidence intervals (C. I.) did not overlap, or when an LD_{50} value was greater than 1.0. LD_{50}s were determined in 1983, 1985 to 1988 and 1990-1991 on larvae from

Table 1. Insecticide toxicity (µg/larva after 48 or 72 h) against a laboratory strain of tobacco budworm.

Insecticide	Number treated	Slope + SE	LD_{50}	(95% Confidence Level)
Cypermethrin[1]	740	2.40 + 0.38	0.01	(0.004 - 0.019)
Permethrin[2]	1130	3.05 + 0.25	0.003	(0.0004 - 0.019)
Zeta Cypermethrin	637	0.86 + 0.10	0.002	(0.001 - 0.003)
Cyfluthrin[3]	NA	NA	0.003	NA
λ-Cyhalothrin	140	1.73 + 0.33	0.006	(0.003 - 0.013)
Deltamethrin[3]	NA	NA	0.001	NA
Bifenthrin	122	1.84 + 0.66	0.017	(0.005 - 1.32)
Fenvalerate[4]	NA	NA	0.011	NA
Esfenvalerate	247	1.12 + 0.27	0.018	(0.005 - 0.055)

[1]Determined in 1976 and 1980.
[2]Determined in 1974, 1978 and 1979.
[3]Taken from Wolfenbarger and Harding (1982).
[4]Taken from Harding *et al.* (1977).
NA = Not available.

the Winter Garden Area, 1979-1988 and 1991-1992 on larvae from the Lower Rio Grande Valley, Texas and from 1991-1993 and 1995 on larvae from the Lower Rio Grande Valley in northeast Tamaulipas, Mexico.

RESULTS

In 1974, the LD_{50} for permethrin was 0.000097 µg/larva (Davis *et al.* 1975) in the susceptible laboratory strain, while in 1974, 1978 and 1979 the LD_{50} was 0.0028 mg/larva (Table 1). This 29-fold difference indicates variation in strain susceptibility. LD_{50}s of permethrin and cypermethrin were statistically similar (Table 1). Zeta cypermethrin, the most toxic isomer of cypermethrin, was significantly more toxic to budworm than cypermethrin (Table 1). Cyfluthrin was equally toxic to budworm as permethrin, cypermethrin, bifenthrin, esfenvalerate and zeta cypermethrin (LD_{50} = 0.003 mg/larva) (Wolfenbarger *et al.* 1982). In 1975, the LD_{50} for fenvalerate was 0.011 µg/larva for a laboratory strain (Wolfenbarger & Harding 1977). Meanwhile, esfenvalerate was the least toxic pyrethroid tested against the susceptible strain (LD_{50} = 0.018 mg/larva) (Table 1). Deltamethrin was the most toxic insecticide tested against this strain (LD_{50} < 0.001 mg/larva) (Table 1). There was a 30-fold difference between the highest and lowest LD_{50} values for this susceptible strain. We consider this to be range of variation in susceptibility.

LD_{50} values for six pyrethroids were determined from 23 field collections of budworm in the United States and Mexico (Tables 2 - 4). LD_{50} values varied from year to year. For the Lower Rio Grande Valley, Texas, LD_{50}s for cypermethrin ranged from 0.014 to 0.15 from 1979 to 1988 (Wolfenbarger *et al.* 1982, Staetz *et al.* 1988, Staetz *et al.* 1989). However, a LD_{50} of 1.22 µg/larva for cypermethrin was determined for a population sampled in 1993 from cotton near Matamoros, Mexico (Table 3). We estimate that this population was 87-fold resistant to cypermethrin and statistically different from any other LD_{50} value determined over a 15-year period.

In 1992, cypermethrin LD_{50} values from ten populations of budworm from the Lower Rio Grande Valley ranged from 0.066 to 0.78 µg/larvae (Table 2). Three LD_{50}s were within the C. I. values for cypermethrin determined between 1979 and 1991 while the other five LD_{50}s were greater than any interval from previous years. In 1995, LD_{50}s were determined from three additional budworm populations

Table 2. Toxicity (µg/larva after 72 h) of various insecticides against larvae of the tobacco budworm. Lower Rio Grande Valley, TX, U.S.A. and Mexico. 1992.

