Resistant Pest Management Newsletter

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

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Table of Contents

Letter from the Editors

We are pleased to present our eleventh issue of the Newsletter. This issue is international in scope with a sweep of resistance related topics. We thank all the contributors. We remind the subscribers that all news, reviews, abstracts, etc. are unreferred and should be recognized as such.

Newsletter News:

1. The posting of the Newsletter onto an E-mail bulletin board has met with extremely favorable responses. We thank all those who wrote, phoned or faxed their thoughts on this recent development. Our sincere thank you is also extended to Michael Caprio at Mississippi State University and Doug King, system operator of the IPMnet Bulletin Board, for all their time and effort to get the Newsletter up and running on computer networks. Currently, this option will result in easy access for anyone with the equipment to access Internet. Instructions for accessing the Newsletter through Internet can be found on page 31 of the Newsletter.

2. Both new and current subscribers are asked to fill out and return the application form found on page 31 of the Newsletter. This information will allow us to discern those subscribers who need hard copies and those subscribers that will be accessing the Newsletter through Internet.

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Resistance Management from around the Globe

Circumstantial Evidence Supports Insect Resistance in Bangledesh

Heliothis armigera (Hubn.) (Noctuidae: Lep.) was considered as a minor pest of cotton in Bangladesh during the mid 1980's. Following the rapid introduction of synthetic pyrethroids, this insect emerged as a major pest of cotton in the country. Last year a trial was conducted to screen synthetic pyrethroids (10 different insecticides) against *H. armigera*. None were found effective against the test insect. These results indicate that *H. armigera* may have developed resistance against the synthetic pyrethroids in Bangladesh. This work is under further investigation.

Leucinodes orbanalis Guen. (Pyraliade: Lep.) is the key insect pest of eggplants (brinjal) in Bangladesh. For years, this pest has been subjected to heavy selective pressures by different groups of chemical insecticides. For the last couple of years no other insecticide except synthetic pyrethroids were effective against this insect. In 1993 trials showed that even the synthetic pyrethroids did not give sufficient control of *L. orbanalis.*

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The Basic Research of Resistance Management in Cotton Bollworm (Lepidoptera: Noctuidae)

The results of monitoring pyrethroids resistance
In the 1970s, control of cotton bollworm, *Heliothis armigera* Hubner, was mainly dependent on Organochlorine and Organophosphate chemicals. In China, resistance to DDT, carbaryl and parathion has been reported.

The results of monitoring insecticide resistance in this insect collected from 24 locations of 6 provinces including Shandong, Hebei, Henan, Jiangsu, Anhui and Xinjiang in China from 1983 to 1992 showed that this insect has developed high levels of resistance to fenvalerate (40-1361x), deltamethrin (26-115x), and low-moderate levels of resistance to esfenvalerate, cyhalothrin and fenpropathrin (<40x) in cotton growing areas of Shengzhi, Raoyang County, Hebei Province and Yanggu County, Shandong Province.

Basic research on resistance management
A knowledge of cross-resistance spectrum, resistance mechanisms, genetic basis of resistance was considered fundamental to pest resistance management strategy. The results of monitoring resistance indicated the level of resistance to fenvalerate in *Heliothis armigera* H. was the highest. Therefore selection for fenvalerate resistant and susceptible strains, studies on the cross resistance spectrum, the resistance mechanism and the genetics of fenvalerate resistance were carried out in our laboratory.

Selection for fenvalerate resistant and susceptible strains
A fenvalerate resistant strain (YG-R) of cotton bollworm was developed by laboratory selection of a colony (YG) collected from Yanggu County,Shandong Province in 1990. YG-R strain showed about 2000-fold resistance to fenvalerate compared with susceptible Dongtai Strain collected in 1983.

A fenvalerate susceptible strain (YS-S) was also obtained by selection of the clones resulted from single-pair matings of a colony (YS) collected from Yanshi County, Hena Province in 1991. Its susceptibility to fenvalerate is close to that of Dongtai susceptible strain.

Cross resistance spectrum of YG-R strain
Since selection with chemicals can result in increased tolerance to related compounds, resistance spectra of YG-R strain (fenvalerate selected) and YG strain (non-selected) were tested. The results showed that YG-R strain possessed moderate levels of cross-resistance to fenpropathrin (25.9-fold), low levels of cross-resistance to deltamethrin (5.9-fold) and cypermethrin (2.7- fold),

no cross-resistance to cyhalothrin, permethrin, methomyl, methamidophos and monocrotophos, and probably negative cross- resistance to methyl parathion (0.3-fold). This can provide a scientific basis for insecticide rotation and mixture in a resistance management strategy.

Preliminary analysis on fenvalerate resistance mechanisms

The synergism of fenvalerate plus three synergists (PB, DEF and TPP) was investigated by bioassay against fenvalerate- resistant strain (YG-R, YG9221, YG9241). DEF and TPP did not significantly increase the toxicity of fenvalerate to these resistant strains. However, about 70-90% of fenvalerate resistance can be overcome by PB, which implied that mfo was the main factor contributing to fenvalerate resistance in cotton bollworm.

Studies on penetration of 14C-fenvalerate in the 3rd instar larvae of YG-R and YS-S strain showed that the percentage of 14C- fenvalerate that penetrated 2, 4, 6 and 8 hours following topical application were significantly higher in YS-S strain than that in YG-R strain. So decreased fenvalerate penetration may contribute to the resistance in YG-R strain.

YG-R strain was more resistant to DDT than YG strain because of selection with fenvalerate. This implies that the YG-R strain possesses cross resistance to DDT. Nerve insensitivity may be another minor resistance mechanism.

Thus, there may be three mechanisms contributing to fenvalerate resistance: mfo factor, decreased penetration and nerve insensitivity. The mfo factor is the major mechanism. The other two factors are not very important, conferring only about 20-30% of fenvalerate resistance.

Genetic analysis of fenvalerate resistance

The mode of inheritance of resistance to fenvalerate was evaluated from log dosage-probit mortality curves constructed from the response of 3rd instar larvae to fenvalerate treatment. YG-R and YS-S were used as the resistant and susceptible strains respectively. The results indicated that fenvalerate resistance in cotton bollworm appeared to be controlled by three autosomal genes and the major gene(s) involved was incompletely dominant (dominant degree of progeny from F1 cross was 0.41). Results from the resistance mechanism study also suggest that the mfo gene, primarily responsible for fenvalerate resistance, was incompletely dominant.

Suggested strategy for insecticide resistance management of cotton bollworm in China

(a) The strategy must be based on restricting the use of pyrethroids and pyrethroid mixtures. Pyrethroids should be used only on the first-second instar larvae and in only one generation of the season. In North China, the use of pyrethroids, such as fenvalerate, against cotton bollworm must be stopped in those areas with high levels of resistance.

(b) Rotation of chemical groups is an important tactic, especially those chemicals that show no cross resistance.

(c) Mixtures. In North China, especially in the areas of high level of resistance to pyrethroids, cotton farmers like to use insecticide mixtures for controlling cotton bollworm, because mixtures are more effective than using one insecticide alone.

According to our results obtained from laboratory and field experiments and basic research on resistance management in cotton bollworm, guidelines for the proper use and mixtures of insecticides are as follows:

- Cotton bollworm populations to be controlled should be susceptible or have low levels of resistance to each insecticide used in the mixture. Under such conditions, the frequency of multiple resistance genotypes in the population may be maintained at lower levels.
- Each insecticide of a mixture should exhibit no cross resistance to populations of resistant cotton bollworm.
- The mixtures selected should have significant synergism to reduce the selection pressure of insecticide to pest, and delay resistance development.
- The Mammalian toxicity of mixture. Insecticides which have lower mammalian toxicity should be used as the candidate compounds for mixtures in order to decrease overall mammalian toxicity of mixture. Frequently mixtures with higher toxicity to insect produces the phenomenon of increasing toxicity to mammals.

Insecticide mixtures are one of the tactics for resistance management strategy. In a broad sense, the mixture also is chemicals. If any pesticide is used extensively, there will be a danger of selecting for resistance in pest insects.

(d) Non-chemical tactics of IPM for the control of *Heliothis armigera* should be enforced, such as attracting and killing adult moths with pheromone and high-voltage mercury lamps, cultivation and irrigation in winter to destroy diapausing pupae, removing top

foliage of cotton plant with eggs of cotton bollworm in the cotton late growing season. (Table 1)

Table 1. A suggested example of resistance management for the cotton bollworm in China.

Generation	1	2	3	4
Low Level resistance - susceptible area			Py/mixtures* (2 times)	
Moderate Level resistance area		Bt, Endosulfan	Mixtures*/Py (2-3 times)	Carbamate OP Mixtures
High Level resistance area	Bt	Bt, Endosulfan Mixtures Systemic OP (Smeared on sotton stem or top)	Mixtures* (2-3 times)	

*Mixtures containing pyrethroids

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Resistance of California Red and Yellow Scale in the San Joaquin Valley of California

California red scale, *Aonidiella aurantii* (Maskell), and yellow scale, *A. citrina* (Coquillet), are pests of citrus worldwide and key pests in the San Joaquin Valley of California. California growers attempted to eradicate California red scale when it was first introduced, however, scale populations developed resistance to hydrogen cyanide by 1912 (Quayle 1938). Since the development of parathion in the late 1940s, citrus growers have used primarily organophosphate and carbamate insecticides for scale insect control (Carman 1977, Riehl 1990). When these insecticides first came into use, growers in the San Joaquin Valley were able to maintain economic control of armored scale with one spray application of parathion every two to three years. However, more recently, many growers now use one to three broad spectrum insecticide applications yearly (Carmean 1988). It has been observed that yellow scale, which was displaced by California red scale for many years, has become the dominant species in some orchards in Tulare County. Insecticide resistance in California red scale and yellow scale populations was a suspected cause of these changing patterns of insecticide use and armored scale species distribution. Insecticide resistance among California red scale populations has been documented in South Africa (Georgala 1975, Nel et al. 1979) and Australia (Abdelrahman 1973). Organophosphate and carbamate insecticides have not provided effective control of many armored scale populations in several citrus growing regions of these countries since the mid 1970s.

The following study evaluated the distribution and severity of resistance among California red scale and yellow scale populations in the San Joaquin Valley during 1990-1993. This study utilized two discriminating concentrations of the organophosphates Supracide (31.6 and 100 ppm methidathion) and Lorsban (10 and 100 ppm chlorpyrifos) and the carbamate Sevin (1000 and 3162 ppm carbaryl) to survey a wide number of orchards. Concentrations resulting in 90% mortality of the susceptible strain of California red scale (Walker et al. 1991) were chosen for the lower discriminating concentrations. Scale infested fruit were collected from 102 orchards during 1990-1993 and bioassayed for resistance. In both California red scale and yellow scale, many of the scale populations had low to high levels of chlorpyrifos resistance (as defined by <80% mortality of the low discriminating concentration) (Table 1). Resistance to methidathion was less common initially, but nearly as

common in 1993. Carbaryl resistance in scale was found less frequently than for the other two insecticides. A similar level of resistance was found in both California red scale and yellow scale species. The percentage mortality response of individual scale populations to chlorpyrifos was significantly correlated with the percentage mortality response to both methidathion and to a lesser extent carbaryl, suggesting cross-resistance between these insecticides.

Table 1. Percentage of citrus orchards with resistant California red scale and yellow scale as defined by < 80% mortality of a discriminating concentration in the laboratory bioassay.

	Chlorpyrifos (10ppm)		Methidathion (31ppm)		Carbaryl (1000 ppm)	
Survey Year	% Sites with <80% Mortallity of Scale	# Sites Tested	% Sites with <80% Mortallity of Scale	# Sites Tested	% Sites with <80% Mortallity of Scale	# Sites Tested
California Red Scale						
1990	86	21	-	0	25	16
1991	25	16	17	12	6	16
1992	56	34	11	27	14	28
1993	71	31	60	30	7	29
Califrnia Yellow Scale						
1990	100	5	-	0	0	5
1991	100	4	100	2	0	4
1992	75	4	50	4	25	4
1993	67	3	0	2	33	3

The patterns of resistance seen are closely related to the extent of use of the three insecticides. Chlorpyrifos is the most commonly used pesticide of the three tested because it is less expensive than the other two insecticides and has a shorter pre- harvest and re-entry interval than methidathion. Additionally, chlorpyrifos is used to control other pests of citrus, such as Lepidoptera, katydid, soft scale and citrus thrips, while methidathion and carbaryl are used primarily for armored scale control. The most resistant populations were found in eastern Tulare and Kern Counties and were located in areas in which eradication programs for California red scale using hydrogen cyanide and parathion were active for many years.

We required a heavy infestation of armored scale in order to collect enough scale-infested fruit for a bioassay; therefore, our sampling was biased towards collecting resistant scale populations. Since many armored scale populations were still susceptible to the registered insecticides, even those located near highly resistant populations, our data suggest that a resistance management program is still feasible. Future research will focus on determining how long it takes for resistance to diminish in the absence of insecticide treatments or in the presence of insecticide rotations. We have conducted bioassays for several seasons on scale-infested fruit from orchards where growers have stopped using organophosphate insecticides for scale control and switched to using oils, carbaryl or leaving their orchards unsprayed. These bioassays indicate that organophosphate resistance frequencies in California red and yellow scale do not noticeably decrease the year after use is halted.

