MANAGEMENT OF PYRETHROID RESISTANCE IN THE TOBACCO BUDWORM[1]: ROLE OF INSECTICIDE PERSISTENCE AND INSECTICIDE MIXTURES

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ABSTRACT

To develop a pesticide mixture strategy for managing resistance in *Heliothis virescens (F.)*, several insecticides (chlordimeform, methomyl, sulprofos, permethrin, fenvalerate, cypermethrin, EPN-methyl parathion, and chlorpyrifos) were tested alone or in two- or three-way combinations at full and reduced rates in laboratory and small plot field experiments. While several two- and three-way mixtures (especially chlordimeform-methomyl-permethrin and chlordimeform in combination with several pyrethroids) were as effective at reduced rates as full rates of pyrethroids alone, the residual activity of pyrethroids was often longer than that for the other compounds tested. However, residual activity in the terminal bud region of cotton is reduced as a result of plant growth. This minimizes the effects of differences in pesticide decay rates for insecticides deposited in this region of the plant, the target area for most insecticide applications directed against *H. virescens*, and supports the role of mixtures in resistance management.

Although differences in residual activity were observed for the different insecticides studied, some of the mixtures still showed good potential for control of pyrethroid resistant populations of the tobacco budworm.

INTRODUCTION

The pyrethroids are extremely effective insecticides. In a review article discussing the future of pyrethroids for insect control, Elliott et al. (1978) advocated use patterns which would guard against resistance development and increase the useful lifetime of the compounds. The tremendous value of pyrethroids, particularly in regard to control of bollworm (*Helicoverpa zea* (Boddie)) and tobacco budworm (*Heliothis virescens* (F.)) was immediately recognized following introduction of these compounds into production agriculture.

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Resistance monitoring efforts (see Sparks 1981 and Staetz 1985 for summaries) were implemented at several sites in the United States. The potential impact of pyrethroid resistance in tobacco budworm on cotton production in Mississippi encouraged researchers in the early 1980's to implement studies on methods of delaying the development of resistance and preserving the efficacy of pyrethroid insecticides for cotton production (Roush et al. 1983).

One approach to delaying the evolution of resistance is the use of insecticide mixtures. Although controversy exists on the theoretical degree of benefits from using mixtures versus using other management options (Knipling and Klassen 1984, Curtis 1985, Mani 1985, Comins 1986, Roush 1989), the practical advantages of mixtures warranted consideration. Cotton growers are often faced with insect complexes that respond differently to different classes of insecticides. Mixtures could be implemented readily with little change in traditional insect management techniques.

Specific information was needed on the persistence and efficacy of reduced and full use rates of insecticides that could be used in mixtures. The mixture approach would be more readily adopted because of its cost effectiveness if mixtures of insecticides could be identified which were efficacious when reduced rates of each component insecticide was included in the mixture. Described here are: (1) field studies conducted to measure the relative efficacy of reduced rates of pyrethroids applied alone and in combination with reduced rates of insecticides with other modes of action; and (2) laboratory studies designed to measure the effects of various insecticides applied alone and in combination with pyrethroids on mortality of pyrethroid resistant and susceptible strains of *H. virescens*.

MATERIALS AND METHODS

Both laboratory assays and small-plot field experiments were conducted in the early 1980's prior to the documented presence of pyrethroid resistance in natural populations of *H. virescens* in Mississippi (Luttrell et al. 1987). The pyrethroid-susceptible strain of *H. virescens* (Luttrell et al. 1987) used in laboratory assays originated from insects collected on cotton in Mississippi during the late 1970's and early 1980's. The pyrethroid-resistant strain (Payne et al. 1988) was the US-82 strain maintained by ICI Americas, which had been exposed to pyrethroid selection in the laboratory. Insect rearing methods were essentially the same as those described by Roush and Wolfenbarger (1985).

Field Plot Studies. Several small-plot field studies were conducted at the Plant Science Research Farm, Mississippi State and at the Delta Branch Experiment Station, Stoneville. Depending on the study, plot size varied from 4 to 8 rows wide and from 15 to 30 m long. Row width varied from 91 to 102 cm. All treatments were replicated a minimum of four times in a randomized complete block design. Applications were made with ground equipment equipped with compressed-air spray systems. The spray volume, nozzles, and ground speed varied with different experiments, but all treatments were applied at total spray volumes between 45 and

90 L/ha with two TX-4 or two TX-6 nozzles per row. Application pressure ranged from 200 to 280 kPa. Applications were made at weekly intervals during detectable periods of bollworm - tobacco budworm infestation based on weekly scouting of field plots. The number of applications varied from 5 to 12 depending upon the goals of the specific study, the age of the crop and the length of the infestation period. To control pests other than bollworm and tobacco budworm, all tests were routinely over-sprayed with recommended insecticides (dimethoate (0.224 kg ai/ha) for thrips and plant bugs, and azinphosmethyl (0.28 kg ai/ha) for boll weevils). In general, the natural population densities of bollworm and tobacco budworm were low during the studies. Plots were monitored on a weekly basis following treatment by making 10 whole plant observations in each plot. Yield was measured by mechanically harvesting the center two rows of each plot.

