MONITORING PYRETHROID RESISTANCE IN THE TOBACCO BUDWORM[1] IN MISSISSIPPI: IMPLICATIONS FOR RESISTANCE MANAGEMENT

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ABSTRACT

Annual measurements of pyrethroid resistance levels in field populations of *Heliothis virescens (F.)* in Mississippi indicated that resistance has been present since 1986. Direct measurements of resistance levels were made by topical, adult vial, and sprayed plant assays with insects collected from field populations. Indirect measurements included annual small plot efficacy studies conducted throughout cotton growing areas of the United States.

Results of topical assays indicated that variation existed in dosage-mortality responses prior to 1986. Since 1986, some field colonies collected each year exhibited higher LD-50's and LD-90's than those for known susceptible strains. Sprayed plant assays indicated that pyrethroid resistance was high enough to significantly reduce mortality of third instar larvae on treated cotton. Adult assays indicated that pyrethroid resistance tends to increase in Mississippi as the growing season progresses and that resistance is spreading geographically. Trends for reduced larval control were identified in small plot data summarized for Alabama, Georgia, Louisiana, Mississippi, and Texas.

In general, all four monitoring techniques were effective in documenting the presence of insecticide resistance. Limitations and advantages of the different techniques are examined.

INTRODUCTION

Reduced efficacy of pyrethroid insecticides in Mid-South cotton was associated with the presence of pyrethroid resistance in field populations of *Heliothis virescens (F.)* in 1986 (Leonard et al. 1987, Luttrell et al. 1987, and Plapp et al. 1987). One year earlier pyrethroid resistance had

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been reported in field populations of *H. virescens* in Texas (Plapp and Campanhola 1986). Although variation in mortality had been observed with standard topical monitoring techniques prior to the mid-1980's (Davis et al. 1977, Harding et al. 1977, Crowder et al. 1979, Twine and Reynolds 1980, Plapp 1981, Martinez-Carrillo and Reynolds 1983, Staetz 1985), reduced efficacy in field environments had not been correlated with higher dosage-mortality responses.

Field control failures are influenced by pest density as well as frequency of resistant individuals. Roush and Miller (1986) discussed sampling problems associated with early detection of resistance in field populations. It is unlikely that available monitoring procedures can detect the presence of insecticide resistance at frequencies less than 1 to 10%. This complicates effective implementation of resistance management strategies. Grower cooperation, financial support, and development of viable management strategies are closely related to problem recognition.

As a reaction to the 1986 observations of reduced pyrethroid efficacy against *H. virescens*, resistance management strategies were developed and recommended in the Mid-South (Anonymous 1986) and Texas (Frisbie and Plapp 1987). Plapp and Campanhola (1986) developed an adult monitoring technique that produced results closely correlated with those obtained with larval assay techniques (Roush and Luttrell 1987, Roush and Luttrell 1989). This adult monitoring technique, which was readily accepted by entomologists because results could be easily obtained in a timely manner, facilitated cooperation and region-wide monitoring throughout the Mid-South and Texas. The level of cooperation among scientists across and within states was high. Industry actively supported the project and PEG-US (Pyrethroid Efficacy Group, United States) was particularly instrumental in information transfer. Plapp et al. (1990) recently summarized results obtained in region-wide monitoring programs. Numerous additional reports (Graves et al. 1988, Leonard et al. 1988, Luttrell et al. 1988, Riley 1988, Simonet et al. 1988, Campanhola and Plapp 1989, Kitten et al. 1989, Riley 1989) have described results of adult monitoring efforts. Based on the amount of information generated and the number of scientists involved, reaction to the problem of pyrethroid resistance in *H. virescens* was rapid once the problem was documented.

The effectiveness of resistance management strategies in delaying development of pyrethroid resistance in *H. virescens* is impossible to quantify experimentally. The scope of adoption of a recommended strategy by growers is difficult to measure. Even if it could be accurately measured, one would need to know what would happen in the absence of resistance management to have a valid empirical basis for comparison of changes brought about by adoption of the strategy. Therefore, this paper is not to be a commentary on the degree of success of resistance management, but is to summarize data collected during the 1980's in Mississippi which documents detection of pyrethroid resistance in *H. virescens*. This historical data base may be useful to others interested in improving detection of insecticide resistance and in efficiently implementing resistance management strategies.

MATERIALS AND METHODS

Techniques used during the 1980's in Mississippi to monitor insecticide resistance in *H. virescens* included standard topical assays of larvae from field collected strains (Anonymous 1970), exposure of larvae from field collected strains to treated cotton terminals (Luttrell et al. 1987), exposure of adult males captured in pheromone traps to residual deposits of insecticide in scintillation vials (Plapp et al. 1987), and small-plot efficacy studies conducted annually. Various strains were obtained from a diversity of crop production environments with assistance of many cooperators. Brief descriptions of the collection sites and known relationships to insecticide treatments are summarized in Table 1. A minimum sample size of 400 to 500 insects was the goal for establishing viable strains for these studies. However, actual numbers of insects collected and subsequent survival to reproduction in the laboratory varied tremendously with density of insects available and levels of contamination by micro-organisms. Several strains which were collected over the 9-year period failed to survive the colonization process. Most laboratory strains listed in Table 1 were established from a minimum of 50 reproducing females.

TABLE 1. Historical Information on Laboratory and Field Strains of *H. virescens* used in Topical and Sprayed Plant Assays.[a]

Strain	Historical Information
	1981 Studies
MSU-LAB	Originally collected on cotton in Mississippi, but maintained in the laboratory for several years without introduction of new insects, considered to be very susceptible to most insecticides.
TUC-SUS	A laboratory colony originally collected in Tucson, AZ. Often used as a reference strain for insecticide susceptibility.
STN-MS	Collected from cotton in late August at the Delta Branch Experiment Station, Stoneville, MS. No indications of field control failures were associated with the collection.
LEF-MS	Collected from late-maturing cotton in Leflore County near Greenwood, MS. High densities of larvae were found in a late-maturing cotton field that was planted behind wheat and irrigated by center-pivot. Reduced levels of control were not associated with the collection.
ARS-LAB	Obtained from the mass-rearing unit of the USDA/ARS facility at Stoneville, MS.
	1982 Studies
IMP-CAL	Originally collected from the Imperial Valley of California in regions where high dosage-mortality values were reported (Martinez-Carrillo and

TABLE 1. (Continued)

Reynolds 1983). The insects had been in laboratory culture for several generations prior to study at Mississippi State University.

LEF-MS -- Collected from cotton in Leflore County near Greenwood, MS during August. No apparent control failures were associated with the colony.

STN-MS -- Collected from cotton near the Delta Branch Experiment Station, Stoneville, MS. No apparent control failures were associated with the colony.

PSF-MS -- Collected from cotton on the Plant Science Research Farm at Mississippi State University. Large numbers of laboratory reared insects are annually used to infest research plots at this location.