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An ANOVA Procedure Used to Compare German Cockroach Resistance Ratios: Results and Interpretations

BACKGROUND: The two tests most commonly used for detecting insecticide resistance in the German cockroach (*Blattella germanica*, L.) are topical applications and jar tests. Topical applications have been used to monitor resistance in numerous insect species (ffrench-Constant & Roush 1991), while the jar test (Keller et al. 1956) was specifically developed for use with the German cockroach. Topical applications place a known volume of insecticide upon either the ventral thorax or abdomen of test insects. The jar test

utilizes tarsal contact with insecticide deposits in 473 ml (1 pt) glass jar, and is the accepted, worldwide standard method for detecting resistance in the German cockroach (W.H.O. 1970). Several researchers have examined these two test methods using numerous insecticides: propoxur and diazinon (Collins 1975); chlorpyrifos (Milio et al. 1987); cypermethrin (Zhai & Robinson 1992), and; propoxur, bendiocarb, chlorpyrifos, fenitrothion, pyrethrins, and cypermethrin (Chapman et al. 1993). Irrespective of insecticide, the conclusions from these studies support the following generalizations: (1) jar tests are more realistic than topical applications because they rely upon tarsal contact with insecticide deposits; and (2) compared to the more precise topical applications, jar tests are not sensitive enough to define the magnitude of resistance, because they always produce smaller resistance ratios.

In this study, we examined topical-thoracic applications, jar tests, and an additional procedure for makingg topical applications to the tarsal pads. In doing so, our objectives were (1) to understand how resistance detection is impacted by the tarsal site using resistance ratios, and (2) to statistically examine three factors contributing to the production of resistance ratios in these three methods using an ANOVA procedure.

METHODS: Topical applications (thoracic and tarsal) were executed using an Arnold Automatic Micro-applicator (Burkhard Mfg. Co., Ricksmanworth, England). Adult, male cockroaches of each strain were anesthetized and then treated with a range of doses of insecticides dissolved in acetone. After treatments, the cockroaches were placed in 10 cm petri dishes with food and harborage for observation.

The thoracic method was executed by placing a 1.0l droplet of insecticide upon the ventral, meso-thorax of anesthetized test insects. For the tarsal method, the cockroaches were mounted (via the pronota) onto wooden applicator sticks by a melted wax product. Test insects were allowed to "grab" two 0.5l droplets of insecticide with their tarsi and lower legs after being maneuvered into position with the applicator stick. Approximately 1 hour post application the test insects were removed from the applicator sticks and held for observation. In each topical method, post-treatment mortality observation was terminated at 72 hours, with moribund insects considered dead.

The jar test was executed by treating the bottom surface of 1 pint jars with 2.5 ml of the appropriate insecticide dissolved in acetone. Test insects were placed in the jars and monitored over time. Insects remained in their respective jars until at least 90% mortality was achieved, with moribund insects considered dead.

Chlorpyrifos (an organophosphate) and cypermethrin (a pyrethroid) were used to observe the effects of the three exposure methods. Three strains of cockroaches were utilized: (1) the Muncie '86 strain ("M'86"; resistant), (2) the Las Palms strain ("LP"; resistant) and (3) the Johnson Wax-lab strain ("JWax"; susceptible).

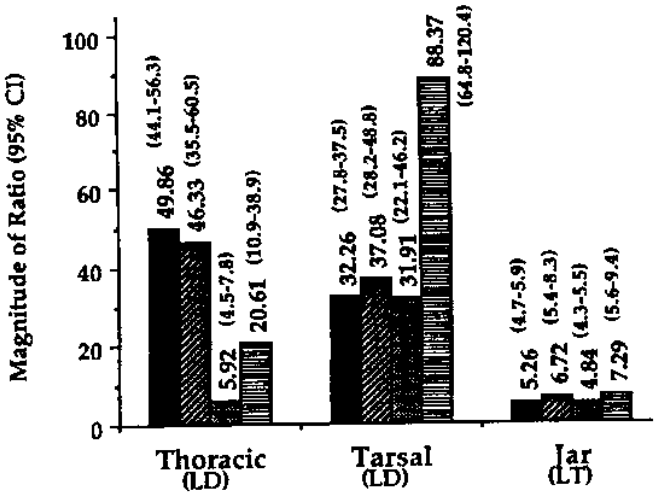
Concentration and time-mortality data for each strain-insecticide combination were analyzed by probit analysis (SAS Institute 1990). Incidences of control mortality were automatically corrected by Abbott's transformation. After probit analyses, resistance ratios with 95% confidence intervals (CI's) were calculated for each insecticide and application method. This was done by comparing probit responses from resistant (M'86 or LP) strains to corresponding regressions from the susceptible (JWax) strain with the methods described by Robertson & Preisler (1992). This procedure requires the following components from the regression analyses: slope, intercept, and the three values of the estimated covariance matrix. With this test, whenever the 95% CI of a ratio is greater than 1.0 at its lower limit, significant differences existed between the probit responses which were compared (Robertson & Preisler 1992).

An ANOVA procedure was developed to compare the main effects of strain (3 levels), insecticide (2 levels), bioassay (i.e. exposure method: 3 levels), and their respective interactions for the purpose of defining their relative significance in the production of the observed resistance ratios. This routine required data of two types: theta(), and the variance of . Values of were obtained during resistance ratio determinations as described by Robertson & Preisler (1992), and are equal to the Log of an LD or LT value. These values were used to generate a poly-factorial 2 analysis which functioned as an ANOVA. When the p-value for a particular effect or interaction was >0.1000, that component of the model was considered non-significant.

RESULTS and DISCUSSION: The JWax strain had the lowest thoracic LD, tarsal LD, and jar LT values at both 50 and 95% levels, reflecting its susceptibility to chlorpyrifos and cypermethrin. For both insecticides, LD and LT values of the M'86 strain, at the 50 and 95% levels, were intermediate between those of the JWax strain and the LP strain. Resistance ratios obtained for the M'86 and LP strains over the JWax strain have shown the LP strain (Figure 2) to be more resistant to chlorpyrifos and cypermethrin than M'86 (Figure 1) by all exposure methods. Jar tests resulted in the smallest resistance ratios for either strain. Topical applications resulted in larger resistance ratios, with tarsal resistance ratios being the largest in most instances. With respect to each conventional exposure

method (topical thoracic and jar test), the results did agree with findings by other researchers in that jar tests produced much smaller resistance ratios (Collins 1975; Milio et al. 1987; Zhai & Robinson 1992; and Chapman et al. 1993).

Figure 2. Las Palms resistance ratios.



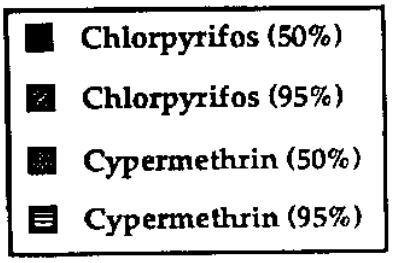
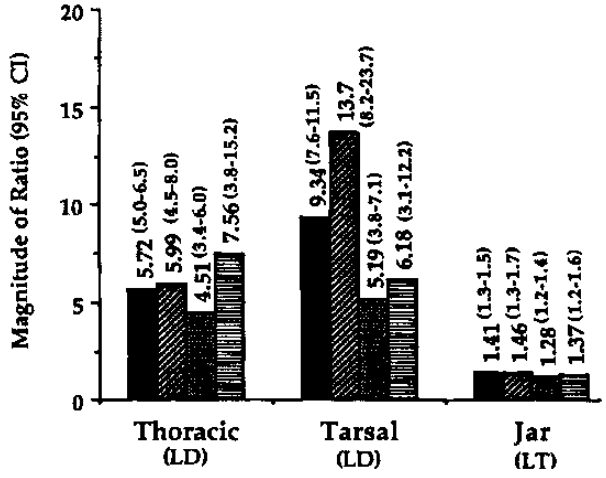


Figure 1. Muncie'86 resistance ratios.



The ANOVA procedure was conducted separately for both 50 and 95% levels of the resistance ratio (Table 1). At the 50% level, all effects in the model were significant ($p < 0.0500$), with strain and bioassay combining for the majority of total model variability. At the 95% level, the main effect of insecticide and its interaction with bioassays were not significant ($p > 0.1000$). As with the 50% model, in the 95% model the effect of strain followed by bioassay, combined for the majority of the total resistance ratio variability. Because strains would be expected to be highly variable across locations, bioassay should be considered as the most important component of this research model. This assumption indicates that a greater emphasis should be placed upon bioassay (exposure method) selection, while keeping other variables as much in control as possible in this type of resistance research.

Table 1. ANOVA results for the model RR= I + B + I*B + S + I*S + B*S + I*B*S (I = insecticide; B = bioassay; and S = strain).

		RR50 Analysis			RR95 Analysis.		
Effect	df	X^2	% total X^2	p-Value	X^2	% total X^2	p-Value
I	1	10.67	0.52	0.0109	1.23	0.11	0.2268
B	2	803.11	38.9	<0.0001	406.27	36.92	<0.0001
IxB	2	6.7	0.32	0.0351	3.84	0.35	0.1469
S	1	1155.69	55.98	<0.0001	575.07	52.27	<0.0001
IxS	1	9.55	0.46	0.002	31.1	2.83	<0.0001
BxS	2	63.48	3.07	<0.0001	18.29	1.66	0.0001
IxBxS	2	15.26	0.74	0.0005	64.49	5.86	<0.0001
Totals	**14**	**2064.46**	**100**	-	**1100.29**	**100**	-

In resistance research intended to generate resistance ratios, goal-oriented decision rules based on the needs of the researcher are recommended for selecting an exposure method. Situations demanding less labor input can be executed using the jar test, while in situations where labor input is not limiting and sensitivity is desired, topical applications can be best utilized. The alternative to a goal-oriented approach, of course, is the implementation of both topical applications and jar tests (or other surface contact methods) which may be the better protocol for fully understanding the resistance profile in a strain of German cockroach.

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Study on the Cotton Aphid Resistance to Three Pesticides

During 1980-1993, we studied resistance management of cotton aphid, *Aphis gossypii* Glover in the Siaocheng cotton area (100,000 hectares), Shandong province, China, a region displaying high levels of aphid resistance to pesticides. At present there are three pesticide groups used to control these cotton aphids displaying resistance: pyrethroids (Pyr.), Organophosphates (Op.) and Carbamates (Carb.). We measured cotton aphid resistance to these insecticides representing each pesticide group: Decis, Monocrotophos and Methomyl.

We measured pesticide resistance in cotton aphid with the topical application assay recommended by FAO (1980) (Table 1). We also measured and compared field efficacy of several insecticides on cotton aphid in 1985 and 1993 (Tables 2 and 3).

1. Decis (Pyr.) - Resistance to Decis suddenly increased in 1983 with an estimated resistance ratio of 54.8 (Table 1). By 1988, resistance peaked at 10,384-fold higher than baseline estimates taken in 1980. After 1989 the quantity of Decis used decreased in most cotton areas. Resistance levels declined distinctly, especially after resistance management was initiated in 1990. Resistance levels of declined 3-fold by 1993. We show a direct relationship between decreasing resistance levels and cotton aphid resistance management.

Table 1. Changes in cotton aphid resistance to three pesticides from 1980 to 1993

Year	Decis			Monocrotophos			Methomyl		
	LD_{50} (μg/ml)	Ratios		LD_{50} (μg/ml)	Ratios		LD_{50} (μg/ml)	Ratios	
		S	Y		S	Y		S	Y
1980 (Base Line)	0.000011	1	1	0.00175	1	1	0.000469	1	-
1981	0.000011	1	1	0.0875	50	1	..	..	..
1982	0.000072	7	7	0.203	116	2.3	..	..	..
1983	0.000952	359	54.8	0.7429	424	3.6	..	..	..
1984	0.009281	843	2.3	1.0195	582	1.4	0.0093	19	..
1985	0.035219	3201	3.8	0.713	407	0.7	0.0101	21	1.1
1986	0.09209	8371	2.6	0.8426	481	1.2	0.0077	16	0.7
1987	0.084736	7703	0.8	1.1415	652	1.3	0.0673	143	8.7
1988	0.11423	10384	1.3	3.5138	2009	3.1	0.0177	38	0.3
1989	0.07623	6930	0.7	2.7927	1595	0.8	0.0177	38	1
1990	0.067177	6107	0.9	0.8181	467	0.3	0.0531	113	3
1991	0.061169	5560	0.9	0.0844	48	0.1	0.078	166	1.5
1992	0.045889	4171	0.7	0.3216	183	3.8	0.0178	38	0.4
1993	0.037724	3429	0.8	0.3005	171	0.9	0.0465	99	2.6

Notes: S: LD_{50}/LD_{50} Baseline Y: LD_{50}/LD_{50} previous year

2. Monocrotophos (OP.) - Highest levels of resistance were detected in 1988 (Table 1). Unlike Decis, there was no sudden increase in resistance levels. After resistance management was initiated in 1990, the quantity of monocrotophos used decreased as did cotton aphid resistance to monocrotophos.

3. Methomyl (Carb.) - Methomyl was used experimentally during 1986-1988. The

quantity of methomyl used was small and little resistance was detected in the cotton aphid (Table 1). After 1989, methomyl was imported from foreign countries. The cost of methomyl increased constantly and demand decreased quickly. The high price of this pesticide reduced its utility to resistance management of cotton aphid.

Tables 2 and 3 show that reduced usage of some pesticides after 1985 lead to increased efficacy of those pesticides in 1993 (ie., Decis and Cyfluthrin).

Table 2. Study on control effect of cotton aphid in Liaocheng fields (July 1985).

Pesticide Treatment	Application Rate (g/hec)	Initial aphid density per plant	Post spray aphid density			
			1st day		3rd day	
			Density per plant	% reduction	Density per plant	% reduction
2.5% Decis	150.1	1000	1744	20.3	2785	-15.9
10% Cypermethrin	123.1	1009	1812	17.9	3185	-32.6
2.5% LambdaCyhalothrin	123.1	1058	2398	-3.6	3256	-29.3
5.7% Cyfluthrin	123.1	1038	1526	32.8	2586	-4.7
40% Monocrotophos	245.9	1048	154	93.3	31	98.7
CK	water	1020	2231	-	2427	-

Table 3. Study on control effect of aphid in Liaocheng fields (July 1993).