The initial field experiment was designed to measure the efficacy of reduced rates of a formamidine (chloridmeform), a carbamate (methomyl), an organophosphorous (sulprofos), and a pyrethroid (permethrin) insecticide applied alone and in combination for control of bollworm and tobacco budworm in small plots of cotton. Each insecticide was evaluated at 0.25X, 0.5X, and 1X the label recommended field rates. Ovicide rates of chlordimeform (0.14 kg ai/ha) and methomyl (0.14 kg ai/ha) were considered as 1X field rates. The 1X rates of sulprofos and permethrin were 0.84 and 0.112 kg ai/ha, respectively. Formulations of the insecticides used were Fundal 4E (NOR-AM Chemical Co., Wilmington, DE), Lannate 1.8L (E. I. du Pont de Nemours & Co., Inc., Wilmington, DE), Bolstar 6E (MoBay Corp., Kansas City, MO), and Pounce 3.2E (FMC Corp., Philadelphia, PA). Reduced rates of the insecticides were evaluated in two- and three-component mixtures. Two-component mixtures included all possible paired combinations of the four insecticides applied at 0.5X rates. Three-component mixtures included all possible three-way combinations of the four insecticides applied at 0.25X rates. The experiment was repeated four times (Mississippi State - 1982, Mississippi State - 1983, Mississippi State - 1984, Stoneville - 1984). Data were combined for the four experiments and studied by analysis of variance. Means were separated by Duncan's (1955) multiple range test.

Several additional experiments were conducted from 1981 to 1984 to evaluate the efficacy of reduced rates of pyrethroids applied alone or in combination with ovicide rates of chlordimeform (0.14 kg ai/ha) and methomyl (0.14 kg ai/ha). The pyrethroids tested included permethrin (Pounce 3.2 E or Ambush 2 E), fenvalerate (Pydrin 2.4 E), and cypermethrin (Ammo 2.4 E or Cymbush 3 E). Field plot procedures and data analysis for individual experiments were the same as those described for the initial study.

Laboratory Assays. Eggs, third instar larvae, and adults from the pyrethroid susceptible and resistant colonies were exposed to cotton terminals treated with formulated insecticides to estimate mortality rates of tobacco budworm exposed to insecticide mixtures. Insecticides tested were fenvalerate (0.056 and 0.112 kg ai/ha), chlordimeform (0.14

kg ai/ha), methomyl (0.14 kg ai/ha), chlorpyrifos (Lorsban 4E, Dow Chemical Comp.) (0.56 kg ai/ha), fenvalerate (0.056 kg ai/ha) + chlordimeform (0.14 kg ai/ha), fenvalerate (0.056 kg ai/ha) + methomyl (0.14 kg ai/ha), and fenvalerate (0.056 kg ai/ha) + chlorpyrifos (0.56 kg ai/ha).

Gravid females (ca. 10 per replication) from susceptible and resistant strains were caged individually on separate cotton plants in the greenhouse for egg assays. Leaves with eggs attached were collected from the caged plants the following morning and pinned to a plywood board. The board holding the pinned leaves was then sprayed on a motorized spray table calibrated to deliver 88.9 L/ha total volume with a single TX-4 nozzle. Nozzle pressure was 207 kPa. Immediately following treatment, the leaves with the eggs were removed from the plywood board and allowed to dry for ca. 15 min. A small disk of leaf (28.3 mm^2) holding each egg was cut from the leaves with standard paper-hole punchers. Each leaf disk holding an individual egg was transferred to individual cells in cardboard sheets lined with adhesive tape on the back, similar to the procedures routinely used to measure egg parasitism by *Trichogramma* spp. (Hoffman et al. 1970). The eggs and leaf disks inside the cells were covered with transparent tape and observed under a microscope for mortality at 96 h posttreatment. Eggs were considered dead if the chorion of the egg was not broken. Treatments were replicated four times and each replication included 25 eggs.

Larval assays were conducted by applying insecticides to cotton field plots with a CO_2 powered back-pack sprayer. Applications were made at a spray volume of 79.8 L/ha using two TX-4 nozzles per row and application pressure of 242 kPa. Terminal buds were cut from the field plots and placed in water-pics as described by Luttrell et al. (1987) at 0, 24, 48 and 72 h posttreatment. Third instar larvae from both colonies were placed individually on the treated terminals, caged with a ventilated styrofoam cup, and placed in walk-in environmental chambers maintained at $28\pm2°C$ (Bell and Luttrell 1985). Larval mortality was recorded at 48 h posttreatment. All treatments included four replications in time. Each treatment replication included 25 larvae.

Adult assays were conducted in field plots similar to those described for larval assays, except plots were sprayed late in the afternoon to prevent solar degradation of the treatments prior to caging of adults. After treatments had dried on the plants, 10 moths were placed in nylon mesh bags and the bag was secured over the top 40 cm of the terminal region of a single plant. Five bags (50 moths) were used for each treatment replication. All treatments were replicated four times. The following morning, bags were removed and mortality was recorded. Fresh moths were also caged at 24, 48 and 72 h posttreatment to measure residual activity of the treatments against adults. Caging procedures and mortality observations were the same as those at 0 h posttreatment. Mortality data (%) were transformed to arcsin $\sqrt{\text{percentage}}$ and studied by analysis of variance for all assays. Means were separated by Duncan's (1955) multiple range test.