Location	Insecticide	Number treated	Slope + SE	LD_{50}	(95% Confidence Interval)
Brownsville	Cypermethrin	143	1.39 + 0.23	0.066	(0.045 - 0.10)
La Blanca (Field 1)[1]	Cypermethrin	188	0.91 + 0.30	0.40	(0.1 - 33.67)
	Fenvalerate	90	1.12 + 0.31	0.87	(0.06 - 3.25)
La Blanca (Field 2)[1]	Cypermethrin	183	1.37 + 0.20	0.076	(0.051 - 0.12)
	Bifenthrin	85	2.09 + 0.40	0.068	(0.046 - 0.098)
La Blanca (Field 3)[1]	Cypermethrin	209	-0.24 + 0.09	0.41	(0.016 - 341.74)
	Cyfluthrin	174	1.08 + 0.23	0.19	(0.079 - 0.48)
	Cypermethrin	68	0.20 + 0.13		
La Blanca (Field 4)[1]	Cypermethrin	209	0.20 + 0.09		
La Blanca	Cypermethrin	87	0.83 + 0.15	0.78	(0.31 - 2.15)
San Perlita[1]	Cypermethrin	164	0.58 + 0.25	0.67	(0.11 - 4.59)
Rio Bravo (June 1)[2]	Bifenthrin	149	1.22 + 0.21	0.055	(0.036 - 0.088)
	Cypermethrin	253	1.83 + 0.22	0.19	(0.15 - 0.25)
Rio Bravo	Fenvalerate	83	0.93 + 0.40	0.24	(∞∞ -∞∞)
	Bifenthrin	192	1.30 + 0.20	0.014	(0.009 - 0.019)
	Cypermethrin	279	0.56 + 0.10	0.086	(0.044 - 0.17)
Valle Hermoso[2]	Esfenvalerate	321	0.62 + 0.09	0.017	(0.0083 - 0.029)

[1]Norman *et al.* (1993).
[2]Vargas-Camplis & Teran-Vargas (1993).

Table 3. Toxicity of cypermethrin (µg/larva after 72 h) against tobacco budwom. Lower Rio Grande Valley, Tamaulipas, Mexico. 1993 and 1995.

Location	Year	Number Treated	Slope ± SE	LD_{50}	(95% Confidence Interval)
Matamoros	1993			1.22[1]	
	1995	542	1.47 ± 0.25	0.18	(0.11 - 0.44)
San Fernando	1995	89	2.77 ± 1.03	0.41	(∞∞ - ∞∞)
Rio Bravo	1993			0.37[1]	
	1995	96	1.96 ± 0.45	0.16	(0.11 - 0.34)

[1]Vargas-Camplis & Wolfenbarger (1994).

Table 4. Toxicity of pyrethroids (µg/larva after 48 h) against tobacco budworm. Winter Garden Area, Uvalde, TX. 1990-1991.

Year	Insecticide	Number Treated	Slope ± SE	LD_{50}	(95% Confidence Interval)
1990	Cypermethrin	83	1.61 ± 0.37	0.081	(0.049 - 0.10)
1991	Cypermethrin	228	0.58 ± 0.11	0.035	(0.0067 - 0.088)
	Esfenvalerate	301	0.59 ± 0.076	0.049	(0.015 - 0.11)
	Permethrin	316	0.47 ± 0.062	3.83	(1.83 - 9.47)

were sampled in the Lower Rio Grande Valley. These C.I.s ranged from 0.16 to 0.41 µg/larva (Table 3) and overlapped with the five high LD_{50}s found in 1992. The LD_{50}s of all pyrethroids tested against Lower Rio Grande Valley strains were greater than those for the laboratory strain. There were no clear patterns in relative toxicity between these pyrethroids to these budworm populations.