Pesticide Treatment	Application Rate (g/hec)	Initial aphid density per plant	Post spray aphid density					
			1st day		3rd day		5th day	
			Density per plant	% reduction	Density per plant	% reduction	Density per plant	% reduction
2.5% Decis	150.1	1003	614	45.3	--	--	--	--
10% Cypemethrin	123.1	1010	798	29.3	--	--	--	--
2.5% Lambda-Cyhalothrin	123.1	1009	789	30.1	--	--	--	--
5.7% Cyfluthfin	123.1	1000	47	95.8	64	97.6	65	97.5
40% Monocrotophos	245.9	1007	83	92.6	18	99.3	8	99.6
CK	water	1006	1125	--	2632	--	2592	--

CONCLUSION: Pesticides with reduced efficacy due to serious resistance will regain their efficacy if their use is stopped for 3-5 years, if IPM is practiced and growers are provided with inexpensive pesticide alternatives.

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Comparison of Two Bioassay Techniques for Resistance Monitoring in *Heliothis armigera* and *Plutella xylostella*

Insecticide resistance is becoming a major concern in insect pest management in China. The insect pests with most severe resistance problems in China include the cotton bollworm (CB), *Heliothis armigera*, the diamondback moth (DBM), *Plutella xylostella*, and several species of aphids. Resistance monitoring is essential for resistancemanagement programs. Most of the current standard methods for insecticide resistance monitoring used in China, which were recommended by General Station of Plant Protection, Ministry of Agriculture in 1991, were by topical application.

Topical application is not easy and practical to use by most of the extension services in China. The development of alternative technique is desirable. We compared a more practical larvae immersion method with the standard topical application method for resistance monitoring in *H. armigera* and *P. xylostella*.

H. armigera

A laboratory strain (Lab) and four field populations (BD, FX, HS, HD) of CB from different regions of Hebei Province in June and July 1993 were used. Third instar at 7-13 mg/larva was used for bioassays. Fenvalerate was technical grade or Sumicidin 20%EC (Sumitomo Chemical Co.) for topical application or larvae immersion, respectively. After treatment, larvae were reared individually with diet and examined for mortality at 48 h.

The resistance ratios (RR) in field populations were 6.1-- 18.4-folds compared with the Lab strain, and the

slope values of the four field populations were 1.19-1.39 in topical application (Table 1). The larvae immersion bioassay showed 12.0--38.4-folds of RR with the slope values at 1.67-2.12 in the four field populations. The results showed a significant correlation (r=0.966, P=0.01) of resistance ratios between the two bioassay methods. Both the slopes of dose-mortality lines and the RRs were increased in larvae immersion than in topical application for the four fenvalerate-resistant CB population, indicating larvae immersion could improve discrimination between genotypes in traditional bioassays for resistance monitoring (ffrench-Constant & Roush 1990).

Table 1. A comparison of different methods for the monitoring of fenvalerate resistanc in cotton bollworm.

Population	Slope ± SE	LD_{50} (μg/g) or LC_{50} (mg/l)	95% FL	Resistance Rati
Topical application (LD_{50})				**A**
2Lab	2.18+/-0.27	1.47	1.04-2.08	1.0c
BD	1.34+/-0.25	8.96	6.21-12.93	6.1b
FX	1.39+/-0.21	15.69	11.20-21.98	10.7ab
HS	1.22+/-0.25	17.74	11.31-27.83	12.1ab
HD	1.19+/-0.24	27.02	17.88-40.83	18.4a
Larvae immersion (LC_{50})				**B**
Lab	1.31+/-0.27	23.46	11.20-49.16	1.0c
BD	1.67+/-0.26	281.89	213.47-372.27	12.Ob
FX	1.77+/-0.29	449.8	337.25-599.90	19.2ab
HS	2.12+/-0.28	665.51	528.12-838.64	28.4a
HD	1.80+/-0.26	742.69	573.16-962.35	31.7a
			rA.B=0.966>r 0.01(=O.959)	

1 Resistance ratios within the column of same method by the same letter are not significantly different (P=0.05).

P. xylostella

Three strains (Lab, WA and WH) of DBM and fourth instar at 2- 3 mg/larva were used. Alphacypermethrin was technical grade or Fastac 3%EC (Shell Co.) for topical application or larvae immersion, respectively. Bioassay methods were similar to Zhao & Grafius (1993) and Zhao et al. (1993).

RRs were increased in larvae immersion than in topical application for two resistant DBM strains, with a same conclusion on the susceptibility of the three strains to alphacypermethrin (Table 2).

Table 2. Susceptibility of diamondback moth strains to alphacypermetrin.

Strain	Slope ± SE	LD_{50} (μg/g) or LC_{50} (mg/l) (95% FL)	Resistance Ratio[1]
Topical application (LD50)			
Lab	1.14+/-0.16	4.04 (2.16-7.57)	1.00c
WA	1.64+/-0.24	13.95 (8.92-21.83)	3.45b
WH	1.74+/-24	160.75 (105.61-244067)	39.79a
Larvae immersion (LC_{50})			
Lab	1.25+/-16	0.59 (0.32-1.08)	1.00c
WA	1.50+/-0.20	3.94 (2.62-5.47)	6.68b
WH	1.68+/-0.23	45.57 (29.61-70.13)	77.23a

[1] Resistance ratios within the column of same method by the same letter are not significantly different (P=0.05).

Conclusion

Larvae immersion method was more quick and practical for resistance monitoring both in H. armigera and P. xylostella, with potential in other lepidopteran larvae, especially for the use by extension services in China.

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Study on the Cotton Aphid Cross-Resistance to Some Pesticides

To practice effective resistance management of cotton pests, it is important to establish cross-resistance patterns between pesticides towards resistance pests. This information can be used to select the proper pesticides to use in pesticide mixtures and rotations that extend effective control measures. In this study we collected susceptible cotton aphids, Aphis gossypii Glover, from the Yimong mountain area of China. We reared these aphids in cages on cotton and selected for resistant aphid populations by spraying aphid 30 times/year with high concentrations of insecticides. We selected three populations of cotton aphid with resistance to esfenvalerate, monocrotophos, and parathion. We then measured the cross-resistance patterns of each resistance strain to 18 different pesticides. Table 1 shows the cross- resistance patterns of the three resistant cotton aphid populations to nine of these pesticides.

Table 1. Studies on the cotton aphid cross-resistant to some pesticides.

Pesticide	LD_{50} Susceptible (YiMong)	Monocrotophos Resistant Population		Parathion Resistant Population		Esfenvalerate Resistant Population	
		LD_{50}	R	LD_{50}	R	LD_{50}	R
Decis	0.00068	0.090515	13.3	0.077648	11.4	2.1066	209.7
Esfenvalerate	0.00392	0.52304	133.4	15.4085	3930.7	18.9283	4828.6
Monocrotophos	0.0014	1.192	851.4	0.0945	67.5	0.1274	91
Dimethoate	0.0467	28.0618	600.8	77.0154	150.2	6.2392	133.6
Parathion	0.0253	1.108	43.7	15.1145	579.4	13.3218	526.5
EPN	0.0129	1.043	80.8	11.5259	893.4	6.1849	479.4
Omethoate	0.0388	1.0847	27.9	4.1741	107.5	0.4951	12.7
Methomyl	0.00042	0.0115	27.4	0.1131	269.2	0.0067	15.1
Carbofuran	0.00016	0.21974	1373	0.05012	313	0.0433	27
R = Resistance Ration (LD_{50}/LD_{50} YiMong Population)							

RESULTS:

The following cross-resistance patterns were identified:

1. Cotton aphids with resistance to moncrotophos showed cross- resistance to esfenvalerate, dimethoate and carbofuran.

2. Cotton aphids with resistance to parathion showed cross- resistance to esfenvalerate, dimethoate, EPN, omethoate, methomyl and carbofuran.

3. Cotton aphids with resistance to esfenvalerate showed cross- resistance to Decis, dimethoate, parathion and EPN.

4. Cotton aphids with resistance to esfenvalerate or monocrotophos showed negative cross resistance to omethoate and methomyl.

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Effects of Synergists on Pyrethroid Resistance in the German Cockroach

The German cockroach, *Blattella germanica* (L.), is a primary pest in the urban environment, and as such is intensively controlled by Pest Control Operators and individual homeowners. A variety of insecticides have been used for that purpose in the past, including cyclodienes, organophosphates, and carbamates. In addition, several other classes of insecticides are currently available. Among them are the pyrethroids which have been used heavily for cockroach control during the past 5-10 years (Fisher 1990).

Resistance to natural pyrethrins in the German cockroach has been known for many years (Keller et al. 1956). More recently resistanceto allethrin and several of the newer pyrethroids has been reported (Cochran 1989). Field populations currently exist that are highly resistant to as many as 12 pyrethroids (Cochran unpubl. date). While populations with such resistance profiles are not wide spread, they provide an indication of what could happen in the future. Should resistance of this magnitude become common, it would likely mean the loss of this entire class of insecticides for cockroach control. Finding replacements for them would not be easy.

A classical way of attempting to circumvent resistance is to combine a synergist with the insecticide (Casida 1970, Wilkinson 1971). This method is predicated on the concept that the action of a synergist is to inhibit a detoxifying enzyme within the insect's body thus preventing insecticide degradation. As a result, the insecticide concentration remains high and kills the otherwise resistant insect. Obviously, a synergist will not be effective if the resistance mechanism is not

metabolic or if the chosen synergist fails to inhibit the pertinent enzyme.

I report here on a study designed to evaluate the effects of the synergists PBO, MGK 264, and DEF on resistance to eight pyrethroids in the German cockroach. Cockroaches from several field-collected strains and a known susceptible strain were exposed to glass surfaces treated with either the pyrethroid alone or in combination with each synergist. The pyrethroids and their concentrations (AI) were: pyrethrins and allethrin (0.3 nl/cm2); permethrin, cyfluthrin, and cypermethrin (0.6 nl/cm2), phenothrin (1.5 nl/cm2), and fenvalerate and esfenvalerate (3.0 nl/cm2). The insecticide/synergist ratio was 1:5 in all cases except with fenvalerate and esfenvalerate where it was 1:2.5. The replicated results from time-mortality tests were pooled and analyzed by probit analysis (SAS 1982). Resistance ratios (RRs) based on LT50 values were calculated for each insecticide alone and with each synergist on each sstrain tested. Changes in RRs in the presence of a synergist were used to evaluate the effects of that synergist.

Two kinds of synergist effects were observed in field strains of the German cockroach tested with pyrethrins. PBO, MGK 264, and DEF all nearly completely negated resistance to this insecticide in some strains, while they had no observable effect in others. RRs either remained very high or were reduced to the 1.0-2.0 range (1.0 indicating no resistance). Cockroaches were resistant only to pyrethrins in those strains in which the synergists reduced resistance. In the strains in which the synergists had no effect, the insects were also resistant to allethrin. Therefore, resistance to pyrethrins appears to be metabolic in nature, whereas we have shown by electro-physiological means that resistance to allethrin is due to a nerve-insensitivity mechanism (Bloomquist et al., unpubl. data). Many strains exist that have resistance only to pyrethrins, while many others occur that have resistance only to pyrethrins and allethrin.

Similar tests with permethrin, cyfluthrin, and cypermethrin have shown that all three synergists reduced the RRs of the strains tested with these insecticides to the 1.0-3.0 range. The strains tested were also resistant to pyrethrins and allethrin. Since that is the case, these findings indicate that additional types of resistance have developed in these strains that appear to be metabolic in nature, but are independent of the metabolic resistance to pyrethrins.

When fenvalerate, esfenvalerate, and phenothrin were tested with the three synergists, variable results were obtained. With some strains the RRs were reduced to the 1.0-3.0 range, particularly with fenvalerate and esfenvalerate. With other strains, the RRs were only partially reduced and in some cases one synergist worked better than the others. The results with phenothrin were the most variable in that RRs were greatly reduced in one strain, while the synergists had no effect in another strain. Several other strains showed moderate reductions in RRs with one synergist and no effect with the others.

While the results of this study are not definitive, they provide a clear indication that several pyrethroid-resistance mechanisms are present in this insect. The actual number of mechanisms remains in doubt, but field strains exist with well defined resistance profiles. For example, pyrethrins resistance alone; resistance to pyrethrins and allethrin; resistance to pyrethrins, allethrin, and permethrin; resistance to pyrethrins, allethrin, and phenothrin; resistance to pyrethrins, allethrin, and fenvalerate; and phenothrin resistance that can be synergized in some strains but not in others. Strains like these suggest strongly that several mechanisms are present in this insect.

One of the most surprising outcomes of this work is that the three synergists typically produced very similar results. This is in spite of the fact that PBO and MGK 264 are known mixed- function oxidase inhibitors, whereas DEF is an esterase inhibitor (Casida 1970, Wilkinson 1971). A possible explanation is that all three are attacking a common receptor site the result of which is the rapid induction of a suite of detoxifying enzymes (Plapp 1984). Obviously, other explanations are also possible.

This work has serious implications for cockroach control. It showed that synergists are not likely to provide a cure-all for the pyrethroid-resistance problem in this insect. In some populations all three synergists worked well against most of the pyrethroids tested. In other cases these synergists were totally or partially ineffective as demonstrated with allethrin and phenothrin, respectively. It is clear that other means of dealing with resistant populations must be found.

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Methoprene Resistance in the Cigarette Beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) from Tobacco Storages in the Southeastern United States

The cigarette beetle, *Lasioderma serricorne* (F.), is the most important insect pest on stored tobacco. It is capable of damaging both the raw material and the finished product. Tobacco may be stored for 2 years or longer before use in manufacturing. During this period it must be protected against damage by the cigarette beetle. In the past, a variety of insecticide treatments have been used including: synergized pyrethrins, dichlorvos, methyl bromide, and phosphine (anonymous 1972). Methoprene (Kabat) has been registered for use on tobacco for over 12 years. Because of the effectiveness and safety of methoprene, its large scale use in the tobacco industry has expanded to include both applications to tobacco as a protectant and applications as space or crack & crevice spray to control cigarette beetle populations in manufacturing areas.