Additional studies comparing the residual activity of fenvalerate (0.112 kg ai/ha), permethrin (0.112 kg ai/ha),

and EPN-methyl parathion (0.56 + 0.56 kg ai/ha) were conducted because of the importance of insecticide persistence to the value of the mixture strategy. Procedures were the same as those described for third instar larvae above. The only differences were that this additional study was conducted on more mature cotton plants which were growing less rapidly and the study included assays with first, third, and fifth instar larvae. Data from both studies were studied by regression analysis (Freed et al. 1986) to describe the residual activity (log % mortality) as a function of time posttreatment (day + 1).

RESULTS AND DISCUSSION

Field Plot Studies. The density of *H. virescens* in field plots varied from year to year and from experiment to experiment within each year, but the overall trend was for low- to moderate-densities. In the initial experiment conducted over a 3 year period to measure the efficacy of reduced rates of chlordimeform, methomyl, sulprofos, and permethrin applied alone and in two- and three-component mixtures, data were summarized across the four separate field studies (Table 1). Based on these averages, the full rate of permethrin (0.112 kg ai/ha) reduced densities of larve by 59.7%, reduced square damage by 45.2%, and increased yield 39.9% over that observed in the untreated check. Larval density data were significantly ($P=0.05$) correlated with damage data (correlation coefficient (r)=0.44) and yield (r=-0.65). Damaged square data were also signficantly related to yield (r=-0.48). The highest yield (2676 kg seed cotton/ha) was obtained with the three-component mixture of chlordimeform (0.035 kg ai/ha), methomyl (0.035 kg ai/ha), and permethrin (0.028 kg ai/ha). Treatments which produced statistically similar yields to the highest yielding treatment were two rates (0.21 and 0.84 kg ai/ha) of sulprofos applied alone, all three rates (0.028, 0.056, and 0.112 kg ai/ha) of permethrin applied alone, and all two- and three-component mixtures.

In the studies conducted to measure the efficacy of reduced rates of pyrethroids applied alone and in combination with ovicidal rates of chlordimeform (0.14 kg ai/ha) and methomyl (0.14 kg ai/ha), yields of plots treated with 1X rates of pyrethroids were 41.8 to 119.3% higher than those observed in untreated checks (Table 2). Two-component mixtures of chlordimeform and 0.5X rates of pyrethroids produced the numerically highest yields in the three experiments. However, no significant differences ($P \leq 0.05$) in yield between 0.5X and 1X rates of pyrethroids applied alone and between 1X rates of pyrethroids and 0.5X rates applied in mixtures with the ovicides were detected. In the 1984 study, cotton plots receiving the 0.5X rate of fenvalerate (0.056 kg ai/ha) had significantly higher densities of larvae than those treated with the 1X rate of fenvalerate (0.112 kg ai/ha). Included in the collective data presented in Tables 1 and 2 are five direct comparisons between 0.5X rates of pyrethroids applied in mixtures with chlordimeform (0.125 kg ai/ha) with 1X rates of pyrethroids applied alone where each single component (0.5X rate of pyrethroid and ovicidal rate

TABLE 1. Efficacy of Reduced Rates of Chlordimeform (CLD), Methomyl (MTH), Sulprofos (SLP), and Permethrin (PER) Applied Alone and in Combination for Control of Bollworm and Tobacco Budworm in Cotton Small Plots in Mississippi: 1982-1984.[a]

Treatment (kg ai/ha)	Number of Larvae/ha (x 10^3)	% of Squares Damaged	Yield in kg Seed Cotton/ha
CLD (.035)	12.4 ab	7.5 a-c	2055 c-f
CLD (.070)	9.4 ab	6.4 a-c	2153 b-f
CLD (.140)	12.6 ab	7.0 a-c	2141 b-f
MTH (.035)	13.8 ab	7.4 a-c	2168 b-f
MTH (.070)	14.8 a	8.4 ab	1931 ef
MTH (.140)	10.9 ab	6.6 a-c	2035 d-f
SLP (.210)	10.1 ab	6.6 a-c	2478 a-d
SLP (.420)	10.4 ab	5.2 a-c	2186 b-f
SLP (.840)	9.6 ab	5.1 a-c	2495 a-d
PER (.028)	7.4 ab	5.5 a-c	2349 a-e
PER (.056)	6.9 ab	4.5 c	2426 a-d
PER (.112)	6.2 ab	5.1 a-c	2593 ab
CLD+MTH (.070+.070)	7.4 ab	4.5 c	2462 a-d
CLD+SLP (.070+.420)	6.7 ab	3.9 c	2482 a-d
CLD+PER (.070+.056)	7.4 ab	4.8 bc	2562 ab
MTH+SLP (.070+.420)	13.3 ab	5.7 a-c	2327 a-e
MTH+PER (.070+.056)	5.4 b	4.5 c	2561 ab
SLP+PER (.420+.056)	7.9 ab	5.3 a-c	2466 a-d
CLD+MTH+SLP (.035+.035+.210)	7.2 ab	9.5 a	2298 a-f
CLD+MTH+PER (.035+.035+.028)	8.2 ab	6.1 a-c	2676 a
CLD+SLP+PER (.035+.210+.028)	7.4 ab	5.9 a-c	2535 a-c
MTH+SLP+PER (.035+.210+.028)	5.7 b	4.6 bc	2453 a-d
Untreated Check	15.3 a	9.3 a	1854 f