HUM-MS -- Collected from cotton in Humphreys County during late August. A reported control failure was associated with the collection, but application and timing factors were suspected.

1983 Studies

MSU-LAB -- Same colony as the 1981 strain, except that a limited number of males collected in the Delta region had been incorporated into the colony.

1984 Studies

MSU-LAB -- Same colony as the 1983 strain.

STN-MS -- Collected in early September from burcucumber (*Sicyos angulatus*) growing on ditch banks near the Delta Branch Experiment Station, Stoneville, MS.

1986 Studies

MSU-LAB -- Same colony as the 1984 strain.

BEL-MS -- Collected from high densities of larvae remaining in a cotton field treated twice with pyrethroids within a 10 d period. The field was located near Belzoni, MS and the collection was made in early September. Control failure did not seem to be related to timing and application factors. (Luttrell et al. 1987).

INV-MS -- Collected from soybean in early September following multiple applications of pyrethroids to a large-plot field experiment. The field was located near Inverness, MS, in the same general region as the BEL-MS site. The lack of control observed was surprising and could be related to application factors. (Luttrell et al. 1987).

GLA-MS -- Collected from cotton in July near Glen Allen, MS. Less than adequate control of *H. virescens* had been oberved following pyrethroid application (Luttrell et al. 1987).

CG-AZ -- Collected from cotton near Casa Grande, AZ in 1985. The colony had been in laboratory culture for ca. 1 year. (Luttrell et al. 1987).

HRN-TX -- Collected as eggs from a late-maturing cotton field near Hearne, TX during August. This strain

TABLE 1. (Continued)

had exhibited resistance in laboratory studies at Texas A&M University. (Luttrell et al.1987).

1987 Studies

INV-MS -- Collected as eggs from a cotton field in July near the site of of the 1986 INV-MS collection. Eggs were collected immediately prior to the initial application of pyrethroids. Extremely high densities of eggs (>50% of plants infested) were observed. The initial application was a mixture of cypermethrin and chlordimeform, and the grower obtained effective control.

COAH-MS -- Collected from cotton in the southern region of Coahoma County in mid-July. A control failure had been reported, but timing and application factors may have been involved.

RANK-MS -- Collected from cotton in Rankin County, MS in June. Less than adequate control was observed following application of pyrethroids with ground equipment. Timing and application factors did not seem to be involved.

FPT-MS -- Collected as eggs from cotton near Friar's Point, MS in late August. Although control failures had not been reported, agricultural consultants considered efficacy of pyrethroids to be less than that historically observed.

STN-MS -- Collected in early September from a late-maturing cotton plot at the Delta Branch Experiment Station, Stoneville, MS. Reduced pyrethroid efficacy was not associated with the collection.

PAR-RR -- A laboratory strain (ICI-resistant) that was originally based on field collections from several sites in the United States experiencing field control problems. Resistance had been maintained in the colony with routine exposure to pyrethroids in the laboratory.

QUIT-MS -- Collected from cotton during early August in Quitman County, MS. A control failure was associated with the collection but application and timing factors may have been involved.

1988 Studies

STN-MS -- Collected from cotton during July at the Delta Branch Experiment Station, Stoneville, MS. Control failures were not associated with the collection.

PPEA-MS -- Collected as eggs and larvae from an experimental plot of pigeon pea (*Cajanus* spp.) at the Delta Branch Experiment Station, Stoneville, MS. The collection was made during late-August.

FPT-MS -- Collected as eggs from the same site as the 1987 FPT-MS collection. The collection was made during late-August. As with 1987 FPT-MS, control failures were not associated with the collection, but agricultural consultants believed that

TABLE 1. (Continued)

efficacy of the pyrethroids had decreased slightly.

1989 Studies

DUP-RR -- Same strain as the DuPont-84R which was originally collected from the Imperial Valley of California in 1984 and had been routinely exposed to pyrethroid selection in the laboratory.

RANK-MS -- Collected from cotton in Rankin County during early-July. Less than acceptable levels of control were observed with pyrethroid and carbamate insecticides.

OKT-MS -- Collected during May from wild geranium (*Geranium* spp.) growing on road sides and ditches near Mississippi State University, Starkville, MS.

HUM-MS -- Collected from cotton in northern Humphreys County, MS in late-July. Control problems had been reported following multiple applications of pyrethroids, but the application may have been poorly timed. Densities of larvae remaining were higher than one would expect. It appeared that the oviposition period had spanned several days and the population of larvae remaining was composed of several instars.

GLA-MS -- Collected from cotton in mid-August near Glen Allen, MS. The field had received a total of 4 pyrethroid applications within a 2 week period. Larvae remaining in the field were late instars found primarily in flowers and bolls.

LEF-MS -- Collected as eggs and larvae from a large-plot study area in Leflore County, MS during early September. The field had been monitored weekly for the presence of pyrethroid resistance in adult males (see the article by Micincki et al. in this issue of Southwest. Entomol.) and high survival in the adult tests were concurrently observed.

[a]If not stated, strains were established by collecting larvae in cotton.

Insect rearing procedures were essentially the same as those described by Roush and Wolfenbarger (1985). The need to conduct assays against progeny from the first generation following colonization was emphasized throughout the study, but assays were often conducted against progeny two to three generations removed from original collection.

Topical Assays. Procedures were similar to those recommended by the Entomological Society of America (Anonymous 1970) and the same as those reported by Luttrell et al. (1987). Early third instar larvae (18±5 mg) were topically treated with 1-ul volumes of solution. Larvae were held individually in diet cups following exposure to insecticide solutions, and mortality was measured at 24 h posttreatment. Technical grade permethrin (FMC Corp., Philadelphia, PA and ICI Americas, Wilmington, DE),

fenvalerate (Shell Development, Modesto, CA and E. I. du Pont de Nemours & Co., Inc., Wilmington, DE), cypermethrin (FMC Corp. and ICI Americas), methomyl (E. I. du Pont de Nemours & Co.), and methyl parathion (Monsanto Agricultural Co., St. Louis, MO) were used to prepare treatment solutions. All dosage-mortality relationships were established with a minimum of four dosages. Each dosage was replicated four times and the number of individuals per replicate was generally greater than 18. In some cases, fewer insects were used because of limited reproduction in the laboratory strains. Dosage-mortality relationships were calculated by probit analysis using the POLO procedure (Robertson et al. 1980). Resistance ratio's (RR) were calculated by dividing the LD-50 or LD-90 in the test strain by the LD-50 or LD-90 in 1984 MSU-LAB strain. Although the MSU-LAB strain was tested several years, the 1984 MSU-LAB strain gave results closer to a known susceptible strain, 1981 TUC-SUS.