Cypermethrin LD_{50} values among budworm populations collected in the Winter Garden Area ranged from 0.013 to 0.34 µg/larva between 1983 and 1988 (Staetz *et al*. 1988, Staetz *et al*. 1989). Broad overlaps in C.I. indicate that this is a range of susceptibility. In 1986, pyrethroid LD_{50} values ranged from 0.018 to 1.12 mg/larva in these budworm populations (Sparks *et al*. 1988). Deltamethrin was the most toxic pyrethroid and permethrin the least toxic. In 1991, permethrin was again the least toxic and nearly 1,400 higher than the LD_{50} of the laboratory strain (Table 4). These LD_{50} values indicate budworm resistance to permethrin. Budworm populations in this area appear to be more susceptible to bifenthrin and less susceptible to fenvalerate than the Lower Rio Grande Valley populations.

CONCLUSIONS

We conclude that 87% (20 of 23 LD_{50}s) of populations sampled were not resistant to any of the pyrethroid insecticides in the Winter Garden Area and the Lower Rio Grande Valley. Larvae sampled near Matamoros and treated with cypermethrin in 1993 and larvae sampled near the Winter Garden Area and treated with permethrin in 1986 and 1991 may be resistant. Thus, resistant populations of tobacco budmoth can be found in the Winter Garden area of South Texas and the Lower Rio Grande Valley in South Texas and Tamaulipas, Mexico.

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Abstracts

Detection and Inheritance of DDT and Pyrethroid Resistance in Maize Weevil (*Sitophilus zeamais*) from Brazil

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Failure to control the maize weevil, *Sitophilus zeamais*, (Coleoptera: Curculionidae) with deltamethrin in Brazil led us to investigate the resistance of this stored grain pest to DDT, pirimiphos-methyl and to three pyrethroids (deltamethrin, cypermethrin and permethrin) with and without the synergist piperonyl butoxide (PBO). DDT is not registered in Brazil, but was widely applied in the past against this pest. Pirimiphos-methyl and PBO-synergized deltamethrin are recommended for maize weevil control, but cypermethrin and permethrin are not used despite their efficiency against this pest.

Bioassay tests were carried out on insecticide-impregnated filter paper as recommended by FAO. The discriminating concentrations for each insecticide, with and without PBO, were established based in the LC_{99} and LC_{9} of a standard susceptible population. With this bioassay, the cross-resistance spectra were verified in six populations of *S. zeamais* from four different states (Minas Gerais, Paraná, Goiás and Rio Grande do Sul) to DDT and pyrethroids. One population from Cachoeiro do Itapemirim (state of Espírito Santo) showed resistance to DDT and deltamethrin only, while three other populations from Sete Lagoas and Viçosa (Minas Gerais) and Ponta Grossa (Paraná) showed resistance to DDT only (Guedes *et al.* 1995).

We also used the insecticide-impregnated filter papers to analyze the inheritance of deltamethrin resistance in one DDT/pyrethroid resistant strain of maize weevil (Guedes *et al.* 1994). At the LC_{50}, the responses of the F_1 hybrid reciprocal crosses were very different and c^2 analyses of the observed responses for the F_1 and F_2-backcross progenies provided evidence that a single gene was responsible for resistance. Deltamethrin resistance seems to be controlled by a single recessive sex-linked gene (Guedes *et al.* 1994).

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Increased Cytochrome P450 Activity in an Insecticide Resistant Strain of German Cockroach

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The Munsyana strain (R-MA) of German cockroach (*Blatella germanica* L.) displayed 80-fold resistance to the pyrethroid insecticide, cypermethrin. The strain possessed a 4.5-fold greater total cytochrome P450 content and a 2.5-fold greater cytochrome P450-mediated N-demethylation of the substrate 4-chloro-N-methylanaline when compared to the susceptible Johnson Wax (S-JW) strain. Immobilized artificial membrane high performance liquid chromatography (IAM-HPLC) of microsomal proteins from the R-MA strain enriched cytrochrome P450 activity greater than 7-fold. Following purification, we detected a single protein band of M_r = 49 kDa (P450 MA) by silver-staining SDS PAGE gels.