[a]Means within a column not followed by a similar letter differ significantly ($P \leq 0.05$) according to Duncan's multiple range test.

of chlordimeform) of the mixture was also tested. The mean (std. dev.) reduction in numbers of larvae for chlordimeform, 0.5X rates of pyrethroids, 1X rates of pyrethroids, and 0.5X rates of pyrethroids + chlordimeform were 17 (34.6), 33 (28.7), 75 (12.3), and 64.7 (20.5)%, respectively.

The high efficacy of pyrethroids against bollworm-tobacco budworm in the early 1980's in Mississippi is illustrated by the yield increases observed in studies conducted to compare rates of pyrethroids (Table 3). Maximum yield increases in these studies ranged from a low of 22.6% over the untreated check in the 1984 study to a high of 146% in a 1981 study. Variation in yield increases was likely due to different amounts of insect pressure. Few differences were detected between rates of pyrethroids. Reduced rates were often as effective as 1X rates. In the collective data presented in Tables 1, 2, and 3 there are 15 direct comparisons between 0.5X and 1X rates of pyrethroids.

TABLE 2. Efficacy of Reduced Rates of Pyrethroid Insecticide Applied Alone and in Combination With Ovicide Rates of Chlordimeform and Methomyl for Control of Bollworm and Tobacco Budworm in Cotton Small Plots: Mississippi 1981-1984.[ab]

Treatment (kg ai/ha)	Larvae Per ha (X 10^3)	% Squares Damaged	Yield in kg Seed Cotton/ha
	1981 Study		
chlordimeform (.14)	14.3 ab	3.9 a	1735 ab
methomyl (.14)	13.3 ab	1.9 a	1986 ab
permethrin (.112)	9.1 ab	3.3 a	2557 a
permethrin (.056) + chlordimeform (.14)	3.2 b	1.5 a	2689 a
permethrin (.056) + methomyl (.14)	3.5 b	4.2 a	2209 ab
untreated check	24.5 a	3.8 a	1323 b
	1983 Study		
chlordimeform (.14)	2.0 b	13.0 a	1964 bc
fenvalerate (.056)	9.9 ab	5.0 b	2961 ab
fenvalerate (.112)	1.7 b	3.5 b	2682 ab
fenvalerate (.056) + chlordimeform (.14)	6.9 a	8.5 ab	3058 a
untreated check	12.4 a	13.0 a	1223 c
	1984 Study		
chlordimeform (.14)	9.6 a	3.6 ab	2694 a
cypermethrin (.0224)	5.9 ab	2.1 bc	2433 a
cypermethrin (.0448)	2.7 b	1.2 bc	2570 a
fenvalerate (.056)	10.4 a	2.1 bc	2555 a
fenvalerate (.112)	3.0 b	0.8 c	2401 a
permethrin (.056)	7.2 ab	2.8 b	2463 a
permethrin (.112)	1.7 b	1.3 bc	2648 a
cypermethrin (.0224) + chlordimeform (.14)	1.2 b	0.6 c	2678 a
fenvalerate (.056) + chlordimeform (.14)	0.7 b	0.8 c	2893 a
permethrin (.056) + chlordimeform (.14)	4.7 b	1.2 bc	2863 a
untreated check	9.1 a	7.7 a	1693 b

[a]Data presented are seasonal means.

[b]Means for a study within a column not followed by a similar letter differ significantly ($P \leq 0.05$) according to Duncan's (1955) multiple range test.

Average (std. dev.) reductions in larval densities over those observed in untreated checks were 57 (25.5) and 82 (12.3)% for 0.5X and 1X rates of pyrethroids, respectively.

Laboratory Assays. The ovicides, chlordimeform and methomyl, applied alone or in combination with larvicides caused more mortality of eggs than did the larvicides fenvalerate and chlorpyrifos applied alone (Table 4). Fenvalerate (0.112 kg ai/ha) caused significantly higher egg mortality than chlorpyrifos (0.560 kg ai/ha) which was

TABLE 3. Efficacy of Reduced Rates of Pyrethroid Insecticides for Control of Bollworm and Tobacco Budworm in Cotton Small Plots: Mississippi 1981-1984.[ab]