Sprayed Plant Assays. Third instar larvae from field collected strains were exposed to cotton terminal buds treated with recommended field rates of formulated insecticide. Procedures were the same as those described by Luttrell et al. (1987). Cotton terminals (ca. upper 15 cm of plant) were cut from greenhouse or field grown plants. Excised plant tissue was placed in water-pics and sprayed on a motorized spray table (56.1 L of total spray volume/ha) with selected insecticide treatments. All applications were made with formulated insecticides. After treatments had dried on plant tissue, larvae were individually caged on the terminal buds using ventilated styrofoam cups. Larvae were allowed to remain on treated terminals for 48 h before mortality data were collected. Treatments were replicated a minimum of four times and each replicate included a minimum of 20 larvae. Mortality in untreated checks was seldom greater than 5%. All data were corrected for mortality in untreated checks (Abbott 1925) prior to study by analysis of variance. In experiments with significant treatment effects, means were separated by Duncan's (1955) multiple range test.

Adult Vial Assays. Adult males captured in pheromone traps were exposed to cypermethrin treated scintillation vials following procedures developed by Plapp and Campanhola (1986) and widely adopted by other researchers (Graves et al. 1988, Luttrell et al. 1988, Simonet et al. 1988). Insects collected at numerous sites in Mississippi from 1987 to 1989 were included in the study, but intensive bi-weekly monitoring programs were established in Washington, Leflore, and Monroe Counties from May to September of each year. These counties are geograpically located in diverse cotton production areas. Washington County is located near the Mississippi River in the center of the "Delta" region. Intensive, high-input agriculture is a characteristic of the area and insecticide use is usually high. Leflore County represents another location in the "Delta" region but it is geographically located near the more diversified and less agronomically intensive "Hill" region. Monroe County is a major cotton producing area in the "Hill" region of Mississippi. Insecticides are routinely used in "Hill" cotton production, but fields are generally smaller than those in the "Delta" region and widely distributed among

large areas of natural vegetation, pasture, and other crops. Additional assays were conducted with adults captured in pheromone traps in Union, Rankin, Tallahatchie, Sunflower, Humphreys, Bolivar, and Coahoma counties.

Most assays were conducted with moths obtained from one-night trap captures. Vials treated with four concentrations of technical grade cypermethrin (5, 10, 30, and 100 ug/vial) were included in 1988 and 1989 studies. Only two concentrations (5 and 10 ug/vial) were used in 1987. Attempts were made to test a minimum of 20 moths at each dosage level on a given sample date. When low numbers of insects were captured, lower dosages were given priority over higher dosages. During the 3-year period, a total of 43,101 male moths was captured in pheromone traps and assayed. Sample sizes for years, months within years, locations, and dosages varied widely. Of the total number tested, 94.2% were associated with studies in the "Delta" region of the state. The 5.8% associated with the "Hill" region were predominatly associated with studies in Monroe County. Although studies were conducted from April to September of each year, only a small percentage of the moths were assayed in April (0.6%) and May (7.4%). June, July, August, and September assays accounted for 22.4, 24.5, 24.7, and 20.4% of the total number of moths assayed, repectively. More moths were assayed at the 5 ug/vial dose (32.4%) than at any other dose. Untreated checks accounted for 25.5% of the moths assayed. Doses of 10, 30, and 100 ug/vial included 23.7, 11.7, and 6.7% of moths assayed, respectively. Studies conducted in 1988 included 44.4% of the total moths studied. Studies in 1987 and 1989 accounted for 24.5 and 31.2%, respectively, of the total number of moths.

Mortality observations were made 24 h posttreatment as described by Luttrell et al. (1988). Data were corrected for mortality in the check (vials treated with acetone alone) treatments and converted to percent survival. Trends in percent survival were routinely monitored and seasonal and geographic shifts in survival rates were reviewed annually (Luttrell et al. 1988, Kitten et al. 1989).

Trends in Small Plot Efficacy Tests. Insecticide efficacy is annually measured in small-plot field studies throughout the cotton producing areas of the United States. In Mississippi, these studies are usually conducted at Mississippi State University and the Delta Branch Experiment Station. Insect control recommendations in Mississippi are largely based on the results of these studies and similar studies conducted in other states. Data reported in the Entomological Society of America's Insecticide and Acaricide Tests were summarized from 1978 to 1987 to study trends in the efficacy of pyrethroids for control of bollworm (*Helicoverpa zea* (Boddie)) and tobacco budworm (*H. virescens*) on cotton. Reports from Alabama, Georgia, Louisiana, Mississippi, and Texas that included information on the efficacy of fenvalerate (0.112-0.123 kg ai/ha) or cypermethrin (0.056-0.067 kg ai/ha) were summarized. Data were recorded as efficacy ratios (e.g. square damage with fenvalerate/square damage in untreated check) for reported information on larval densities, square damage, and yield. A few data sets did not include information on square damage or

yield. For fenvalerate comparisons, the number of experiments summarized with larval density, square damage, and yield data were 28, 28, and 25, respectively. Cypermethrin data on larval density, square damage, and yield included the results of 31, 30, and 30 experiments, respectively.

Data were converted to percent control of larvae, percent reduction in square damage, and percent increase in yield over untreated checks and plotted across time. Trends in the data were studied with the CORR procedure of PC-SAS (SAS Institute, Inc., 1985).

RESULTS AND DISCUSSION

Topical Assays. Toxicity of permethrin, cypermethrin, fenvalerate, methomyl, sulprofos, and methyl parathion to the various larval strains exposed to topical assays is summarized in Table 2. During the 9-year period, resistance ratio's (RR's) based on comparisons to the LD-50 of the 1984 MSU-LAB strain ranged from 0.3 to 9.3 for permethrin, from 0.4 to 11.1 for cypermethrin, from 0.2 to 5.6 for fenvalerate, and from 0.3 to 11.7 for methomyl. Ranges of RR's at the LD-90 were 0.9 to 11.9 for permethrin, 1.0 to 25.6 for cypermethrin, 0.4 to 10.9 for fenvalerate, and 0.1 to 74.6 for methomyl.