An antiserum to this purified protein from the MA strain was produced in mice. The antiserum, Anti-P450 MA, inhibited cytochrome P450-mediated N-demethylation by 4-fold in both R-MA and S-JW

strains. In Western blots of microsomal proteins, anti-P450 MA differentiated single R-MA and S-JW individuals by recognizing the 49 kDA protein band in the R-MA strain only. We were able to detect the 49 kDa in the S-JW strain in a Western blot analysis following induction with pentamethylbenzene (PMB). PMB induction increased N-demethylation by 2.6 and 8.0-fold in the R-MA and the S-JW strains, respectively. These results are consistent with the hypothesis that insecticide resistance in this R-MA strain of German cockroach is due to over-expression of cytochrome P450.

Laboratory Selection for Insecticide Resistance in the Leafminer Natural Enemy, *Ganaspidium utilis* Beardsley (Hymenoptera: Eucoilidae)

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A laboratory strain of *Ganaspidium utilis* Beardsley (Hymenoptera: Eucoilidae), a parasitoid of *Liriomyza* leafminers, was selected for fenvalerate resistance. This strain was reared for 50 generations with 22 generations treated with fenvalerate. The LC_{50} for females and males from this selected strain (Select) to fenvalerate was 8,891 and 9,976 mg active ingredient (AI) fenvalerate/L, respectively. Compared with an unselected baseline colony (Base), the LC_{50} in the Select females was increased about 5-fold. This new LC_{50} is roughly 37-fold higher than the recommended rate of fenvalerate applied in the field.

At 2, 24, 48 and 72 hours after treatment, *G. utilis* adults were exposed to bean foliage treated with various rates of fenvalerate (0.056, 0.112, 0.224 and 0.448 kg AI fenvalerate/ha) applied with a boom sprayer. Results showed significantly higher mortality in the Base strain compared to the Select strain in most treatments. For example, when exposed to bean foliage 2 hours after fenvalerate treatment at the recommended field rate of 0.224 kg AI/ha, the Select strain exhibited significantly higher survivorship (72%) compared with the Base strain (27%).

Assessment of parasitoid susceptibility to esfenvalerate, malathion, oxamyl and methomyl in plastic cups showed significantly higher LC_{50}s (2.5-fold higher) for esfenvalerate and malathion in the Select strain compared with the Base strain.

The Select strain exhibited normal progeny production but the sex ratio was highly biased towards males (1.00:0.58 M:F) when exposed to 3,000 ppm fenvalerate for 24 h. At this rate of fenvalerate exposure, the number of offspring per female was 37% less than normal. No other reduction in fitness was found in the Select strain. Genetic back crosses suggest an additive, polygenic mode of inheritance for fenvalerate resistance in the Select strain.

If established in the field, the Select strain may reduce pest resurgence and secondary pest outbreaks associated with pesticide applications directed towards *Liriomyza* leafminers.

Multiple Mechanisms Conferring Organophosphate Resistance in Greenbugs

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The greenbug, *Schizaphis graminum* (Rondani), is a major insect pest of sorghum, wheat and other small grain crops worldwide. In midwestern United States, the infestations of greenbugs are common and organophosphate insecticides are often applied in greenbug management programs. As a result, greenbugs developed high resistance to these insecticides (Sloderbeck *et al.* 1991). Resistance in greenbug is attributed to an increase in esterase activity and a decrease in acetylcholinesterase (AChE) sensitivity (Siegfried & Ono 1993, Ono & Siegfried 1995). In a recent study, we examined the inter- and intra-strain variations in esterase and AChE activities. We also examined AChE sensitivity to inhibition by paraoxon in individual aphids from an organophosphate susceptible (OSS) and three resistant (OR-0, OR-1 and OR-2) greenbug strains. Our findings are sum-

marized as following:

1. The resistance factors for parathion in the OR-0, OR-1 and OR-2 strains of greenbugs were 1.6, 32 and 42, respectively, based on a glass-vial residual contact bioassay.

2. There was no significant difference in the general esterase level between the OSS and OR-0 strains, but the enzyme activities in the OR-1 and OR-2 strains were 1.9- and 2.4-fold higher, respectively, than that of the OSS strain when a-naphthyl acetate was used as substrate.