Treatment (kg ai/ha)	Larvae Per ha (X 10^3)	% Squares Damaged	Yield in kg Seed Cotton/ha
	1981 Study		
cypermethrin (.0448)	7.2 a	2.1 a	2919 a
cypermethrin (.134)	3.2 a	1.2 a	3258 a
permethrin (.112)	0.0 a	3.3 a	2557 a
untreated check	5.4 a	3.8 a	1324 b
	1981 Study		
fenvalerate (.056)	2.0 b	1.2 b	2522 b
fenvalerate (.112)	0.5 b	0.3 b	2718 ab
cypermethrin (.0336)	0.0 b	1.5 b	2395 b
cypermethrin (.0448)	0.0 b	2.2 b	2877 ab
cypermethrin (.067)	0.5 b	0.5 b	3060 a
cypermethrin (.134)	0.0 b	1.0 b	2458 b
permethrin (.056)	1.5 b	2.8 b	2167 b
permethrin (.112)	2.0 b	2.1 b	2824 ab
untreated check	6.4 a	10.7 a	1666 c
	1982 Study		
cypermethrin (.0336)	---[c]	2.4 ab	3100 ab
cypermethrin (.067)	---	1.1 b	3644 a
permethrin (.056)	---	3.1 ab	2897 b
permethrin (.112)	---	3.0 ab	2992 b
fenvalerate (.112)	---	1.8 b	3619 a
untreated check	---	4.3 a	1860 c
	1983 Study		
cypermethrin (.0224)	7.4 a	11.7 ab	2790 ab
cypermethrin (.0448)	3.0 ab	9.0 b	2292 b
cypermethrin (.067)	4.0 ab	9.0 b	2920 a
permethrin (.056)	2.5 ab	7.0 b	2790 ab
permethrin (.112)	1.7 b	10.4 ab	2745 ab
untreated check	8.2 a	30.6 a	1575 c
	1983 Study		
cypermethrin (.056)	1.7 b	3.0 b	2390 ab
cypermethrin (.067)	2.7 b	9.0 ab	2740 a
cypermethrin (.134)	0.7 b	5.5 b	2396 ab
fenvalerate (.112)	0.5 b	6.5 b	1953 b
untreated check	7.7 a	14.5 a	1348 c
	1984 Study		
cypermethrin (.0224)	5.9 b	2.1 b	2324 a
cypermethrin (.0448)	2.7 b	1.2 b	2433 a
cypermethrin (.067)	1.2 b	0.8 b	2570 a
fenvalerate (.056)	7.9 b	0.9 b	2555 a
fenvalerate (.112)	0.7 b	0.8 b	2401 a
fenvalerate (.168)	2.7 b	0.8 b	2354 a
permethrin (.056)	7.2 b	2.8 b	2463 a
permethrin (.112)	2.0 b	1.3 b	2648 a
permethin (.168)	1.2 b	0.8 b	2509 a
untreated check	15.8 a	7.7 a	1693 c

TABLE 3. (cont.)

	1984 Study		
fenvalerate (.056)	6.4 b	1.2 b	2962 a
fenvalerate (.112)	1.0 c	0.8 b	2936 a
cypermethrin (.0448)	1.5 c	0.8 b	3128 a
cypermethrin (.056)	1.2 c	1.1 b	2728 a
cypermethrin (.067)	0.7 c	0.4 b	2844 a
untreated check	35.1 a	7.9 a	2551 a

[a]Data presented are seasonal means.
[b]Means for a study within a column not followed by a similar letter differ significantly ($P \leq 0.05$) according to Duncan's (1955) multiple range test.
[c]Data were not collected.

significantly ($P \leq 0.05$) higher than egg mortality in the untreated check (14%). Against third instar larvae, fenvalerate (0.112 kg ai/ha), methomyl (0.140 kg ai/ha), and two-component mixtures which included a 0.5X rate of fenvalerate plus chlordimeform (0.14 kg ai/ha), methomyl (0.14 kg ai/ha) or chlorpyrifos (0.56 kg ai/ha) caused higher mortality than chlordimeform (0.14 kg ai/ha) or chlorpyrifos (0.56 kg ai/ha) applied alone at 0 h posttreatment. Mortality from the 0.5X rate of fenvalerate was statistically equal to that from the 1X rate of fenvalerate. Chlordimeform (0.14 kg ai/ha) alone did not cause significantly ($P \leq 0.05$) more mortality of larvae than that observed in the untreated check (1%). Methomyl (0.14 kg ai/ha) treatments resulted in the highest mortality of adults (90%). The 0.5X rate of fenvalerate applied in combination with methomyl caused less mortality than methomyl alone (possibly due to repellency by fenvalerate, e.g. Virgona et al. 1983, Forrester and Cahill 1987) but more than all other treatments at 0 h posttreatment. Fenvalerate in combination with chlordimeform or chlorpyrifos was more active against adults than the 0.5X rate of fenvalerate alone. Both rates of fenvalerate alone were more active than chlordimeform or chlorpyrifos but mortality from chlordimeform and chlorpyrifos treatments was not significantly ($P \leq 0.05$) greater than that observed for adults in the untreated check (0%).