Six strains (RR's) (1982 IMP-CAL (3.8), 1982 STN-MS (2.5), 1986 BEL-MS (3.0), 1987 INV-MS (7.5), 1987 COAH-MS (2.5), 1987 UVL-TX (9.3)) exhibited LD-50 values for permethrin significantly higher than that of the 1984 MSU-LAB strain. At the LD-90, five strains (1982 IMP-CAL (4.6), 1986 BEL-MS (7.8), 1987 INV-MS (11.6), 1987 FPT-MS (9.3), 1987 UVL-TX (11.0)) had values significantly higher than the 1984 MSU-LAB strain. With cypermethrin studies, fifteen strains (RR at LD-50, RR at LD-90) (1986 INV-MS (8.9, 13.3), 1986 BEL-MS (11.1, 20.8), 1986 HRN-TX (10.2, 16.9), 1987 COAH-MS (0.8, 3.2), 1987 RANK-MS (1.5, 2.9), 1987 INV-MS (2.8, 6.1), 1987 PAR-RR (4.2, 25.6), 1988 STN-MS (4.2, 25.6), 1988 FPT-MS (3.9, 7.9), 1988 PPEA-MS (2.1, 5.6), 1989 DUP-RR (6.3, 21.1), 1989 GLA-MS (1.8, 3.6), 1989 RANK-MS (1.4, 2.8), 1989 GLA-MS (1.8, 3.6), 1989 LEF-MS (4.9, 12.5)) expressed levels of resistance at either the LD-50 or the LD-90. Only four strains (RR's) (1986 HRN-TX (5.6), 1987 INV-MS (3.1), 1987 QUIT-MS (3.4), 1987 RANK-MS (2.4)) had LD-50's for fenvalerate higher than that for 1984 MSU-LAB, but ten strains (1982 LEF-MS (2.6), 1982 PSF-MS (2.7), 1986 GLA-MS (4.8), 1986 INV-MS (4.9), 1986 BEL-MS (10.9), 1986 HRN-TX (9.0), 1987 INV-MS (6.9), 1987 COAH-MS (2.7), 1987 QUIT-MS (4.6), 1987 RANK-MS (4.4)) had higher LD-90's.

Two strains expressed some resistance to methomyl. Resistance ratio's at the LD-50 and LD-90 for the 1984 STN-MS and 1986 BEL-MS strains were 6.8 and 8.0, and 11.7 and 74.6, respectively. These dosages are similar to those previously reported for insects considered to be resistant to methomyl in Mississippi by Furr (1978) and at other locations in the United States by Wolfenbarger et al. (1987). The LD-50's were 12 to 21 fold greater than susceptible colonies used in comparisons by Furr (1978).

TABLE 2. Toxicity of Permethrin, Fenvalerate, Cypermethrin, Methomyl, and Methyl Parathion to Strains of Tobacco Budworm in Topical Assays in Mississippi: 1981-1989.[a]

YEAR	COLONY	N[b]	SLOPE(SE)	LD_{50} (95%CI)[c]	RR[d]	LD_{90} (95%CI)	RR
				permethrin			
1981	TUC-SUS	630	2.11 (0.13)	0.050 (0.034-0.071)	1.0	0.201 (0.129-0.424)	1.2
1981	MSU-LAB	800	1.32 (0.10)	0.015 (0.009-0.023)	0.3	0.145 (0.086-0.334)	0.9
1982	IMP-CAL	500	2.18 (0.17)	0.195 (0.170-0.233)	3.8	0.756 (0.612-0.994)	4.6
1982	GRWD-MS	500	2.58 (0.20)	0.086 (0.064-0.118)	1.6	0.270 (0.179-0.576)	1.6
1982	STNV-MS	600	2.55 (0.17)	0.131 (0.087-0.202)	2.5	0.417 (0.255-1.150)	2.5
1982	PSF-MS	500	2.70 (0.21)	0.086 (0.054-0.144)	1.6	0.256 (0.150-1.100)	1.6
1983	MSU-LAB	800	1.45 (0.11)	0.056 (0.012-0.131)	1.1	0.427 (0.168-29.10)	2.6
1984	STNV-MS	576	2.45 (0.17)	0.062 (0.051-0.075)	1.2	0.207 (0.160-0.294)	1.2
1984	MSU-LAB	576	2.56 (0.18)	0.052 (0.034-0.078)	---	0.165 (0.104-0.387)	---
1986	BEL-MS	360	1.40 (0.15)	0.156 (0.116-0.209)	3.0	1.283 (0.831-2.370)	7.8
1987	INV-MS	400	1.87 (0.17)	0.390 (0.320-0.480)	7.5	1.912 (1.44-2.7700)	11.6
1987	COAH-MS	396	2.07 (0.28)	0.130 (0.100-0.160)	2.5	0.550 (0.420-0.840)	3.3
1987	RANK-MS	320	2.87 (0.30)	0.082 (0.048-0.144)	1.6	0.221 (0.136-1.030)	1.3
1987	FRT-MS	311	1.13 (0.17)	0.114 (0.069-0.174)	2.2	1.534 (0.974-3.110)	9.3
1987	UVL-TX	286	2.20 (0.23)	0.482 (0.155-0.961)	9.3	1.822 (0.912-13.50)	1.0
				cypermethrin			
1984	MSU-LAB	648	2.86 (0.21)	0.029 (0.020-0.037)	---	0.076 (0.053-0.140)	---
1984	STNV-MS	648	2.13 (0.15)	0.030 (0.023-0.039)	1.0	0.121 (0.087-0.193)	1.6
1986	INV-MS	360	2.16 (0.25)	0.258 (0.123-0.643)	8.9	1.011 (0.465-16.20)	13.3
1986	BEL-MS	522	1.86 (0.20)	0.323 (0.256-0.413)	11.1	1.583 (1.095-2.700)	20.8
1986	HRN-TX	189	2.01 (0.28)	0.297 (0.119-0.546)	10.2	1.290 (0.682-5.057)	16.9
1986	MSU-LAB	324	1.77 (0.20)	0.014 (0.010-0.018)	0.5	0.074 (0.052-0.120)	1.0
1987	INV-MS	139	1.69 (0.28)	0.080 (0.051-0.123)	2.8	0.464 (0.273-1.200)	6.1
1987	PAR-RR	261	1.07 (0.17)	0.122 (0.082-0.214)	4.2	1.942 (0.801-9.910)	25.6
1987	COAH-MS	510	1.29 (0.14)	0.022 (0.012-0.054)	0.8	0.244 (0.902-6.440)	3.2

TABLE 2. (Continued)