Resistance to methoprene has been shown to develop in several insect species. Since Diptera has been controlled by methoprene for the longest period, methoprene resistance is more fully documented in this group (Brown & Brown 1974, Brown & Hooper 1979, and Wilson & Fabian 1986). Only a few references have been made to methoprene resistance in Coleoptera. In 1972, a low level resistance was described in the stored- product insect, *Tribolium castaneum*, by Dyte (1972). In 1978, a 4- fold resistance was shown in another stored-product beetle, *Tribolium confusum*, by Brown et al. (1978). There are no reports of methoprene resistance in cigarette beetles nor has methoprene resistance been reported in any insect population in the field.

This study was initiated to assess the development of methopreneresistance in several field populations of cigarette beetles.

MATERIALS and METHODS: Two reference strains of cigarette beetles that were never exposed to insecticides during laboratory culture were used as controls to assess the methoprene resistance in cigarette beetles collected from tobacco facilities (field populations). Reference strain A was obtained from the USDA laboratory (Richmond VA, laboratory reared for 50 generations) in 1982, and strain B was isolated from a can of George Washington pipe tobacco in 1982. Field populations of cigarette beetles were obtained from following locations: (1) tobacco storages in Winston-Salem, North Carolina, (2) through Dr. Dennis W. Keever, USDA-ARS, Oxford, NC (CRL18, 20, and CRL4), and (3) through Dr. J. Larry Zettler, USDA- ARS, Savannah, GA (22 and LL). Three field populations (413, 449, and 454) from Winston-Salem were collected by light trapping in three tobacco storages where the tobacco was treated with methoprene. Each population was collected from a tobacco stemmery, storage complex, or manufacturing facility. Three populations (20, 22, & CRL4) were collected in other areas of North Carolina, CRL18 was collected in Virginia, and LL was collected in Pennsylvania. The previous exposure to methoprene of these populations was not certain. All field cigarette beetles were reared in the laboratory for 2-3 generations on untreated flue-cured tobacco containing approximately 12% water.

The study included 4 experimental parts: (1) Three concentrations of methoprene were tested on reference strains A and 454 to preliminary assess the methoprene resistance in a field strain and to select an optimal diagnostic dose of methoprene to be used in the following resistance study. (2) The resistance study was conducted in all beetle populations described above. A standard test procedure was developed to evaluate methoprene resistance based on the diagnostic dose method (Zettler et al. 1989). Briefly, 25 insects, not separated by sex, were placed into a 118 ml rearing jar containing 25 grams either untreated (served as an internal control for that population) or methoprene containing (1.7 ppm) tobacco. Each beetle population was replicated at least 4-6 times. Jars were incubated at 27C and 70% RH for approximately 8 weeks until adults were emerged. Surviving F1 adults were

counted, the emergence rate of cigarette beetles reared on treated tobacco was compared to those on untreated tobacco, and overall resistance for the field populations was assessed by comparison to the reference strains. (3) Methoprene resistant study was further carried out in the resistant populations determined by part 2 of this study. A six dose regiment of methoprene was tested among strains 413, 454, and 449. (4) Fecundity tests were undertaken in strain 413 to examine the fertility of F1 in this methoprene-resistance population. In this study, 25 adults of the F1 that emerged from methoprene treatments in part 3 were reared on untreated tobacco, surviving F2 were counted and emergence rate was compared to those emerged from F1 without treatment. The statistical significance in these experiments was determined by ANOVA using MINITAB ($P < 0.05$).

RESULTS and DISCUSSION: Field strain 454 demonstrated a significant increasing in emergence rate compared to reference A when those beetles were reared on tobacco treated with 1.3 ppm of methoprene (Table 1) suggested this field strain was resistant to methoprene treatment. The effectiveness of methoprene to control beetle population in the laboratory strain was similar to those reported previously (Benezet & Helms 1986). A concentration of 1.7 ppm methoprene selected as the diagnostic dose for the resistance study provided a good baseline control in the laboratory strains, therefore any resistance to methoprene existing in field populations would be easily detected.

Table 1. Preliminary evaluation of Methoprene toxicity in a field-collected population.

Dose	Reference A	Field 454
0.7 ppm	49.5% (SD 4.8)	42.0% (SD 11.5)
1.3 ppm	7.5% (SD 0.8)	78.3% (SD 15.6)
3.1 ppm	1.2% (SD 0.5)	56.9% (SD 3.7)

Among the field populations, only 3 strains (413, 454, & 449) collected from tobacco storages with confirmed methoprene treatment showed significant resistance to methoprene (Table 2). This observation strongly suggested field application of methoprene induced resistance in cigarette beetles.

Table 2. Comparison of Methoprene resistance in cigarette beetles by exposure to treated tobacco rearing media.

Strains	0 ppm Number Emerged (SD)[1]	1.7ppm Number Emerged (SD)	1.7ppm Percent Emergence (SD)
Laboratory			
Reference A	534 (50)	0	0
Reference B	471 (135)	0	0
Field Collected			
413	230 (101)	225 (32)	98 (14)
454	177 (71)	67 (7)	38 (11)
449	112 (29)	67 (8)	60 (12)
22	268 (30)	0	0
CRL 1 8	328 (101)	18 (1.5)	5.5 (8.5)
20	284 (33)	0	0
CRL4	188 (69)	0	0
LL	92 (26)	4 (0.7)	4.5 (1.6)

[1] Standard deviation

Resistance developed in three field strains (413, 454, & 449) illustrated a strong inverse dose relationship of methoprene treatment (Table 3). Although methoprene concentrations normally applied for cigarette beetle control in the storage was no longer effective in these three fields strains under the laboratory test conditions (Table 3), an effective control by methoprene can be achieved when using 14.1 ppm of methoprene (Table 3).

Table 3. Methoprene resistance in three field-collected cigarette beetle population.

	413		454	449
Dose, ppm	F_1 Emergence (SD)[1]	F_2 Emergence (SD)	Emergence (SD)	Emergence (SD)
0 (Control)	230 (101)	180 (32)	117 (71)	122 (29)
1.7	98% (14)	61% (25)	38% (11)	60% (12)
3.5	88% (19)	58% (29)	65% (21)	85% (15)
5.5	75% (21)	53% (33)	74% (31)	54% (21)
7.5	60% (25)	63% (23)	81% (22)	38% (17)
9.7	17% (5)	37% (14)	49% (12)	13% (13)
14.1	7% (4)	23% (21)	13% (3)	3% (3)

[1] Standard deviation

Methoprene application may also impact on the effectiveness in developing fertility in F1. Comparing results with a previous study (Benezet & Helms 1985), our data obtained from fecundity tests in the 413 strain suggest the low dose application of methoprene will further reduce the effectiveness of methoprene to control F2 (Table 3).

Methoprene resistance may be initiated through several mechanisms such as enhanced metabolism. A small (1.2-fold) but significant increase in esterase activity was observed in the larvae of one of the resistant strains by Dr. R.M. Roe (North Carolina State University, personal communication). Theoretically, the resistance could be caused by enhance metabolism of methoprene due to the increased titer of JH esterase found in the resistance insects (Brown & Hooper 1979). Further studies are required to substantiate this theory.

Methoprene is a safe and effective pesticide and it is an important part of a cigarette beetle control program. In order to control the cigarette beetle and reduce the development of resistance, methoprene must be applied consistently at an appropriate level. This can be technically difficult. Repeated application of sub-effective concentrations could lead to the development of a resistant population. An integrated pest management program must be developed to manage resistance development.

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Insecticide Induced Carboxyl Esterase (EC.3.1.1.1) Activity in *Helicoverpa* (*Heliothis*) *armigera* Hubner

Over the forty years a great deal of emphasis has been given to the physiological, biochemical and genetic processes through which insects and related arthropods overcome the intoxication characteristically produced by insecticide action (Georghiou and Saito, 1983; Ambrose and Regupathy, 1992; and Pasupathy and Regupathy, 1993). A number of increasingly generalized mechanisms for insecticide resistance have been identified in terms of specific genetic regulation. Metabolic resistance involves detoxification of the insecticides by enzymatic processes including esterases, microsomal oxidases, glutathione -S-transferases and epoxide hydrolases.

With this basis, we had attempted to study the carboxyl esterase (CE) activity in the field-collected TNAU *H. armigera* Hubner population showing fairly high resistance to fenvalerate.

The field-collected eggs were placed individually onto the side well of a 7.5 ml cell in a 12-well tissue culture plate (Linbro ICN, Flow Ltd) containing chickpea-based artificial diet (Armes et al. 1994). Third and fourth instars weighing 30-40 mg were used for the study. Serial dilutions of technical grade insecticides viz., fenvalerate and quinalphos were made in analytical grade acetone. Discriminating doses of fenvalerate (0.2 g/ml = LD 99 for susceptible strain calibrated in Australia (Forrester and Cahill, 1987) and quinalphos (0.75 g/ml = LD 99 for Natural Resources Institute, UK laboratory susceptible strain as adopted by Armes et al.1994) and their combinations were dosed topically to the thoracic dorsum of each third instar using single needle guided plunger (SNGP) to deliver 1 g/ml drop. Control larvae were treated with acetone only. The treatments were: (1) fenvalerate 0.2 g (2) quinalphos 0.75 g (3) fenvalerate 0.2 g + quinalphos 0.75 g (4) fenvalerate 0.1 g + quinalphos

0.37 g (5) Control (acetone alone). For each treatment 50-100 larvae were used.

Observations on mortality were recorded for six days. Individual larvae surviving 3 hours, 3 and 5 days after the application were assayed for carboxyl esterase activity following Devonshire (1977) and Bradford (1976).

The mean carboxyl esterase activity was 89.9 nmole naphthol released min-1mg-1 of protein in untreated larvae. There was gradual increase in the level of enzymes over days irrespective of the treatments including untreated larvae. Topical application of fenvalerate alone induced carboxyl esterase activity (Table 1). The activity was 109.7, 237.8 and 277.7 nmole naphthol released min-1 mg-1 of protein 3 hours and 3 and 5 days after application when compared to 89.9, 131.7 and 218.7 nmole naphthol released min-1 mg-1 of protein respectively in untreated larvae. Application of quinalphos alone reduced the CE activity. The inhibition was more pronounced up to three days. When quinalphos was applied with fenvalerate, reduced activity of CE was observed. The activity varied from 27.0 to 31.4 nmole naphthol released min-1 mg-1 of protein. The CE activity in larvae treated with quinalphos either alone or combined with fenvalerate was comparable to that of untreated on day 5 after application.

Table 1. Induction of Carboxyl Esterase (EC.3.1.1.1) in insecticide treated *H.armigera* Hubner.

S. No.	Treatment	Dose µg/L3	Dose after Insecticide Application	*Mean CE activity n mole α naphthol released min-1 mg-1 of protein	Percent mortality after	
					48h	72h
1	Fenvalerate	0.2	(3hr)	109.7	12.9	12.9
	Fenvalerate	0.2	3	237.8	-	-
	Fenvalerate	0.2	5	277.7	-	-
2	Quinalphos	0.75	(3hr)	31.4	51.6	54.8
	Quinalphos	0.75	3	99.6	-	-
	Quinalphos	0.75	5	277.77	-	-
3	Fenvalerate + Quinalphos	0.2 + 0.75	(3hr)	27	54.8	51.6
	Fenvalerate + Quinalphos	0.2 + 0.75	3	112.4	-	-
	Fenvalerate + Quinalphos	0.2 + 0.75	5	218.5	-	-
4	Fenvalerate + GPS	0.1 + 0.37	(3hr)	27.6	22.6	22.6
	Fenvalerate + GPS	0.1 + 0.37	3	121.2	-	-
	Fenvalerate + GPS	0.1 + 0.37	5	226.9	-	-
5	Check		(3hr)	89.9	-	-
	Check		3	131.7	-	-
	Check		5	218.7	-	-

* Mean of five observations

** 0 day sampling after 3 hours after application L_3 Third Instar

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Some Resistance Problems in Brazil

Little research data can be found about resistance of fungi to fungicides in Brazil on temperate zone fruits. Toledo (1974) and Zambolin & Chaves (1983) published the following table (Table 1) about field resistance of fungi to fungicides.

Table 1. Field resistance of some fungi to fungicides.

Fungus	Fungicides
Penicillium italicum	difenil, nitrobenzenes, clorados
Penicillium digitatum	difenil, nitrobenzenos, clorados
Rhizoctonia solani	PCNB
Sclerotium cepivorum	dicloran
Tiletia faetida	hexaclorobenzeno
Botrytis cinerea	benomyl, thiabendazole, tiof. metilico
Cercospora arachidicola	benomyl
Cercospora beticola	benomyl
Cercospora musae	benomyl
Erysiphe graminis	benomyl
Fusarium oxysporum	benomyl
Mycosphaerella fragariae	benomyl
Penicillium italicum	thiabendazole
Penicillium digitatum	thiabendazole

Studies developed by Melzer (1986 not published) in the laboratory of the Experimental Station of Cacador, SC show that from 35 orchards evaluated, 17 presented resistance of *Venturia inaequalis* to one or more benzimidazole fungicides (Table 2). Since the beginning of the 80's these fungicides became less utilized in Santa Catarina because of resistance problems.

Table 2. Study of the resistance of *Venturia inaequalis* to Benzimidazole fungicides.