Residual activity of the insecticides in the terminal bud region of cotton plants followed similar trends for both larval and adult assays (Table 4). Treatments including fenvalerate exhibited longer residual activity than treatments that did not include a pyrethroid. Against third instar larvae some residual activity of pyrethroids was still detectable at 72 h posttreatment. However, residual activity at 48 h posttreatment was less than 50% of the original activity measured at 0 h posttreatment. Residual activity of methomyl declined more rapidly. At 0 h posttreatment, methomyl was more toxic to adults than fenvalerate but by 24 h posttreatment fenvalerate was just as active as methomyl. Chlorpyrifos also exhibited a rapid decline in residual activity. Residual activity of the mixtures seemed to be

TABLE 4. Residual Activity of Fenvalerate (FEN), Chlordimeform (CLD), Methomyl (MTH), and Chlorpyrifos (CPS) Applied Alone and in Combination Against Tobacco Budworm Eggs, Third Instar Larvae, and Adults.[a]

Treatment (kg ai/ha)	Mean % Mortality Time Posttreatment 0 h	24 h	48 h	72 h
		Eggs		
Untreated Check	14 e	---[b]	---	---
FEN (.056)	42 b-d	---	---	---
FEN (.112)	46 bc	---	---	---
CLD (.140)	91 a	---	---	---
MTH (.140)	87 a	---	---	---
CPS (.560)	32 d	---	---	---
FEN+CLD (.056+.140)	94 a	---	---	---
FEN+MTH (.056+.140)	90 a	---	---	---
FEN+CPS (.056+.560)	49 b	---	---	---
		Third Instar Larvae		
Untreated Check	1 d A	2 d A	2 c A	2 c A
FEN (.056)	91 bc A	65 b B	31 b C	5 ab D
FEN (.112)	99 a A	81 a B	46 a C	9 a D
CLD (.140)	12 d A	9 cd A	2 c B	1 b B
MTH (.140)	100 a A	15 c B	4 c C	2 b C
CPS (.560)	86 c A	17 c B	3 c C	2 b C
FEN+CLD (.056+.140)	100 a A	63 b B	38 b C	3 b D
FEN+MTH (.056+.140)	96 ab A	65 b B	38 b C	8 ab D
FEN+CPS (.056+.560)	98 a A	66 b A	32 b C	3 b D
		Adults		
Untreated Check	0 e A	2 d A	1 d A	3 a A
FEN (.056)	31 d A	25 c AB	10 bc BC	4 a C
FEN (.112)	36 cd A	33 bc A	18 a B	6 a B
CLD (.140)	1 e A	1 d A	2 cd A	2 a A
MTH (.140)	90 a A	26 c B	2 cd C	1 a C
CPS (.560)	12 e A	7 d AB	1 d B	2 a B
FEN+CLD (.056+.140)	50 c A	37 ab B	11 ab C	2 a C
FEN+MTH (.056+.140)	74 b A	46 a B	16 ab C	0 a D
FEN+CPS (.056+.560)	48 c A	28 bc B	10 bc B	5 a C

[a]Means within columns not followed by similar lower case letters and within rows not followed by similar upper case letters differ significantly ($P \leq 0.05$) according to Duncan's (1955) multiple range test.
[b]Observations were not made.

influenced predominantly by the pyrethroid component. In assays with third instars, two-component mixtures of fenvalerate (0.056 kg ai/ha) with chlordimeform (0.14 kg ai/ha) or chlorpyrifos (0.56 kg ai/ha) resulted in more larval mortality than fenvalerate (0.056 kg ai/ha) alone. However, by 24 h posttreatment the fenvalerate (0.056 kg ai/ha) alone treatment caused mortality levels equal to those from the two-component mixtures.

In the study conducted with fenvalerate, permethrin, and EPN-methyl parathion (Table 5), levels of residual activity were similar to those reported in Table 4.

TABLE 5. Residual Activity of Fenvalerate (FEN), Permethrin (PER), and EPN-methyl Parathion (EPN-MP) Against First, Third, and Fifth Instar Larvae of the Tobacco Budworm.[a]

Treatment (kg ai/ha)	Mean % Mortality Time Posttreatment 0h	12h	24h	48h	72h
		1st Instar Larvae			
PER (0.12)	98 aA	97 aA	85 abB	62 aC	26 abD
FEN (0.12)	99 aA	98 aAb	90 aB	69 aC	34 aD
EPN-MP (0.56+0.56)	95 aA	91 aA	78 bB	49 bB	25 bcD
		3rd Instar Larvae			
PER (0.12)	76 bcA	67 cB	62 cB	32 cC	15 dD
FEN (0.12)	79 bA	78 bA	67 cB	34 cC	18 cdD
EPN-MP (0.56+0.56)	69 cA	60 cB	43 dC	23 dD	12 deE
		5th Instar Larvae			
PER (0.12)	45 de	28 deB	25 efB	16 eC	13 deC
FEN (0.12)	48 dA	32 dB	29 eB	20 deC	18 cdC
EPN-MP (0.56+0.56)	38 eA	23 eB	20 fB	12 eBC	6 eC

[a]Means within a row not followed by similar lower caseletters and within rows not followed by similar uppercase letters differ significantly ($P \leq 0.05$) according to Duncan's (1955) multiple range test.