1987	RANK-MS	300	1.79 (0.19)	0.043 (0.032-0.052)	1.5	0.221 (0.151-0.354)	2.9
1988	STNV-MS	400	1.40 (0.17)	0.122 (0.103-0.173)	4.2	1.022 (0.601-2.330)	13.4
1988	PPEA-MS	400	1.57 (0.15)	0.062 (0.035-0.203)	2.1	0.424 (0.153-15.90)	5.6
1988	FPT-MS	400	1.74 (0.18)	0.113 (0.091-0.142)	3.9	0.603 (0.412-1.050)	7.9
1989	DUP-RR	300	1.34 (0.20)	0.183 (0.142-0.253)	6.3	1.602 (0.843-5.000)	21.1
1989	RANK-MS	400	1.63 (0.14)	0.042 (0.034-0.042)	1.4	0.212 (0.163-0.313)	2.8
1989	OKT-MS	400	1.68 (0.19)	0.013 (0.012-0.204)	0.4	0.086 (0.067-0.124)	1.1
1989	HUM-MS	400	1.69 (0.14)	0.034 (0.022-0.067)	1.2	0.196 (0.102-0.704)	2.6
1989	GLA-MS	400	1.78 (0.14)	0.052 (0.042-0.062)	1.8	0.273 (0.213-0.403)	3.6
1989	LEF-MS	400	1.53 (0.15)	0.143 (0.117-0.186)	4.9	0.950 (0.612-1.760)	12.5
				fenvalerate			
1981	MSU-LAB	700	1.58 (0.10)	0.007 (0.005-0.013)	0.2	0.048 (0.032-0.085)	0.4
1981	ARS-LAB	800	1.73 (0.10)	0.027 (0.014-0.045)	0.8	0.146 (0.078-0.510)	1.2
1981	STNV-MS	500	1.10 (0.09)	0.010 (0.007-0.013)	0.3	0.144 (0.105-0.216)	1.2
1982	LEF-MS	315	1.44 (0.09)	0.040 (0.032-0.048)	1.1	0.310 (0.227-0.463)	2.6
1982	PSF-MS	500	1.66 (0.15)	0.054 (0.034-0.025)	1.5	0.318 (0.204-0.691)	2.7
1984	MSU-LAB	576	2.49 (0.19)	0.036 (0.029-0.045)	---	0.118 (0.089-0.178)	---
1984	STNV-MS	576	2.38 (0.17)	0.052 (0.038-0.069)	1.4	0.179 (0.124-0.311)	1.5
1986	CG-AZ	219	1.82 (0.24)	0.023 (0.017-0.030)	0.6	0.115 (0.077-0.212)	1.0
1986	GLA-MS	210	1.04 (0.19)	0.033 (0.019-0.052)	0.9	0.573 (0.280-2.200)	4.8
1986	INV-MS	360	1.68 (0.22)	0.099 (0.044-0.172)	2.8	0.574 (0.300-2.630)	4.9
1986	BEL-MS	432	1.19 (0.16)	0.107 (0.041-0.201)	3.0	1.288 (0.587-7.240)	10.9
1986	HRN-TX	268	1.77 (0.28)	0.200 (0.113-0.290)	5.6	1.062 (0.650-3.020)	9.0
1987	INV-MS	333	1.46 (0.16)	0.113 (0.091-0.142)	3.1	0.822 (0.553-1.490)	6.9
1987	COAH-MS	320	1.66 (0.24)	0.054 (0.040-0.073)	1.5	0.314 (0.213-0.564)	2.7
1987	QUIT-MS	397	1.98 (0.19)	0.123 (0.052-0.222)	3.4	0.542 (0.292-2.640)	4.6
1987	RANK-MS	300	1.62 (0.19)	0.086 (0.063-0.113)	2.4	0.522 (0.323-0.965)	4.4
1987	UVL-TX	305	1.99 (0.28)	0.042 (0.031-0.055)	1.2	0.176 (0.131-0.276)	1.5

TABLE 2. (Continued)

				methomyl				
1981	MSU-LAB	500	0.08 (0.14)	0.310 (0.224-0.450)	0.3	12.17	(4.42-87.820)	1.2
1981	ARS-LAB	600	1.50 (0.12)	0.569 (0.385-0.918)	0.6	4.076	(2.05-16.100)	0.4
1982	HUM-MS	500	2.04 (0.17)	0.307 (0.175-0.463)	0.3	1.303	(0.782-4.160)	0.1
1982	STNV-MS	500	1.48 (0.15)	0.882 (0.728-1.110)	0.9	6.45	(4.16-12.300)	0.6
1984	MSU-LAB	720	1.23 (0.09)	0.942 (0.735-1.180)	---	10.40	(7.69-15.120)	---
1984	STNV-MS	720	1.15 (0.08)	6.415 (4.520-9.120)	6.8	82.99	(47.7-181.60)	8.0
1986	BEL-MS	372	0.69 (0.11)	11.054 (3.980-28.60)	11.7	775.50	(182 - 21944)	74.6
1987	COAH-MS	174	0.79 (0.30)	1.004 (0.103-2.880)	1.1	42.40	(8.7- 106034)	4.1
				sulprofos				
1981	MSU-LAB	600	1.69 (0.13)	0.534 (0.379-0.731)	---	3.071	(1.950 -6.47)	---
1981	STNV-MS	500	1.43 (0.15)	1.465 (1.020-2.160)	2.7	11.489	(5.960-40.80)	3.7
1981	LEF-MS	500	2.72 (0.21)	0.594 (0.412-0.811)	1.1	1.762	(1.211 -3.60)	0.6
1982	STNV-MS	800	1.50 (0.11)	1.112 (0.787-1.460)	2.1	7.955	(5.70-12.600)	2.6
				methyl parathion				
1984	MSU-LAB	648	1.68 (0.11)	2.889 (2.29-3.500)	---	16.74	(11.96-26.47)	---
1984	STNV-MS	648	1.33 (0.06)	1.752 (1.065-2.78)	0.6	16.010	(8.51-46.300)	1.0

[a]Early third instars were used in all studies.
[b]Number of larvae tested excluding controls.
[c]Dosages are expressed as micrograms of insecticide per larva.
[d]Resistant ratio's calculated as LD-50 or LD-90 in test strain/LD-50 or LD-90 in MSU-LAB strain.

Field strains collected in 1981 and 1982 appeared to be as susceptible to sulprofos as the laboratory strain. One strain (1984 STN-MS) exposed to methyl parathion was no more resistant than the laboratory strain (1984 MSU-LAB), but both had LD-50's equal to those reported for field strains by Pieters and Boyette (1977) which were considered to be resistant. The LD-50's observed for methyl parathion with the 1984 MSU-LAB and the 1984 STN-MS strains and the values reported by Pieters and Boyette (1977) were 20 to 40 fold higher than those reported by Harris (1972) for insects assayed in 1970.

In general, field strains expressed higher levels of resistance to pyrethroids after 1985 than before 1985. It is important to note that some variation in dosage-mortality responses was present before 1985. Higher LD-50 and LD-90 values associated with the 1982 IMP-CAL and 1982 STN-MS strains corroborate the early concern for resistance reported by Martinez-Carrillo and Reynolds (1983). Field strains collected in Mississippi after 1985 expressed some levels of pyrethroid resistance each year.

Sprayed Plant Assays Mortality of third instar larvae placed on cotton terminals treated with fenvalerate (0.112 kg ai/ha) or cypermethrin (0.056 kg ai/ha) was less for all field strains collected in the "Delta" region of Mississippi than for the MSU-LAB strain (Table 3). Mortality of third instar larvae from the CG-AZ strain, considered a pyrethroid-susceptible strain, exposed to fenvalerate was the same as that observed for the MSU-LAB strain (88%). Mortality of field strains from Mississippi ranged from a low of 16% (1987 INV-MS) to a high of 91% (1987 RANK-MS) for fenvalerate, and from a low of 17% (1987 INV-MS) to a high of 86% (1987 RANK-MS) for cypermethrin. For methomyl (0.45 kg ai/ha), the range in mortality for larval strains collected in Mississippi was 55% (1986 BEL-MS) to 91% (1987 COAH-MS). Only 20 and 24% of the third instar larvae from the 1986 HRN-TX strain, a strain with known levels of pyrethroid resistance, were killed by fenvalerate and cypermethrin, respectively.