LOCAL	Orchards Evaluated	Use of Benzimidazole		Number of Orchards Showing			TOTAL
		Yes	No	A	B	C	
Agua Doce	5	5	-	0	0	4	4
Cacador	6	5	1	1	0	1	2
Campos Novos	1	1	-	0	0	0	0
Curitibanos	1	1	-	0	0	0	0
Fraiburgo	6	6	-	1	1	2	4
Lages	1		-	0	0	0	0
Lebon Regis	2	2	-	0	0	1	1
Sao Joaquin	12	12	-	0	4	2	6
Casteli	1	-	0	0	0	0	0
Totals	**35**	**34**	**1**	**2**	**5**	**10**	**17**

A= benomyl B= thiabendazole C= methyl thiofanate

The resistance of *Venturia inaequalis* to benzimidazoles was first described in Santa Catarina by Akutsu & Tanaka (1977).

In a recent article Forcelini (1992) notes a high tendency of resistance appearing to DMI fungicides, especially to triazols. The first cases of DMI resistance in Brazil were related in the 80's for the following fungi: *Sphaerotheca fuliginea* (mildew of cucurbitaceas), *Erysiphe graminis hordei* (mildew of hordei) and fungi of the genera *Penicillium* (fruit rot, especially on citrus). In this period less sensitive strains of *Venturia inaequalis* were identified.

The control of blue mold in post-harvest has been done with benzimidazole fungicides (Valdebenito & Torres, 1972; Bleicher & Bernardi, 1985). Until the beginning of the 90's the only fungicide registered for post-harvest on apple was thiabendazole. Resistant races of *Penicillium expansum* to thiabendazole were found by Fortes (1985) and Valdebenito-Sanhueza (1986). The resistance of *Penicillium* to this fungicide was so spreadable that all the storage apples during the last years presented significant losses due to blue mold. From 1986 to 1992 some research efforts were performed to have iprodione listed for control of apple rots in post-harvest (Valdebenito-Sanhueza, 1986; Berton, 1990; Bleicher & Berton, 1992). These problems have been greatly reduced by the registration and use of iprodione since 1992.

The cases of resistance of fungi to fungicides in Brazil are present in different situations including different groups of fungicides in many cultures in pre- and post-harvest.

On temperate zone fruits dodine is losing its efficiency in the field to control *Venturia inaequalis* and acaristop shows no effect against *Panonichus ulmi* and *Tetranychus* spp. Nora (1993 personal communication) noticed that fenthion is not controlling *Grapholita molesta*.

Until now there have been no serious problems with DMI fungicides. According to Forcelini (1992); Katsurayama and Bonet; Melzer; Bleicher and Berton (personal communication), the strategies to avoid fungicide resistance in the temperate zone of Brazil are:

- Alternate or associate DMI fungicides with others without cross resistance.
- Use only in critical periods.
- Avoid low doses and spray only when recommended threshold levels are reached. Do not delay when the disease severity and the population of the pathogen is high.
- The mixture of protective and curative fungicides seems to be promising by the increasing of the spectrum of action. In Santa Catarina we have used this strategy for more than 10 years without problems.
- Try to avoid curative and eradicative sprays.
- Monitoring the resistant population.

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Resistance Management News

Status of the National Late Blight Epidemic and Tips for Management in 1994

The following information came from national meetings of potato pathologists at Greensboro, N. Carolina on Dec. 17, 1992 and Portland, Oregon on Dec. 4, 1993 and from published information.

During 1992 and 1993 there were severe epidemics of late blight in many potato production areas of the U.S. -- far worse than anything that had occurred for many years. Unlike most potato diseases, the fungal pathogen that causes late blight does not survive in soil or plant debris. For an epidemic to begin in any one area, the fungus must survive the winter in potato tubers (culls, volunteers), be reintroduced on seed potatoes (or possibly even tomato transplants?), or live spores must blow in with rainstorms. Because of the extensive development of late blight in the U.S. in the last two years, there is a much larger chance that the fungus will survive the winter in large numbers in cull piles and volunteers and be carried in seed tubers. Thus, if cool, moist conditions prevail, severe late blight epidemics may continue into 1994.

An additional problem is the widespread appearance of new strains of the late blight fungus in many areas. Two major strains of the late blight fungus exist called mating types A1 and A2. If both A1 and A2 mating types of the fungus are present, they can combine to form a type of spore called an oospore. Unlike other spores of the late blight fungus that we normally deal with, these oospores may be able to survive for extended periods in the soil. Prior to 1990, only the A1 mating type was known to be present in the U.S., although the A2 type has been known from Mexico for some time and from Europe since the early 1980's. By 1993, however, the A2 mating type had been found in at least six U.S. states and in British Columbia. If the A2 mating type becomes well established in U.S. potato production areas and oospores of the late blight fungus are then formed and can survive the winter in soil, the entire disease cycle of late blight may be changed. As yet, no oospores of the late blight fungus have been reported from the U.S. This is an area of active research and much more study is needed before the complete story is known and the significance of these A2 strains is understood.

In addition to the potential problem of A2 strains, there are an increasing number of strains of the late blight fungus being found that are insensitive to the fungicide metalaxyl (Ridomil). This characteristic is often associated with A2 strains, but has been found in A1 strains as well. Metalaxyl insensitive strains of the fungus have been associated with failures of Ridomil to control late blight when applied to potato fields. The widespread existence of metalaxyl-insensitive strains points out the importance of limiting the use of Ridomil as a management tool for late blight and relying on an integrated program with protectant fungicides.

Editor's Note: Resistant strains of the late blight fungus have been detected in Maine, New York, Florida, North Carolina, Michigan, Wisconsin, Washington, Oregon and Western Canada.

Management Recommendations

Because late blight epidemics have been severe in many areas across the United States in1992 and 1993, it is essential that potato growers SCOUT THEIR FIELDS CAREFULLY for the disease in 1994 and take all necessary precautions:

1. Cull potatoes must be disposed of properly -- Do not make cull piles!! Cull potatoes should be spread on fields not intended for potato production next year. These cull potaoes must be exposed to freezing temperatures during the winter. If this is not possible, they must be destroyed by complete chopping, burial, burning, or by some other method. Any cull piles found in your area should be completely destroyed. Cull potatoes must not be left to sprout next season and continue this epidemic!

2. Growers should plant only certified seed potatoes in 1994. Use of "year-out" seed or seed saved from your own crops is asking for trouble with late blight. Seed sources should be selected very carefully to avoid bringing in late blight on seed tubers, especially new strains of the fungus. Ask a lot of questions of your seed sources!!

3. Volunteer potatoes can be a significant source of spores of the late blight fungus. All volunteers should be destroyed as quickly as possible by herbicides, chopping, or cultivation.

4. Growers should scout fields regularly to look for late blight. Special attention should be paid to early-planted fields because that is where the disease is likely to develop first. Scouting should be concentrated in low areas, areas along creeks or ponds, near the center of center-pivot irrigation rigs, and in areas near woodlots or any area that is protected from wind where the leaves tend to remain wet longer. Also keep an eye on places where it is difficult to apply fungicides such as edges and corners or under power lines if using aerial application.

5. A good protectant fungicide program with mancozeb or chlorothalonil should be initiated in 1994 before flowering. Some applications of copper fungicides could also be included in the spray schedule, if desired. Initiating fungicide applications earlier may be appropriate if there is any indication of late blight in your area. If Ridomil-prepacks are used as part of the fungicide program, they should be applied twice, first at late flowering and again 2 weeks later in place of the protectant fungicide. If late blight is known to be in your area, the two applications of Ridomil might be scheduled even earlier. If Ridomil MZ58 is applied at the 2 lb/A rate, an extra 0.8 lbs of mancozeb (80%) should be added for each two pounds of Ridomil MZ58 applied to bring the total mancozeb applied up to 1.6 lbs of active ingredient per acre. No added fungicide is needed with Ridomil/Bravo. Complete coverage of the foliage is very important. Experience has shown that fungicides applied with high-volume boom sprayers are most effective. If fungicides are applied by air, 10 gal/A should be used to improve coverage.

6. Apply vine killers early enough to ensure that vines have been completely dead for 2-3 weeks prior to harvest. This will kill spores of the late blight fungus that remain on the foliage and thus prevent infection of tubers during the harvesting process.Everyone should keep in mind that late blight is an area-wide problem that affects all potato growers. We all must work together to manage this problem in 1994!

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Genetics of Insect Pest Populations and Insecticide Resistance

Acetylcholinesterase insensitivity (AceIn) in *Heliothis virescens*

- Dominant gene for methyl parathion resistance
- One allele confers ~20-fold resistance with slight overdominance
- Linked 11 crossover units from IDH-2 on chromosome 2 (autosomal)
- Not linked to pyrethroid resistance
- R-allele frequency in cotton fields from 1989-93 was 6% to 19%
- R-allele frequency in 1983 Woodrow, SC (Woodrow83) sample was 89%
- R enzyme activity was resistant to inhibition by methyl paraoxon, propoxur, eserine and fenitrooxon
- R enzyme has increased susceptibility to monocrotophos, N- isopropyl-naphthylcarbamate and 4-nitrophenyl di(2-thienyl)phosphinate
- R enzyme hydrolzyed butyrylthiocholine
- Additional genes are involved in 200-fold resistant Woodrow83

Pyrethroid-resistant *Heliothis virescens*

- Pyrethroid-R larvae with 700-fold resistance derived from ICI- 82
- Resistance maintained by selection of most resistant families
- Crossed to Woodrow83 and F2 families selected six times
- Resistance is incompletely recessive
- Histogram of family survivorship of selecting dose is bimodal
- Synergism 3-fold by aryl propynyl ether synergists
- Piperonyl butoxide, hydrolase inhibitors, formamidines not synergists
- Pirate toxicity to adults negatively correlated to cypermethrin toxicity
- Field surveillance and lab vial test indicated low level cypermethrin resistance in South Carolina in 1992 and 1993 for the first time

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Guidelines for the Prevention and Control of Herbicide Resistant Black-grass (*Alopecurus myosuroides*)

A second edition of these Guidelines has recently been published by the UK Weed Resistance Action Group (WRAG). The Guidelines were written by Stephen Moss (Rothamsted Experiment Station) and James Clarke (ADAS, Boxworth) in consultation with all other organizations represented on WRAG. These include both independent organizations as well as the major agrochemical companies.

The eight page leaflet summarizes recent research results and recommends integrated control strategies for three situations:

1. Fields where consistently good control has been achieved by herbicides.

2. Fields where herbicide performance has been inadequate but the reasons for this have not been identified.

3. Fields where resistant black-grass has been positively identified in tests.

The Guidelines stress the importance of cultural control measures and these are described in detail. The importance of avoiding repeated use of the same herbicide type, especially aryloxphenoxypropionate ('fop') and cyclohexanedione ('dim') herbicides is emphasized. A table of black-grass herbicides grouped according to biochemical mode of action is also included.

The Guidelines were produced with financial support from the UK Home-Grown Cereals Authority and have been distributed widely via agrochemical companies, distributors, consultants and advisors. Farmers having seed samples tested will also be sent a copy directly. The Guidelines have been well received, so additional copies have been printed with financial support from the British Agrochemicals Association, Schering Agriculture and Ciba Agriculture. WRAG is grateful for their support. Formal publication in the journal 'Crop Protection' is also planned.

In addition, the British Agrochemical Association has proposed a statement which companies may voluntarily add to herbicide product labels. This statement is 'The Weed Resistance Action Group has produced Guidelines on avoiding and coping with resistant black-grass. Copies of the Guidelines may be obtained from your distributor, crop adviser or product manufacturer'. This statement has been approved by the UK Pesticide Safety Directorate.

Although the Guidelines relate specifically to black-grass in the UK, the general principles, if not the specific herbicide advise, should also be relevant to the control of resistant weeds generally.

Copies of the Guidelines are available free of charge from either the Home-Grown Cereals Authority, Hamlyn House, Highgate Hill, London N19 5PR or the British Agrochemical Association Ltd., 4 Lincoln Court, Lincoln Road, Peterborough PE1 2RP, United Kingdom.

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WRCC-60 Research Report: Linkage Mapping and Insecticide Resistance in *Heliothis virescens*

We are in the process of developing a linkage map of the tobacco budworm *Heliothis virescens* for the purpose of characterizing the genetic basis of insecticide resistance in this species.

Status of the linkage map: Approximately 150 marker loci are now mapped. Each of the 31 chromosomes is represented by a linkage group, with 2-8 loci on each linkage group. The marker loci consist of about 15 allozyme loci that are studied using starch gel electrophoresis, about 15 RFLPs, and about 120 RAPD loci. (RFLP = restriction fragment length polymorphism; RAPD = randomly amplified polymorphic DNA) Most of the RFLPs are "anonymous," i.e. regions of DNA of unknown function, but a few are known; such as the gene for juvenile hormone esterase, and a sodium channel locus discussed below.

Six loci (or clusters of loci) conferring insecticide resistance have been assigned to linkage groups in Heliothis:

(1) AceIn (for Acetylcholinesterase Inhibition), which controls insensitivity of acetylcholinesterase to organophosphates, is on Linkage Group 2 and is 10 centimorgans from the allozyme locus IDH-2. Mapping of this locus was done in collaboration with Thomas M. Brown (Department of Entomology, Clemson University), who has extensively characterized the properties of resistant and susceptible alleles at this locus.

(2) Hscp (for Heliothis Sodium Channel Para), which is the Heliothis homologue of the para-type of voltage gated sodium channel of Drosophila, is on Linkage Group 10. This is linked to the allozyme locus SODH. In collaboration with Martin Taylor (Department of Entomology, University of Arizona), we have shown that molecular variation at this locus is linked with

pyrethroid resistance, but it accounts for at most half the resistance present in T.M. Brown's "RR" strain.