3. AChEs from all three resistant strains were 2- to 3-fold less sensitive to inhibition by paraoxon, and the reduced AChE sensitivity was mainly due to the decrease in affinity between AChE and paraoxon.

4. Resistance in the OR-0 strain was insignificant and apparently was due to the altered AChE with increased activity and reduced sensitivity. This mechanism appears to be less effective in conferring organophosphate resistance.

5. Resistance in both OR-1 and OR-2 strains was due to multiple resistance mechanisms including an increase of esterase activity and an alteration of AChE with reduced sensitivity to inhibition by paraoxon and increased AChE activity.

6. Increased AChE activity in the resistant strains was positively correlated with increased general esterase activity. However, the interactions between these mechanisms is unknown.

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Available Publications

NEW BOOK World Weeds - Natural Histories and Distribution, by LeRoy Holm Jerry Doll, Eric Holm, Juan Pancho, and James Herberger, (John Wiley and Sons, New-York, $195.00, 1200 pages, 1997). This seminal book is the culmination of four decades of research on the natural history and distribution of 125 weed species in all crops in 100 countries. Each chapter contains up-to-date information on resistance to herbicides, changes in weed flora with the new crop tillage systems, and biological control. Beyond the indication of resistance problems, it has the immense amount of natural biology and history that is needed both to estimate the likelihood a given weed species could easily evolve resistance to herbicides, as well as to understand how an evolved resistant weed might spread. Its massive bibliography contains more than 3000 references. A full botanical description is supplied for each species, supplemented by a full-page illustration. There are 125 detailed maps of the world distribution of these plants, which would tell the resistance researcher the extent a resistant weed might spread. All or most of the known biology has been gathered for each species including habitat, seed production, morphology, ecology, physiology, mammalian toxicity, and rank of importance by crop in each country. Over 3400 common names are indexed and cross- referenced by species and country. This book concludes the search for the 200 worst weeds of the world beginning with the publication of The World's Worst Weeds and The Geographical Atlas of World Weeds by Holm, et al. in 1977 and 1979, respectively.

RESISTANCE MANAGEMENT EDUCATIONAL KIT

An educational kit designed for use as a one-hour short course on strategies and tactics to minimize resistance to insecticides is available from the Insecticide Resistance Action Committee (IRAC US). The kit consists of a video, a slide set and a script, a source of references and a fun quiz. The target audience is Extension Agents, Agricultural Specialists and Consultants who routinely train others on crop protection issues. Supplies are limited, so please request for teaching purposes only. A $10 shipping-and-handling fee should be sent to: IRAC EDUCATIONAL KIT, PO BOX 413708, KANSAS CITY, MO 64179-0424.

A Letter from the Coordinator

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CALL FOR ARTICLES

To ensure future success of the Resistant Pest Management Newsletter, we ask our readers to contribute articles, abstracts, reviews and updates on resistance management. We are currently accepting submissions for the Winter, 1997 issue of the Newsletter (Vol. 9, no.2). The deadline for submissions is October 31, 1997. Your submission can be made on disk (any word processing format), email or hard copy. When sending a submission on disk or via email, we also request a hard copy for our files. All figures should be in back & white or gray tones and can be sent in any format as well.

ERRATA

I would like to bring a few mistakes to your attention.

1. The issue number on the cover of the Winter 1996 Newsletter is incorrect. It should be Volume 8, number 2. I recommend changing this with your favorite editting pen to avoid confusion.

2. On page two, the first sentence of paragraph two in "Fewer Constraints than Proclaimed to the Evolution of Glyphosate-Resistant Weeds" by Jonathan Gressel should read:

"A spate of short papers/abstracts have appeared at national (Bradshaw *et al.* 1995), international (Padgette *et al.* 1995) and at regional meetings, as well as in the pages of this newsletter (Jasieniuk 1995)."

Also, the following reference was omitted form the reference list:

Pratley, J., Baines, P., Eberbach, P., Incerti, M. & Broster, J. 1996. Glyphosate resistance in annual ryegrass. *In* Proc.NSW Grasslands Society Conf. Wagga-Wagga (in press).

I appreciated all the comments in response to my first issue as Newsletter coordinator and welcome any other comments or suggestions.

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