Data from sprayed plant assays were evaluated for correlations with results of topical assays (Table 2). No significant correlations were noted. However, collectively the assays of third instars exposed to cotton terminals treated with recommended field rates of pyrethroids indicates reduced levels of control as compared to known susceptible strains.

Adult Vial Assays. Average monthly survival of moths assayed in the "Delta" region at 5, 10, 30, and 100 ug/vial is summarized across and within years in Fig.1. The maximum weekly average survival (i.e. highest weekly average value observed within each month) is also shown because averaging survival rates across time and multiple test sites tends to minimize high survival rates that may occur for short periods or at only a few locations. In general, trends in average monthly survival were the same as those for maximum weekly averages with each month. Maximum weekly averages result in higher estimates of survival rates. If data were plotted for maximum daily averages, survival rates would be higher than

TABLE 3. Mortality of Third Instar Tobacco Budworm Larvae Exposed to Cotton Terminal Buds Treated With Pyrethroid and Carbamate Insecticides.

Colony (Year)	Mean % Corrected Mortality (std. dev.)[a,b] Fenvalerate	Cypermethrin	Methomyl
MSU-LAB (1982-1986)	88(3)	91(6)	88(12)
GLA-MS (1986)	49(5)	---	---
INV-MS (1986)	57(10)	40(13)	---
BEL-MS (1986)	46(6)	46(12)	5(5)
CG-AZ (1986)	88(4)	---	---
HRN-TX (1986)	20(2)	24(3)	---
INV-MS (1987)	16(14)	17(15)	61(12)
COAH-MS (1987)	59(9)	60(17)	91(14)
FPT-MS (1987)	50(21)	41(16)	76(11)
STNV-MS (1987)	32(14)	35(20)	65(17)
RANK-MS (1987)	91(5)	6(5)	---
UVA-TX (1987)	13(11)	9(8)	30(22)
FPT-MS (1988)	---	61(10)	---
STNV-MS (1988)	---	76(3)	---
PPEA-MS (1988)	---	60(12)	90(4)

[a]Third instars were used in all tests. Mortality was measured at 48 h post-exposure to treated terminal buds.
[b]Application rates were 0.112, 0.056, and 0.504 kg ai/ha for fenvalerate, cypermethrin, and methomyl, respectively.

the maximum weekly averages. Since error rates associated with both over- and under-estimating the frequency of resistant genotypes may be important, data were summarized and presented as two different sets of information in Fig.1.

In general, the frequency of pyrethroid resistance in field populations of *H. virescens* increased throughout the growing season each year (Fig. 1). Some decline in survival rates was observed in September. Measurable survival at higher doses (30 and 100 ug) in 1988 and 1989 suggests that field populations are becoming more difficult to control with pyrethroids. There has been some concern about the most appropriate doses to use in adult vial assays. Data used in Fig. 1 were studied for relationships between the different doses. Average monthly survival values were used in these correlations. These averages are based on a large data set (total of 40,601 moths for all doses). Results obtained at 5 ug/vial were significantly correlated with those for 10 ($r= 0.917$, $P=0.001$), 30 ($r = 0.859$, $P= 0.006$), and 100 ($r=0.853$, $P=0.007$) ug/vial doses. Results obtained at 10 ug/vial were significantly correlated with those obtained in assays with 30 ($r=0.940$, $P=0.001$) and 100 ($r=0.701$, $P=0.052$) ug/vial. Mortality of moths in assays conducted with vials treated at the 30 ug/vial dose were correlated ($r=0.675$, $P=0.066$) with those conducted with 100 ug/vial. Lower correlation coefficients associated with higher doses are probably the result of smaller sample sizes with higher doses.

Figure 2 summarizes trends in survival rates at the 10 ug/vial dose for the diverse cotton growing regions of Mississippi. In 1987, survival rates (and resistance levels)

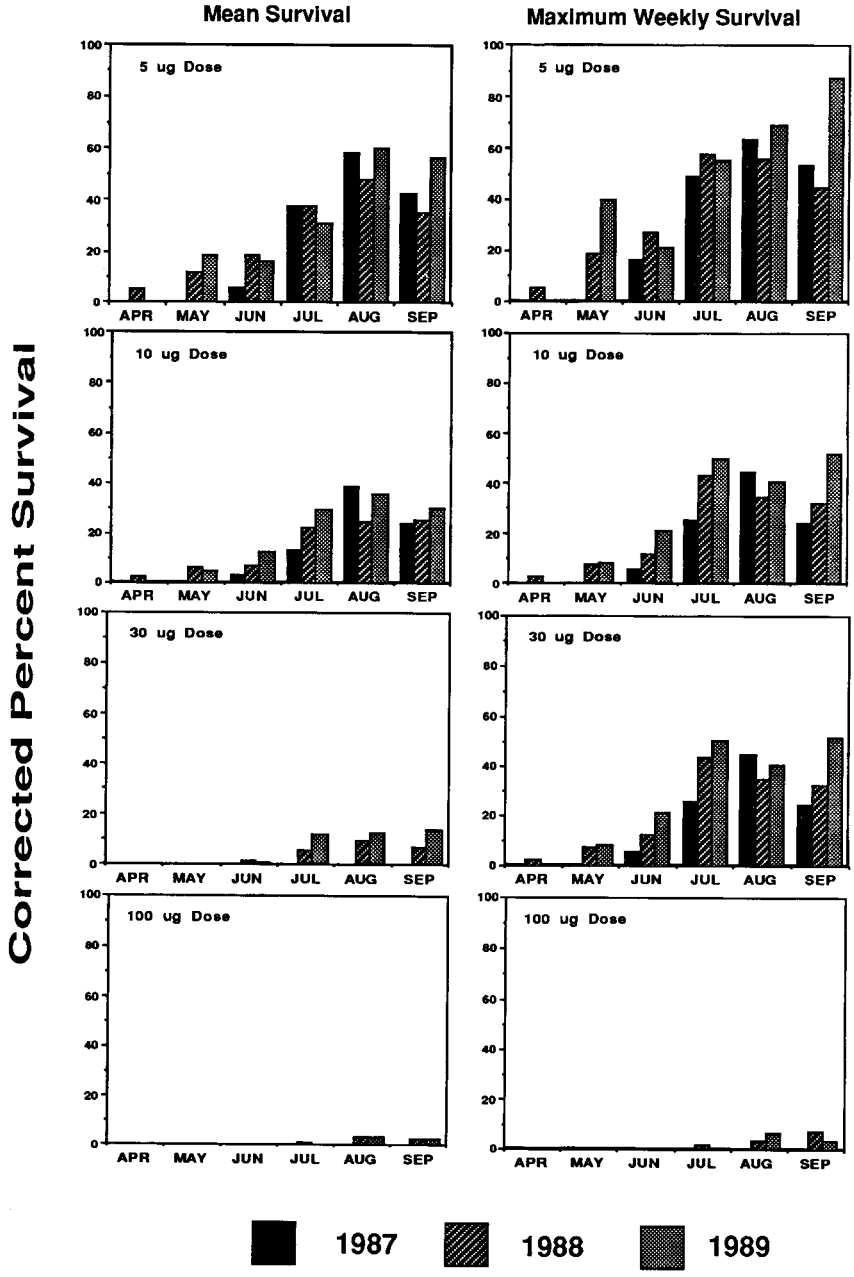

FIG. 1. Average monthly and maximum weekly survival of tobacco budworm moths assayed in vials treated with 5, 10, 30, and 100 µg/vial doses of cypermethrin.