(3 - 6) Bt resistance loci. Four loci (or clusters of loci) conferring resistance to Bt toxin have been assigned to linkage groups. These are called BtR1, BtR2, BtR3, and BtR4 (in order of discovery) and they have been assigned to linkage groups 22, 11, 3, and 9, respectively. The first three were present in a weakly resistant strain developed by Monsanto, the fourth comes from a highly resistant strain selected by Fred Gould (Department of Entomology, North Carolina State University). BtR4 accounts for about 80% of the resistance in this strain, which is about 8000 fold resistant to CryIA(c). Because this resistance locus is so potent, we are trying to localize it within linkage group 9, and are in the process of constructing a detailed map for that chromosome.

Research supported by the USDA Biotechnology Risk Assessment Program, the Bt Management Working Group, and an NSF EPSCoR Grant to the State of South Carolina.

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Research at Texas A&M University

1. Development and field testing of methods to monitor for resistance to nonpyrethroid insecticides in the tobacco budworm. In this research we have determined that combining benzoic acid with biodegradable insecticides and storing treated vials in a freezer allows us to use a monitoring technique for resistance to nonpyrethroid insecticides similar to that developed earlier with pyrethroids. Monitoring data from Texas and mid-South states in 1992 and 1993 has demonstrated resistance to OP, carbamate and cyclodiene insecticides in tobacco budworms. Resistance appears annually, peaks midseason and then declines later in the season.

2. Development and field testing of methods to monitor for resistance to insecticides in the boll weevil. Treated vials have been successfully used to monitor for pyrethroid and OP resistance in boll weevils in Texas and Sonora. Resistance to pyrethroids is widespread; resistance to OPs shows up occasionally at low frequencies.

3. Mechanisms of resistance to nonpyrethroid insecticides in the tobacco budworm: A variety of tests has shown that altered cholinesterase resistance to OPs and carbamates is a major mechanism of resistance to insecticides widely used for tobacco budworm control. Target site resistance is also present with endosulfan. Decreased insecticide uptake also characterizes most field resistant populations.

4. Use of low dose B. thuringiensis as a pest management tool. There is widespread use of low doses of B.t. for tobacco budworm and bollworm control in Texas. At low doses, B.t. slows development rather than killing exposed insects. These slower developing insects are then susceptible to predation. The low dose approach negates the possibility of resistance development since the doses do not produce direct mortality.

5. The use of insecticide mixtures to delay resistance is possible. In 1992 field tests in Texas showed that use of low dose mixtures of cypermethrin and Curacron resulted in less resistance than would be expected in cases where the same insecticides were used at higher rates.

6. Use of juvenoid insect growth regulators and piperonyl butoxide (PBO) for population management of aphids and white flies. In 1992 work we demonstrated that exposure of aphid and whitefly populations to juvenoid compounds resulted in delays in adult emergence and decreased reproduction. Piperonyl butoxide produced identical effects suggesting that it may act as a juvenoid material. The use of juvenoids to manage populations of rapidly reproducing insects such as aphids and white flies is an attractive

alternative strategy to managing these insects with toxic chemicals.

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Abstracts in Resistance Management

Status of Insecticide Resistance in Tobacco Budworm and Bollworm in Louisiana

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Over 4100 male tobacco budworm moths were bioassayed for pyrethroid resistance from May through September 1993 against 10 or 30 g doses of cypermethrin utilizing the adult vial test. Average pyrethroid resistance levels during all months of 1993 except August were higher than previously recorded (1987-1992). Also 854, 595 and 440 male tobacco budworm moths were bioassayed for resistance to profenofos, methomyl and endosulfan using the adult vial test. Although the discriminating doses of profenofos, methomyl and endosulfan selected for these bioassays remain questionable, resistance to all three classes of chemicals was evident. Thus resistance was documented to representative chemical from the four classes of insecticides (carbamates, organochlorines, organophosphates and pyrethroids) primarily used to control tobacco budworms on cotton in the United States. Tobacco budworm population pressure was generally light to moderate in Louisiana during 1993. As a result, few tobacco budworm field control failures occurred although control in most fields was not satisfactory. Over 1700 male bollworm moths were bioassayed against 1, 2, or 5 m g/vial doses of cypermethrin. Data from these bioassays were similar to that obtained 1988-1992 and indicate that bollworms remain susceptible to pyrethroids.

Resistance within Classes of Insecticides in Tobacco Budworm

Strains of the tobacco budworm, *Heliothis virescens* (F.), collected in Mississippi were evaluated in bioassays to four classes of insecticides and a synergist. The temporal development of resistance showed various patterns of cross-resistance among different classes of insecticides. High levels of resistance were found to cypermethrin and endosulfan. Significant levels of resistance were also shown to carbamate and

organophosphorus insecticides, although levels were generally not as high to these classes of insecticides. Tests using piperonyl butoxide did not show synergism of cypermethrin in some cases, nor was synergism seen with thiodicarb or profenofos. Multiple resistance is still apparent and resistance to pyrethroids appears to be increasing.

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Evaluation of Insecticide Resistance and the Effect of Selected Synergists in Tobacco Budworm

Topical diagnostic dose bioassays of representative carbamate, cyclodiene, organophosphate and pyrethroid insecticides were conducted on the LSU laboratory reference colony, a laboratory reference colony from North Carolina and field-collected colonies from Louisiana, Oklahoma and Texas. Comparisons between the LSU and North Carolina colonies showed no significant differences in their responses to these insecticides except for significantly higher susceptibilityto profenofos exhibited by the North Carolina colony. Significant levels of resistance to cypermethrin, profenofos, methomyl and endosulfan were exhibited by the majority of the field-collected colonies in compared to the two reference colonies. Bioassays with piperonyl butoxide (PBO) and cypermethrin combinations resulted in significant increases in mortality for five of the tweelve colonies tested compared to the cypermethrin alone. Significant levels of resistance to thiodicarb were detected in all field- collected colonies using a diet bioassay. Data from a field test with cypermethrin and PBO combinations indicated significant reductions in bollworm/tobacco budworm damage and numbers of live larvae were achieved with Ammo(0.08 lb/acre) + Butacide (1.0 lb/acre) compared to Ammo (0.08 lb/acre) alone. These data suggest that metabolic resistance to pyrethroids is widespread in tobacco budworm populations.

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Biochemical and Physiological Mechanisms of Pyrethroid Resistance in *Heliothis Virescens*

Pharmacokinetic and physiological assays were used to determine mechanisms associated with pyrethroid resistance in *Heliothis virescens* (F.), collected from a single cotton growing region in Louisiana during the four months of the growing season. Reduced neuronal sensitivity was detected in larvae from an early-season (June) collection, but occurred with reduced frequencies in populations sampled later in the season.

Enhanced metabolism of cypermethrin was measured in larval insects and increased in frequency within populations sampled in mid- to late-season. Adult insects also expressed levels of cypermethrin metabolism and excretion that were higher than those measured in a laboratory, reference strain, but the relevance of this result to pyrethroid resistance is not clear because enhanced levels in these insects were not associated with decreased mortality. These results indicate that multiple mechanisms are associated with pyrethroid resistance in this insect, and that levels of expression of these mechanisms in populations of *H. virescens* may fluctuate during the growing season.

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Effects of Single Generation Selection with Insecticides on Resistance Levels in Lousiana Tobacco

Tobacco budworm, *Heliothis virescens* (F.), eggs and larvae were collected at the LSU Agricultural Center's Macon Ridge Research Station (MRS) and separated into four independent colonies labeled as un-selected (UTC), pyrethroid-selected (SP), organophosphate-selected (OP) and carbamate-selected (CRB). Each respective colony was exposed to selecting doses of the pyrethroid, cypermethrin, the organophosphate, profenofos, or the carbamates, thiodicarb/methomyl, using topical application or field sprayed cotton plant terminals. The initial test showed the MRS tobacco budworm colony to possess significant levels of resistance to pyrethroid, organophosphorus and carbamate insecticides. In tests conducted on F1 generation larvae, significant differences in mortality from cypermethrin, profenofos and methomyl were observed between the un-selected (UTC) and insecticide- selected (SP, OP, CRB) colonies. These data suggest that selection with one class of insecticides can influence tobacco budworm susceptibility to insecticides representing different classes in subsequent generations. However, the results do not indicate that a different insecticide use strategy would be more successful than the one currently recommended in the Mid-South region. Alternation among all classes of insecticides recommended for control of tobacco budworm on cotton with a greater emphasis on insecticide mixtures (primary larvacide + ovicide or Bt) will form the basis of the insecticide use strategy for 1994.

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1993 Mid-South Experiences and Insecticide Resistance Management Guidelines for 1994

Tobacco budworm, *Heliothis virescens* (F.), population densities across the Mid-South states of Arkansas, Louisiana and Mississippi during 1993, were lower compared to that reported in 1992. However, above normal infestation levels were observed in geographic regions that have not been consistently associated with control problems in the past. Localized economically damaging populations occurred in the northeastern and central delta counties of Arkansas, in the southern cotton producing parishes and along the Red River in Louisiana, and in the Hill counties of Mississippi. Producers in these areas were not familiar with the implications of insecticide resistance and high population densities. Therefore, numerous instances of unsatisfactory control with recommended insecticides were reported. Tests by state and federal entomologists confirmed substantial levels of resistance to recommended insecticides including carbamates, organochlorines, organophosphates and pyrethroids in tobacco budworms collected from these areas. The guidelines developed to assist cotton producers in managing insecticide resistant tobacco budworm have been revised for 1994 to address current changes in the cotton pest situation and in the availability of the ovicide, Ovasyn 1.5E (amitraz). These guidelines continue to emphasize the production of an early maturing cotton crop using agronomic and integrated pest management practices. A general insecticide use strategy that distributes the risk of tobacco budworm control failures among all available classes of insecticides has been accepted by these entomologists.

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Overview of Fungicide Resistance

Serious problems with fungicide resistance have first been encountered with the benzimidazoles. Here, the mechanism of resistance is based on the point mutation of the target site, resulting in a resistant subpopulation that is clearly separated from the sensitive population. This population is almost insensitive and cannot be controlled at any practical rate of the fungicide. In contrast to other specific fungicides, the sensitivities in wild-type populations are widely distributed and continuous in character, ranging from highly sensitive to resistant. The resistant subpopulation as part of a unimodal sensitivity distribution remains accessible to inhibition at high doses.

The DMIs fenarimol and myclobutanil, both acting as inhibitors of fungal sterol demethylation (DMI), are in use for the control of apple scab. Although considered as fungicides with moderate risk of resistance, previous experiences from Canada and Europe have indicated that slow development of resistance can occur after approximately 10-15 years of use. The development of quantitative monitoring procedures, extensive testing of isolate sensitivities residing in wild-type populations of the scab pathogen (baseline sensitivities), comparison of baseline orchards with a site of documented DMI resistance, and orchard trials have provided the groundrules for anti-resistance strategies. Three separate components were identified: (A) Low disease pressure exerted on the DMIs delays the development of resistance. Comparison of population sensitivities in two experimental orchards with identical DMI use histories but different degrees of disease pressure indicated the predominant importance of this parameter. Low disease pressure under given orchard conditions can be achieved by carefully managing the disease, but also with mixtures of DMIs with contact fungicides such as mancozeb. (B) Restriction of DMI applications to part of the growing season. Use restriction will reduce the number of selection cycles per season. (C) Decreasing the size of the DMI- resistant population delays the development of resistance. This size reduction can be achieved with high application rates. At low doses, proportionally more isolates with reduced sensitivities will not be fully prevented from sporulation and propagation between two consecutive fungicide applications. At a higher dose, a larger proportion of these isolates are fully inhibited, will not propagate and thus will not be selected.

The delay of resistance development at high rates of a fungicide will only be possible for a broad but continuous baseline sensitivity distribution and a resistant subpopulation accessible to a dose-dependent inhibition. It will not be possible for fungicides with insensitive subpopulations based on high-risk target mutations. The high rate concept as preventive anti-resistant tactic for fungicides with broad sensitivity distributions is novel and deserves increased attention and broader validation.

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Insecticide Resistance in Cotton Insects

Pyrethroid resistance continues to increase in frequency in the tobacco budworm, *Heliothis virescens* (F.), in the mid-South. However, in 1993 populations were lower and control problems due to resistance were few in most of the Mississippi delta. Exceptions to this were in the hills of Mississippi, south Louisiana, and some areas in Arkansas where resistance levels reached 70% during late season and populations were extremely high. Spray chamber bioassays confirmed continued resistance to non-pyrethroids, however, the adult vial bioassay failed to confirm resistance to non-pyrethroids in some cases.

Extremely high populations of beet armyworm, *Spodoptera exigua* (Hubner), in Mississippi greatly increased insecticide inputs in 1993. No material gave adequate suppression of these excessive populations. Populations of this magnitude were last seen in 1988 in the mid-South and Southeast. Spray chamber bioassays done on beet armyworms collected in 1993 were

compared with results from the 1993 strains were found with profenofos, sulprofos, and acephate, but not with thiodicarb or methomyl. No insecticide or insecticide mixture tested provided better than 70% control with the exception of the pyrrole AC 303, 630 (American Cyanamid) which gave 100% control of beet armyworms at 0.2 lb(AI)/acre.

Recent evidence indicates pyrethroid resistance in a strain of tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), collected in Schlater, MS in 1993 (G.L. Snodgrass, in press). Resistance to permethrin, as determined by an adult vial test, was 53-fold compared with a strain collected near Stoneville, MS. Resistance to permethrin was also confirmed with a spray chamber bioassay. No resistance to acephate was found, however, resistance to methyl parathion was 18-fold in the Schlater strain (G.L. Snodgrass, unpublished data).

Development of multiple resistance in multiple pests in cotton has placed constraints on economical production and may continue to pose a threat to the industry. New insecticides with novel modes of action, as well as biopesticides, resistance management strategies, and adherence to the principles of integrated pest management, are needed to combat the ever present threat of resistance.