Fenvalerate and permethrin consistently caused more larval mortality than EPN-methyl parathion, although differences among treatments were not detected for first instar larvae at 0 and 12 h posttreatment. First instar larvae were consistently more susceptible than third instars which were more susceptible than fifth instars to all treatments.

Linear relationships (Table 6 and Fig. 1-4) appeared to describe most of the variation associated with residual activity of insecticides in the terminal bud region of cotton. Insecticide decay on inert substrates is expected to follow a linear relationship between log residual activity and time (Matsumura 1975). However, five of the relationships were not significant at $P \leq 0.05$, possibly because of the influence of plant growth and/or experimental variation. Residual activity of fenvalerate was different for the two studies (Study A and Study B in Table 5 and Fig. 4) probably because of different plant growth rates or effects from other variables not measured. Chlordimeform alone had little effect on insect mortality (Table 4) and residual activity of chlordimeform + fenvalerate was similar to that of fenvalerate alone (Fig. 1). The slight difference observed between 0.5X and 1X rates of fenvalerate could not be explained and is probably the result of within experiment variation. Residual activity of methomyl (Fig. 2) and chlorpyrifos (Fig. 3) decreased more rapidly than that of fenvalerate. A comparison of residual activities between the two studies is shown in Fig. 4. EPN-methyl parathion appeared to maintain residual activity in the terminal buds at a rate similar to that of fenvalerate. Methomyl and chlorpyrifos appear to lose activity at a faster rate.

TABLE 6. Regression Statistics for Equations Describing Residual Activity of Insecticides in the Terminal Bud Region of Cotton.[a]

Treatment (kg ai/ha)	Regression Slope(SE)	Regress. Inter.	Corr. Coef.(r)	P
	Study A			
Fenvalerate(0.056)	-0.410(0.103)	2.51	-0.942	0.058
Fenvalerate(0.112)	-0.339(0.099)	2.47	-0.924	0.099
Chlordimeform(0.14)	-0.389(0.067)	1.55	-0.971	0.028
Methomyl(0.14)	-0.568(0.082)	2.44	-0.980	0.020
Chlorpyrifos(0.56)	-0.564(0.093)	2.39	-0.974	0.026
Fenvalerate(0.056)+ Chlordimeform(0.14)	-0.478(0.155)	2.66	-0.909	0.090
Fenvalerate(0.056)+ Methomyl(0.14)	-0.350(0.084)	2.45	-0.947	0.053
Fenvalerate(0.056)+ Chlorpyrifos(0.56)	-0.484(0.142)	2.66	-0.924	0.076
	Study B			
Fenvalerate(0.112)	-0.222(0.037)	2.19	-0.974	0.026
EPN-Methyl Parathion (0.56 + 0.56)	-0.254(0.010)	2.11	-0.999	0.001
Permethrin(0.112)	-0.238(0.010)	2.19	-0.974	0.026

[a]All equations in form Y=a+bX; where Y=log (% mortality), a=intercept, b=slope, and X=days posttreatment +1.

TABLE 7. Mortality of Pyrethroid Susceptible (S) and Resistant (R) Tobacco Budworm Eggs, Third Instar Larvae, and Adults Exposed to Cotton Plant Tissue Treated With Fenvalerate (FEN), Chlordimeform (CLD), Methomyl (MTH), and Chlorpyrifos (CPS) Alone and in Combination.[a]

	Mean % Mortality					
	Eggs		Third Instar Larvae		Adults	
Treatment (kg ai/ha)	S	R	S	R	S	R
Untreated Check	36 ef	32 f	3 g	2 g	2 h	1 h
FEN (.056)	51 b-d	48 cd	87 a-c	10 fg	26 ef	19 fg
FEN (.112)	49 b-d	55 bc	95 a	22 f	41 cd	31 d-f
CLD (.140)	91 a	94 a	3 g	5 g	9 gh	8 gh
MTH (.140)	90 a	90 a	96 a	90 ab	80 a	86 a
CPS (.560)	46 c-e	39 d-f	80 b-d	68 d	20 fg	28 d-f
FEN+CLD (.056+.140)	96 a	92 a	100 a	50 e	55 b	51 bc
FEN+MTH (.056+.140)	90 a	90 a	99 a	90 ab	78 a	82 a
FEN+CPS (.056+.560)	62 b	58 bc	97 a	74 cd	38 de	41 cd

[a]Means within a stage not followed by a similar letter differ significantly ($P \leq 0.05$) according to Duncan's (1955) multiple range test.