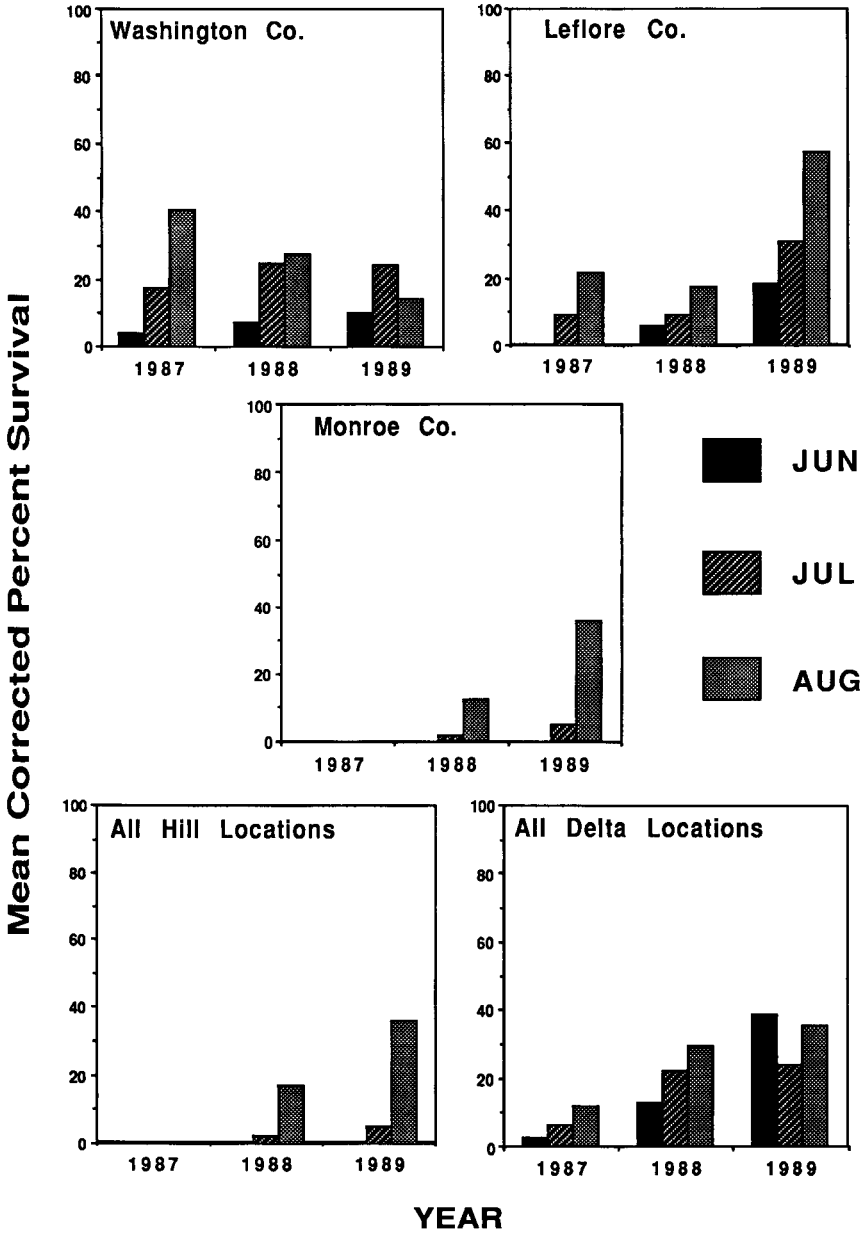

FIG. 2. Average survival of tobacco budworm moths treated at the 10 µg/vial dose during June, July, and August 1987, 1988, and 1989.

were generally higher in Washington County than in Leflore or Monroe Counties. Differences in the 1987 data between Delta and Hill Counties suggests that *H. virescens* populations are different for the different areas. For most areas and as an average across Delta and Hill regions, the relative frequency of pyrethroid resistant individuals in field populations of *H. virescens* is increasing.

The annual decline in survival rates observed during August in Washington County in 1989 was surprising and somewhat unexpected. Reasons for this decline are unknown, but crop maturity and sample error may be involved. In 1988, the cotton crop matured early and the need for insecticide applications in August was reduced. Differences between Washington and Leflore Counties are difficult to explain, but the number of individuals assayed in August of 1989 in Washington County was low in comparison to previous years. Moth captures in pheromone traps reached peak densities in Washington County ca. 5-10 days earlier than those for the Leflore and Monroe County sample sites. Large numbers of moths were captured and high levels of survival were reported from Washington County in the last week of July 1989. Corresponding large samples of moths in Leflore and Monroe Counties were recorded for the first and second weeks of August. Although insecticide use patterns were not accurately measured, there appeared to be a significant shift to organophosphorous and carbamate insecticides alone and in mixtures with pyrethroids in late-July and August 1989. This may have affected selection pressure for pyrethroid resistance in August.

High survival measured in Monroe County and other Hill sites in 1989 indicates that pyrethroid resistance is spreading geographically. Survival at 30 and 100 ug/vial doses was rare prior to 1989 in Delta regions. Survival at the 10 ug/vial dose was relatively rare in the Hill region until 1989.