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Resistance to *Bacillus thuringiensis* CryIC in Beet Armyworm

The use of microbial insecticides such as those based on *Bacillus thuringiensis* (B.t.) is expected to increase drastically in the future. Therefore, increased incidences of documented field resistance to B.t. would not be unexpected. *Spodoptera exigua* Hubner (Leopidoptera: Noctuidae) is a polyphagous insect pest feeding on many economic crops such as tomatoes, celery, cotton, peppers, chrysanthemums, etc. Historically, formulations of B.t. subsp. kurstaki strain HD-1 (such as Dipel, Javelin, Biobit) applied at recommended field rates usually did not provide good economic control against *S. exigua*. Because of this lack of control, high selection pressure was not being applied to *S. exigua*. However, B.t. products containing the CryIC protein (the most toxic Cry toxin against *S. exigua*), recently have been developed and targeted against *S. exigua*. As the potency and use of these new B.t. products against *S. exigua* increases, the likelihood for resistance to develop against these proteins also increases.

Selection for *S. exigua* resistance to B.t. was initiated in 1991 with a laboratory colony that recently had been started from insects obtained from cotton. A spore/crystal preparation of HD-1 and purified CryIC protoxin from HD-133 was used to initiate selection in diet incorporation bioassays. Only a 3-4 fold increase in LC50's was observed for HD-1 after 20 generations. All eggs obtained from the adults of the 20th generation were sterile. However, resistance to the CryIC protoxin was observed after 9-12 generations. Trypsinized CryIC toxin then was used to increase selection. Resistance levels exceeding 50-fold was observed after 21 generations, and over 1000-fold after 32 generations. Bioassays using B.t. formulated materials against CryIC-resistant S. exigua (50-fold resistant) resulted only in a 2-4 fold increase in LC50. Interestingly, the formulated material resulting in a 4-fold increase in LC50 contained exclusively CryIC. CryIC-resistant *S. exigua* also appear to be at least partially cross-resistant to CryIA(b). Preliminary binding studies suggest a 3-4 fold reduction in binding sites, but no apparent difference in Kd. The addition of thuringiensin (- endotoxin) at sublethal rates significantly reduced the level of resistance. Mechanisms of resistance and the likelihood for resistance to develop in the field will also be discussed.

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Larval Age Affects Resistance to *Bacillus thuringiensis* in Diamondback Moth

Neonate larvae are usually more susceptible to pesticides than older larvae. Previous research on insecticide resistance in diamondback moth, *Putella xylostella*, has focused primarily on responses of late instar larvae. The objectives of our study were: 10 to compare susceptibility of neonate and the third instar larvae of diamondback moth to the microbial insecticide,*Bacillus thuringiensis* (B.t.), 2) to compare expression of resistance to B.t. in neonate and third

instar larvae, and 3) to compare post-treatment mortality in susceptible and resistant larvae.

In laboratory bioassays, neonate and third instar larvae from susceptible and resistant colonies ate disks of cabbage leaves that had been dipped in Dipel 2X, a commercial formulation of *B. thuringiensis* subsp. *kurstaki*. After 48 h, larvae were transferred to untreated leaves. Mortality was recorded at 48 h and 120 h. Two bioassays were conducted with each larval stage of the susceptible and resistant colonies. Each bioassay included four replicates of a series of Bt concentrations.

For both susceptible and resistant colonies, neonate larvae had significantly higher LC50 than third instar larvae. This may be due partly to the leaf mining feeding habit of neonate larvae. Resistance to Bt was expressed more strongly in third instar larvae than in neonate larvae. The resistance ratio for third instar larvae was twice as high as that for neonate larvae. Resistance ratios estimated at 120 h (72 h after Bt treatment) were more than double those estimated at the end of the 48 h Bt treatment for both neonate larvae and third instar larvae. This resulted from lower post-treatment mortality in resistant larvae.

In summary, we found that neonate larvae were more tolerant to Bt than the third instar larvae in diamondback moth. Resistance to Bt was expressed more strongly in third instar larvae than in neonate larvae. Resistant larvae had lower post-treatment mortality than susceptible larvae.

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Biochemical Diagnostics Assays for Monitoring Insecticide Resistance in the Tobacco Budworm

Cytochrome P450 levels; P450 activity measured with benzo[a]pyrene, benzphetamine, methoxyresorufin and p- nitroanisolc; esterase activity measured with 1-naphthyl acetate and p-nitrophenyl acetate; and glutathione transferase measured with chlorodinitrobenzene were significantly elevated in the fat body, gut and epidermis of cypermethrin- and thiodicarb-resistant *Heliothis virescens* as compared to a susceptible laboratory strain (Wake, NC State University). The resistant strain was originally collected from Hebert, Louisiana, and additional selection with thiodicarb was conducted in the laboratory. The resistance ratio based on increased metabolism was as high as 37-fold for fat body using methoxyresorufin as a substrate. A novel, microtiter plate (biochemical) assay using the substrate p-nitroanisole, was developed for the detection of elevated cytochrome P450 activity associated with resistance in individual budworms. This kinetic assay was successfully used to investigate resistance in field-collected budworms from several regions of the U.S.

Three resistance-associated esterases (RAEs) (A1, B1 and C1) from the tobacco budworm (Hebert strain) were purified to homogeneity using liquid-column preparative isoelectric focusing and 2-D electrophoresis. Esterase A1 bound DDT, cypermethrin, thiodicarb and methyl-paraoxon but was not inhibited by eserine hemisulfate; was immunologically similar to RAEs from the house fly and tobacco aphid; and shared the greatest N-terminal amino acid sequence homology to that of RAEs from *Drosophila* and *Culex*. A novel slot blot immunoassay was developed to distinguish resistant from susceptible budworms based on increased esterase protein in resistant individuals. The gene for a cytochrome P450 from resistant *H. virescens* was cloned from a cDNA library. A 3' fragment of this clone has been successfully used in Northern blots for the detection of P450 RNA from the tobacco budworm.

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Factors Associated with Herbicide Resistance in Weeds

The major goal of herbicide resistance research is to develop management strategies that will delay, avoid or recover from resistance. In order to achieve this goal an intricate understanding of the interplay between fitness, inheritance and gene flow must be developed.

Herbicide resistance is a result of natural selection for traits that allow weed species to survive specific management practices which would otherwise cause mortality. The phenomenon is no different from the evolutionary changes that have arisen in other pests and pathogens in response to the use of pesticides and fungicides or in plants to industrial pollutants. Fitness is defined as the evolutionary advantage of a phenotype based on its survival and reproductive success. There are two underlying factors that govern the rate of herbicide resistance evolution: (1) heritable variation for the resistance trait and (2) selection intensity. Three components contribute to selection intensity for herbicide resistance; the efficiency of the herbicide, the frequency and duration of use.

These concepts pose some important research questions that are critical for developing management strategies. For example, at what level is resistance present in populations before selection with the herbicide? What are the mutation rates for the resistance traits? How does phenotypic plasticity in fitness impact the relative frequency of mutants with different resistance mechanisms prior to selection? What is the relative risk of selecting cross or multiple resistant weeds with a rotation versus a mixture of herbicides strategy? What factors determine how fast resistance spreads across a field or a region? These questions involve complex interactions among a number of factors. Plant population simulation models can be used to develop specific hypotheses that may be helpful in determining the relative importance of processes linked to resistance evolution and management.

Simulations were conducted with a model to address the above questions. When model simulations were compared to observed scenarios, they indicated that herbicide resistant mutants were probably present at low frequency near the mutation rate at the time when selection begins. There was also strong evidence from simulations that a number of different mutants with different resistance mechanisms could be present in populations when the relative phenotypic plasticity for fitness is varied even a small amount. Further simulations suggested that the herbicide mixture strategy could cause significant delays in resistance evolution to a resistance prone herbicide, but it could increase the potential for selecting a cross or multiple resistant mutant.

The spread of resistance was studied using a wind pollinated outcrossing weed and a self pollinated weed primarily dispersed in the seed stage. The resultant patterns of resistance under field conditions were difficult to interpret.

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Field-based Biological Assays for Rational Planning of Insecticide Resistance Managment in *Thrips tabaci*

Our objectives were to establish an economic threshold for *Thrips tabaci*, the onion thrips, to assess the potential of biological control, and to design a bioassay method to determine insecticide tolerance in thrips populations in the field. The bioassay approach was modeled after that for *Lygus hesperus* (J. Econ. Entomol. 1993. 86:1656-1663).

Contact biological assays in selfclosing plastic bags were used. The insecticide residue was coated inside the bags by spreading, and rapidly drying, a 5 l aliquot of technical gradeinsecticide in acetone. The thrips were captured by aspirators. The plan was to place 5 to 10 adults in each bag but the number ofttен varied more than that. A bit of onion leaf to improve the holding conditions and a small spacer to keep the bag sides apart were inserted into the bag. The temperature was controlled to ca. 20 C in a portable incubator and the bioassay period lasted for 4 hours. For synergism studies, the thrips were confined in short sections of plastic straws that had been coated with piperonyl butoxide, DEF (S,S,S- tributylphosphorotrithioate), or diethyl maleate.

Insecticide toxicities are summarized below in terms of "managed" and "intensive" approaches. The "intensive" approach was five sequential applications of cypermethrin; the "managed" approaches were either three sprays of cypermethrin alone or two sprays of cypermethrin plus one spray of organophosphate. The results demonstrated some concern for increased tolerance to cypermethrin.

Cypermethrin was tested at a range of 200 to 1 g/bag. The mean LC50 from 33 bioassays (on different dates) in "managed" fields was 8.0 g/bag (5.7 to 12.8; 95% confidence limits) while for 16 bioassays in "intensive" fields, the mean LC50 was 16.5 (12.1 to 28.1). Bifenthrin, another pyrethroid, had not been applied to these fields but the LC50 values differed similarly (38.3 g/bag, 29.4 to 56.7, 9 bioassays in managed

fields; 51.6 g/bag, 38.7 79.7, 4 bioassays in intensive fields). Methyl parathion had similar LC50 values between managed (28.8) and intensive fields (20.8) as did malathion (38.2 managed; 47.0 intensive).

Cypermethrin was consistently synergized by piperonyl butoxide suggesting that mixed function oxidases may be involved in the onion thrips' tolerance. An economic threshold of approximately 20 thrips/plant may be appropriate under Utah conditions, and still maintain crop loss below 10%. Less than 1% of 900 thrips specimens examined from 25 onion fields in 1992-1993 were predaceous. Of all phytophagous specimens, approximately 96% were *Thrips tabaci*. Under present circumstances, there was little potential for biological control in these fields.

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Copper Resistance in Bacteria

Resistance to copper bactericides has been described in several plant pathogenic and saprophytic bacteria, including pathovars of *Pseudomonas syringae* and *Xanthomonas campestris*. Genes determining copper resistance in many of these bacteria are conserved and are usually plasmid borne. For the last 10 years, we have investigated copper resistance in *P. syringae* pv. tomato, the cause of bacterial speck inhibited *in vitro* by a commercial formulation of cupric hydroxide (Kocide 101) and for which bacterial speck disease was not controlled in field tests. The resistant operon (*cop*ABCD). A related operon is present in the chromosome of all pseudomonads examined and may be the ancestor of the plasmid-borne resistance operon. The *cop* operon is specifically induced by copper, which requires a two-component signal transduction system encoded by two additional genes in a separate operon, *cop*RS. CopS appears to be a membrane-spanning, copper-sensing protein, and CopR binds to the -35 region of the *cop* promoter and activates transcription. Copper-resistant strains accumulate copper, which is at least in part directly a result of copper-binding proteins produced from the *cop* operon. One of the most abundant copper- binding proteins is CopA, which binds multiple copper atoms and is related to a number of eucaryotic multicopper oxidase enzymes. Whether an oxidase function is part of the resistance mechanism, or whether this reflects an ancestral function as an indigenous chromosomal gene, is not yet known.

Since the level of copper resistance determined by *cop* genes in *P. syringae* is limited (2-4 times the level of sensitive strains), the management of copper resistance may be improved by the use of additives that enhance the solubility and uptake of Cu2+ ions or that interfere with the resistance mechanisms. Additives that have shown some enhancement of copper sensitivity include EBDC fungicides, ferric chloride, zinc chloride, and aluminum nitrate.

The use of nonpathogenic strains of bacteria to competitively displace copper-resistant pathogens is another strategy that might be feasible, given the lack of alternative bactericides. In greenhouse studies, we have demonstrated that nonpathogenic mutants of *P. syringae* pv. tomato can significantly control bacterial speck disease when sprayed onto tomato leaves. Further experiments on this biological control strategy are being pursued by the tomato industry.

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Quantitative Genetics of Behavioral and Physiological Resistance to Insecticides in Diamondback Moth and Colorado Potato Beetle

Insects may survive insecticide applications by physiological mechanisms that permit tolerance of an acquired dose or behavioral mechanisms that reduce the dose acquired. Our work focuses on the genetics of physiological and behavioral aspects of insecticide resistance, and the use of this information for resistance management.

We investigated the genetic basis of physiological and behavioral responses to the pyrethroid permethrin within four diamondback moth *Plutella xylostella* L. populations with different average levels of tolerance. Using a half-sib design, variance components and heritabilities for the two characters, and the genetic correlation between them, were calculated for each population. In all four populations, additive genetic variances and heritabilities for physiological tolerance to permethrin were highly significant. In contrast, heritabilities for behavioral avoidance of permethrin were low and significant in only one population, though additive genetic variances were similar to those for tolerance. The phenotypic and genetic correlations between these two characters varied among the populations, from essentially zero to strongly significant. All correlations were negative in form with behaviorally responsive families tending to be less physiologically tolerant and unresponsive families tending to be more physiologically tolerant. Significant correlations between physiological tolerance and behavioral avoidance occurred in populations with relatively high levels of additive variation for both characters. The presence of significant negative correlations suggests that resistance management through indirect selection for physiological susceptibility may be possible. Our current work involves determining how the genetic correlation arises and tracking changes in it under various selection regimes.