Trends in Small Plot Efficacy Tests. Efficacy of cypermethrin and fenvalerate at recommended rates for control of bollworm - tobacco budworm in small cotton plots is summarized in Fig.3. There were trends for reductions in percent control of larvae and percent reduction in damaged squares over the 10 year period. Percent control of larvae was negatively correlated with time for both fenvalerate ($r = -0.424$, $P = 0.034$) and cypermethrin ($r= -0.447$, $P = 0.013$). Reductions in square damage were also significantly reduced over the 10 year period for fenvalerate ($r = -0.469$, $P = 0.018$) and cypermethrin ($r = -0.529$, $P = 0.003$). Larval density and square damage data were significantly related ($r = 0.705$, $P = 0.0001$ for fenvalerate; $r = 0.380$, $P = 0.018$ for cypermethrin). Yield was not significantly correlated with either variable and significant trends in yield data were not identified. There was a general trend for increased variability in percent control of larvae and in measured yield increases over time (as indicated by standard error bars on graphs in Fig.3). Reasons for these trends are unknown. Based on results of the monitoring studies, the development of resistant populations seems likely. If pyrethroids were reducing overall population densities of *H.*

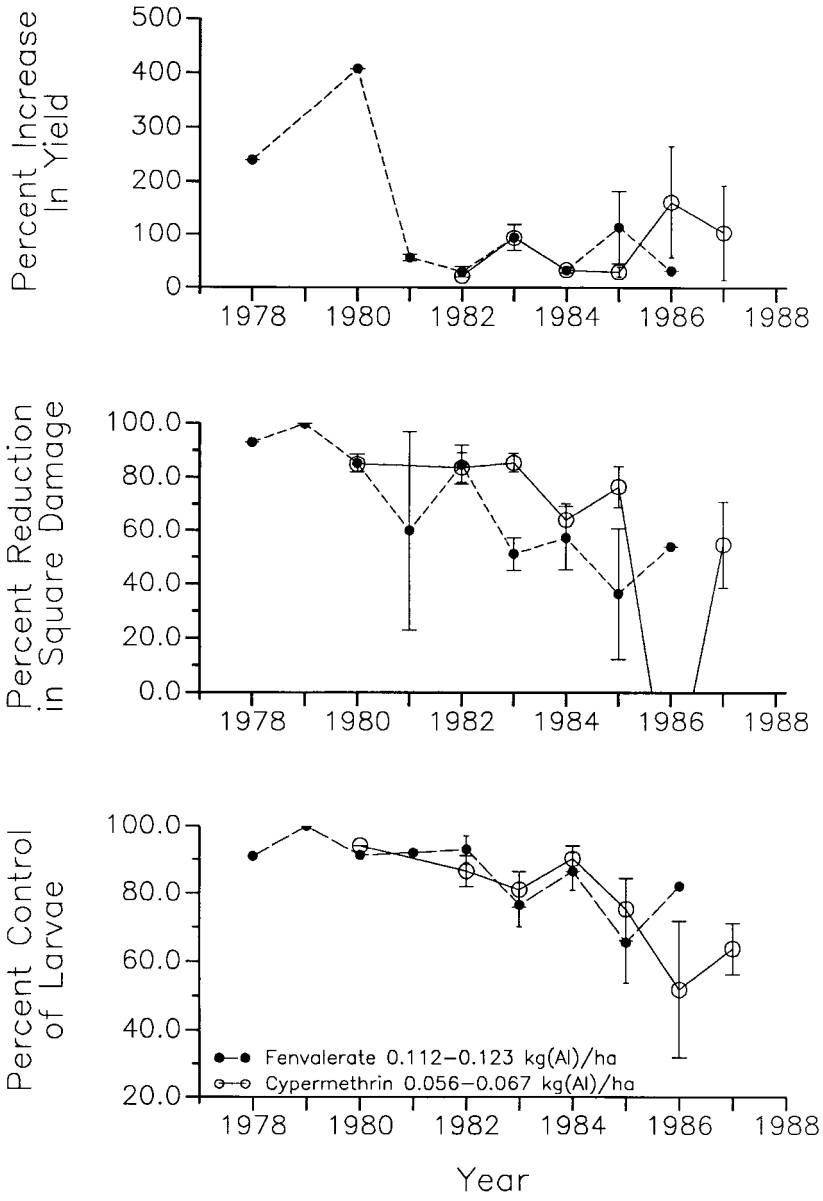

FIG. 3. Trends in efficacy of two pyrethroids for control of bollworm-tobacco budworm in small plot studies conducted in Alabama, Georgia, Louisiana, Mississippi and Texas during 1978-1987.

virescens, differences in yield effect between treated and untreated plots would be reduced.

Results obtained with all four monitoring techniques indicate that pyrethroid resistance has been present in field populations of *H. virescens* in Mississippi since 1986. Trends for increased levels of resistance were difficult to quantify, but the increased survival of moths exposed to cypermethrin in adult assays from 1987 to 1989 indicates that resistance is becoming more of a problem. Trends for reduced control of larvae with pyrethroids in small-plot tests suggests that the resistance problem may be critical for production agriculture.

Although results of sprayed plant assays were not significantly correlated with those of topical assays, earlier studies (Roush and Luttrell 1989) with greater control of experimental error indicated significant relationships between the different monitoring techniques. In the studies reported here, there was some variation in the time required to complete different assays. For example, spray table assays were usually conducted on insects from the first generation removed from field collection. Topical assays often required rearing insects for another two or three generations.

Strong correlations between results obtained in adult assays at 5, 10, 30, and 100 ug/vial doses suggest that differences obtained with different doses are unimportant as long as the data are properly interpreted. A decision to use a low (5 ug) or high (100 ug) dose in monitoring programs capable of only including one dose would be influenced by one's relative concern for Type I and Type II error rates. Including multiple doses in monitoring programs would be preferred given that sample size is large enough (Roush and Miller 1986).

These data indicate that a diversity of monitoring techniques can be used to follow development of insecticide resistance. Methods which can provide results in a timely manner for samples not too far removed from field populations probably have more value in within-season decision making. However, techniques such as standard topical assays and small-plot field tests have traditionally been methods used to document resistance problems. McCutchen et al. (1989) described a treated vial procedure for assaying larvae which may have utility in efficient within-season decision making.

Comparisons of results to previously reported data is an important consideration in studying insecticide resistance. Because of similarity of results obtained across different monitoring techniques in these studies and different benefits associated with different techniques, researchers should consider including multiple techniques in the design of monitoring programs. This obviously increases the costs of such research. As shown by the scope of research reported here, resistance monitoring often receives high priority after a problem is recognized. More timely recognition of resistance problems would greatly improve effective implementation of insecticide resistance management in cotton production.

ACKNOWLEDGEMENT

These studies required dedicated assistance and cooperation from many individuals. B. Head, R. Reid, M. Williams, B. Layton, and other members of the Mississippi Cooperative Extension Service assisted us throughout the course of these studies and can be credited with the success of the project. F. W. Plapp, J. B. Graves, D. Clower, J. Mallet, M. Laster, R. Moody, S. Riley, R. Furr, D. Varner, C. Wilbourn, and J. North are acknowledged for their assistance in obtaining insects and conducting assays. B. Head, H. Pitre, and J. Mallet reviewed early drafts of the manuscripts. This project was largely supported by a special grant from the Director of the Mississippi Agricultural and Forestry Experiment Station. Financial support from and cooperation with PEG-US, FMC Corp., E. I. du Pont de Nemours & Co., Shell Development, and ICI Americas were essential components of the project. This is manuscript J-7633 of the journal series of the Mississippi Agricultural and Forestry Experiment Station.

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