Proposed resistance management strategies for Colorado potato beetle in transgenic potato crops containing *Bacillus thuringiensis* delta-endotoxin include growing a mixture of transgenic and nontransgenic seed. The success of the strategy would be impacted by behavioral responses of Colorado potato beetle to the transgenic foliage in a mixture, and the correlation between behavioral response and physiological resistance. We reared full-sib families of Colorado potato beetle larvae from single mated pairs and measured behavioral response and physiological susceptibility of larvae from each family to transgenic foliage. Significant genetic components were observed in the variation in both characters. Heritability estimates were particularly high for the physiological response. The correlation between the two characters was significant. More behaviorally responsive larvae tended to be more physiologically resistant. The implications for the seed mixture strategy are that larvae moving onto transgenic foliage in a mixture are more likely to return to the nontransgenic foliage and survive if they are more resistant, making the seed mixture strategy potentially counter-productive. We now are comparing the response to direct selection on physiology with the response to indirect selection on behavior.

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Fungicide Resistance in Powdery Mildew of Curcurbits

Strains of the powdery mildew fungus (*Sphaerotheca fuliginea*) that are resistant to benomyl (Benlate) and/or triadimefon (Bayleton) have been found in all fields examined in the United States that were treated with these fungicides; therefore, fungicide resistance is a concern wherever powdery mildew occurs. These are only systemic fungicides registered in the U.S. for this disease. The frequency of Bayleton-resistant isolates ranged from 64% to 100% in fields treated at least twice with this fungicide.

Fungicide usage has a large impact on resistance development during a single growing season. For example, in one commercial field, the proportion of the fungal population resistant to Bayleton was 0% to August 6, 1992, 96% on August 21st after Bayleton had been applied twice, and 100% on September 24th. The proportion of the population resistant to Benlate was 10% on August 6th, 70% on August 21st, and 97% on September 24th. In contrast, fungicide sensitivity did not change in fields that were not treated with fungicides. 86% of the isolates from fungicide-treated fields on Long Island in 1991 and 1992 were able to grow on leaf disks treated with 100 or 200 ppm triadimefon.

Despite rapid development of fungicide resistance, Bayleton has continued to provide good suppression of powdery mildew at least early in disease development. Bayleton has been effective because the frequency of fungicide-resistant strains has been very low at the start of disease development. Bayleton has not controlled powdery mildew adequately late in the growing season, however, the impact on yield probably has been small. The first application of Bayleton, used within a 7-day Bravo spray program initiated after disease detection, contributed much more to disease control and yield protection than the second application.

Although Bayleton-resistant strains do not appear to be able to survive between growing seasons, these strains do seem to be able to compete well with sensitive

strains and to persist in the absence of fungicide during a growing season, since they were found in several non-treated fields located near Bayleton-treated fields.

The frequency of Benlate-resistant strains at the start of disease development has varied; therefore, the effectiveness of this fungicide is difficult to predict. Resistant strains were found before Benlate was used in all fields examined on Long Island in 1991 and 1992. However, in 1993 when fields wre examined throughout the U.S. including Long Island, Benlate-resistant strains were only found in fields that were treated with Benlate or in fields that were near treated fields. This included fields in Florida and Arizona. Growers stopped using Benlate several years ago in these states because of inadequate control which was most likely due to resistance. Therefore, Benlate-resistant powdery mildew strains have not been able to persist in the absence of selection pressure from Benlate applications.

Bayleton-resistant isolates were less sensitive to myclobutanil (Nova) and to propiconazole (Tilt). These fungicides are not registered yet for use in the U.S. These three are DMI fungicides. This level of insensitivity to Nova and to Tilt of Bayleton-resistant isolates is not sufficient to affect the efficacy of these fungicides based on results from a field trial on Long Island in 1993. In contrast with Bayleton, both Nova and Tilt suppressed powdery mildew development throughout the growing season.

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Internet Access

World Wide Web Access

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

To view the newsletter, a WWW browser, such as Mosaic, Cello, Lynx, MacWeb, or WinWeb is required. It is not feasible in a short space to explain the use and installation of such programs (there are books dedicated to this purpose). The most commonly used program, Mosaic, is available free from the NCSA for the X-windows, Macintosh and Windows operating environments, and may be obtained by anonymous ftp from ftp.ncsa.uiuc.edu or from sunsite.unc.edu (the directory at the latter site is: pub/packages/infosystems/www/clients/WinMosiac [or MacMosiac for Macintosh users] directory). Windows users will also need to get the win32s.zip file, which will update their windows system. For those willing to sort out the details, access to the internet over phone lines can be achieved with the trumpet winsock shareware package, available as the file winsock.zip from the previous ftp sites in the socket directory. OS/2 users should be aware that the latest versions of Mosaic are not compatible with winos2, and they should use alternatives such as WinWeb, Cello, or earlier versions of NCSA Mosaic. Several companies are producing all-in-one solutions for internet connections (NetManage's Internet Chameleon and O'Reilly and Spry's Internet in a Box are shipping), and the next releases of OS/2 and Windows are both rumored to include software to enable internet access.

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Michael Caprio
Mississippi State University

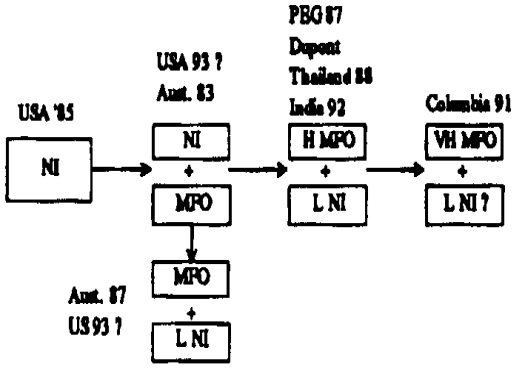
Symposia

IRAC US Cotton Meeting Minutes

The first meeting of 94 was held on the 5th and 6th of January at the Beltwide Cotton Conference in San Diego, California. A portion of the meeting was held jointly with IRAC Cotton (global) and many other guests were in attendance for portions of the meeting. The minutes from the previous meeting were read and accepted. Joe Hope, Rhone Poulenc, led the discussion on the following items:

1. Allan McCaffery - from the University of Reading, whose trip had been sponsored in part by IRAC Cotton, provided an excellent report on his continuing work on electrophysiological studies of mechanisms of action in *Heliothis Helicoverpa*. A portion of the report described the development of a portable electrophysiology unit utilizing a McLab processor, an improved detector, and battery power to provide readings in field conditions. Field and lab data were collected and used to develop the following possible schemes of a general change from nerve site insensitivity to metabolic resistance. Results of US surveys done in conjunction with LSU have suggested that there was very little metabolic resistance during 1991 and 1992. However, in 1993 it was apparent in some populations that metabolic resistance increased late season and nerve insensitivity declined. Complete reports were provided in two papers which will be published in the proceedings of the Beltwide Conference.

Figure 1



NI = Neuronal Insensitivity
L NI= Low Neuronal Insensitivity
MFO= Mixed Function Oxidase
H MFO= High Mixed Function Oxidase
VH MFO= Very High Mixed Function Oxidase

2. Tank Mixes vs. Alternations - Walt Mullins of Miles initiated a lively debate on tank-mixes vs. alternations by initially asking if IRAC US Cotton would support a policy that recommended mixtures particularly where resistance was occurring and asked the question if it would not be a better strategy even with new chemistry. There were many points and counter points made during the discussions but there was a general consensus that the current approach with most insect management programs do not utilize tank-mixes until there is a problem. The group agreed that we do not know for certain that this is the best approach. It was recommended that a symposium be developed to discuss the value of mixtures to stimulate thought in the area.

3. 1994 Action Plans - There was a very lengthy discussion on near term and long term projects for the committee. It was concluded that the committee needs to develop an improved long term plan. A challenge was made that industry is better positioned to set strategy for resistance management and that we are not providing adequate leadership at the present. For the near term it was decided to solicit research proposals, to host a resistance management workshop similar to the 1989 New Orleans event, and to become more active with general education activities.

   Section 18 Discussion: W. Mullins, Miles, related an experience where his company was denied a Section 18 that was requested based on a concern for resistance to current compounds since the criteria of documented 10 fold resistance accompanied by field failures had not been met. He suggested that we invite EPA to discuss the current criteria since they don't address the value of using new compounds early to prevent the complete loss of existing compounds to resistance. This was one of several topics that were suggested for possible discussion with EPA and it was unanimous that the committee needed to identify itself as a resource for EPA.

   Interim Project Report: Joe Hope, Rhone Poulenc, presented the interim results for the Management of Sweet potato Whitefly project by N. Prabhaker, N. Toscano and T. Henneberry, that the committee had partially funded during 1993. The data presented was also included in several presentations at the Beltwide. A final report will be supplied to the committee.

4. Cotton Aphid Resistance - Dave Marsden, DuPont, reported on his assignment to conduct an informal survey on cotton aphids. During 1990 cotton aphids were widespread problems across much of the Cotton Belt. However since then, most infestations have been manageable by avoiding certain compounds early season and by the consistent development of fungal diseases of aphids in most areas. It was recommended that the committee continue to monitor the aphid situation but delay any immediate activity.

   Acaricide Subgroup: John Long, Rohm & Haas, reported that he and representative members from Merck, Uniroyal, DuPont, American Cyanamid and AgrEvo had met and would like to formalize as a subgroup of IRAC US. There was unanimous consensus that the group could work within IRAC US Cotton and meet earlier or later to cover other crops. There was discussion on the committee functioning as IRAC US or IRAC US Cotton with no conclusions or recommendation made.

5. Final Project Report - Monitoring and Managing Tobacco Budworm Resistance to Carbamate, Cyclodiene, Organophosphate and Pyrethroid Insecticides. A 90-page final report which included six individual reports was presented to the committee. Hard copies of the final report were provided to all members present. Abstracts are listed in the Abstracts and Bibliography section of the Newsletter and additional copies of the final report will be provided if requested.

6. Beltwide Conference Workshops - A short discussion was held on how to improve on the workshops which were held at this meeting. Suggestions were made to provide greater awareness of IRAC US in the program. There was some concern that we were not reaching enough of the primary audience (producers). Modifying the title to something like the 92 title "Making it Work on The Farm" was suggested.

   Election of Officers: With 13 voting members present Gary Thompson, DowElanco was elected chair and John Lublinkhof, AgrEvo, vice Chair/Secretary. Frank Carter, National Cotton Council will remain as Treasurer. The terms of the appointments are two years.

   Future Meetings: The next scheduled meeting will be on Tuesday, April 19th 1994 at the National Cotton Council in Memphis. An organized dinner will be arranged for Monday evening. New agenda items should be forwarded to Gary Thompson by March 18.

G.D. Thompson
DowElanco
Vice Chairman/Secretary
Wayside, MS

FAO/IAEA Consultant Group Meeting

The potential for tsetse flies to develop resistance to insecticides

Chemical insecticides are playing an increasingly important role in control of tsetse flies (*Glossina* spp), vectors of human and animal trypanosomiasis in large regions of Africa. Although insecticide resistance has not yet been reported in tsetse, there is no cause for complacency regarding its occurrence in the future. As new reports of insecticide resistance in other disease vectors and agronomic pests continue to accumulate at a rapid rate, it is increasingly clear that no comprehensive approach to tsetse control can afford to ignore the potential resistance problem, as the loss of insecticides from the limited set of options for control would be disastrous. It is likely that one or more of the pyrethroid resistance mechanisms already known from several other species of Diptera will manifest itself in tsetse, in response to the increased selection engendered by the wider adoption of deltamethrin-treated targets in tsetse control at the local level and in eradication efforts. Also, selection for behavioral avoidance of traps and targets could result in decreased control efficiency, although the mechanisms that might cause such behavioral resistance are poorly understood at present.

There is thus an increasingly urgent need for information on the potential for resistance development in tsetse, on accurate and feasible methods for detection, monitoring, and characterization of resistance, on properties of resistant strains, and on appropriate tactics for resistance prevention and management. Because of the extraordinary difficulties in rearing posed by tsetse life history, it is essential that these research efforts get underway immediately. The Consultants Group on the Possibility of Development of Insecticide Resistance in Tsetse has accordingly prepared this report with a consideration of the present state of knowledge, a discussion of the essential elements of a resistance research program, and specific recommendations.

A summary of the recommendations in the Consultants Report is as follows:

- Collection of information on the scope and intensity in past as well as future planned control projects and dissemination of information of insecticides is planned. Information to planners, field operatives, and in training manuals on the possibility and consequences of resistance development in tsetse will be noted.

- Collection of baseline information on current levels of insecticide susceptibility in tsetse, focusing initially on pyrethroids, and incorporating all relevant approaches including bioassay (especially development of discriminating doses), activity assays for detoxifying enzymes, isolation and characterization of genes potentially encoding insecticide resistance, and neurophysiological measurements of nervous system sensitivity to pyrethroids.

- Development of laboratory-selected pyrethroid-resistant strains of tsetse.

- Consideration and evaluation of the resistance-delaying or resistance management benefits of alternative strategies of tsetse control or eradication, especially with regard to inclusion of other compounds to reduce the dependency on pyrethroids.

Each recommendation is discussed in more detail in the Report. The Consultants Group urges its wide dissemination and consideration by all involved in programs of tsetse control and eradication, and by agencies and funding bodies with an interest in science in the service of sustainable development in Africa.

Copies of this report may be obtained from: Dr. Udo Feldmann Insect & Pest Control Section International Atomic Energy Agency Wagramerstrasse 5 P.O. Box 100 A-1400 Vienna AUSTRIA

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