Pyrethroid efficacy was dramatically reduced when treatments were applied to third instars and adults from the (pyrethroid resistant strain (Table 7). Against third

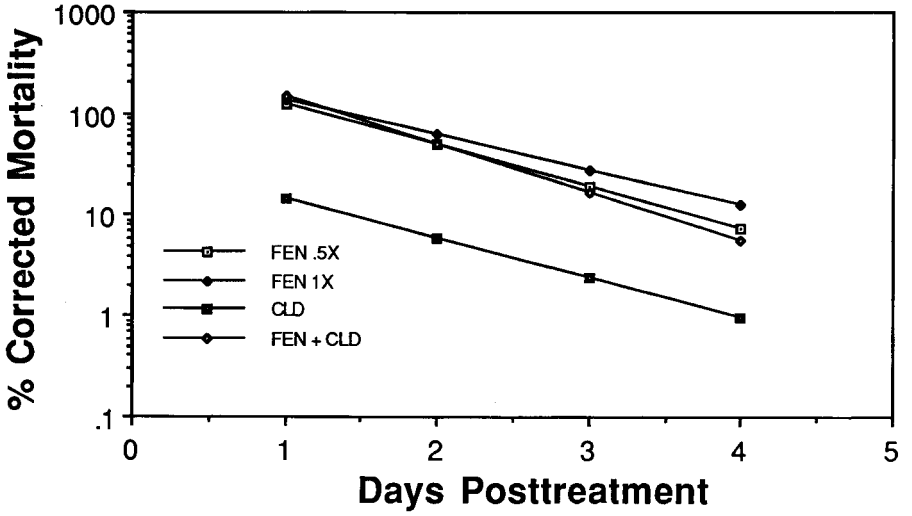

FIG. 1. Persistence of reduced and full rates of fenvalerate (FEN), an ovicide rate of chlordimeform (CLD), and a reduced rate of fenvalerate plus an ovicide rate of chlordimeform (FEN + CLD) on cotton terminals.

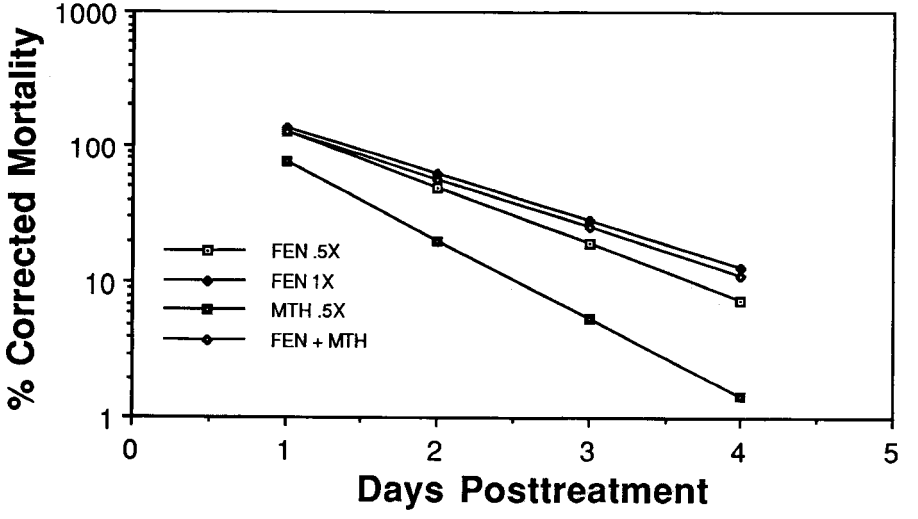

FIG. 2. Persistence of reduced and full rates of fenvalerate (FEN), and ovicide rate of methymyl (MTH), and a reduced rate of fenvalerate plus an ovicide rate of methomyl (FEN + MTH) on cotton terminals.

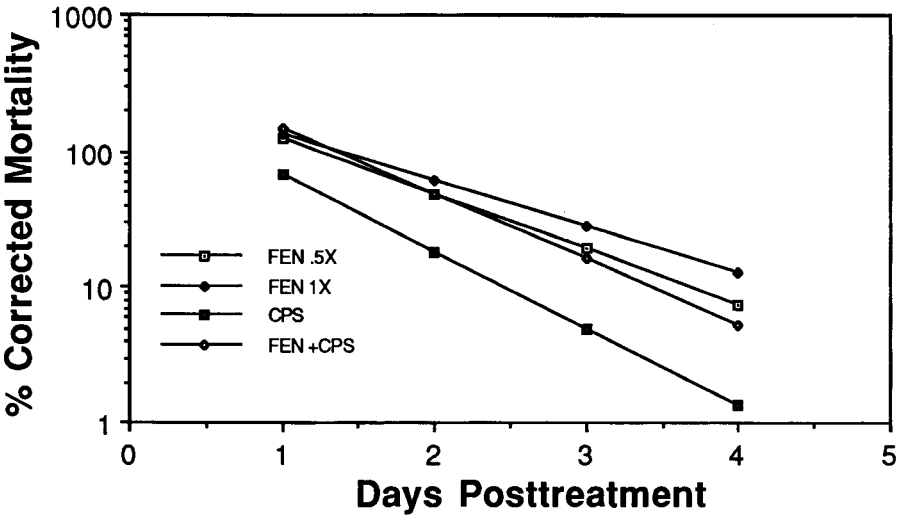

FIG. 3. Persistence of reduced and full rates of fenvalerate (FEN), a reduced rate of chlorpyrifos (CPS), and a reduced rate of fenvalerate plus a reduced rate of chlopyrifos (FEN+CPS) on cotton terminals.

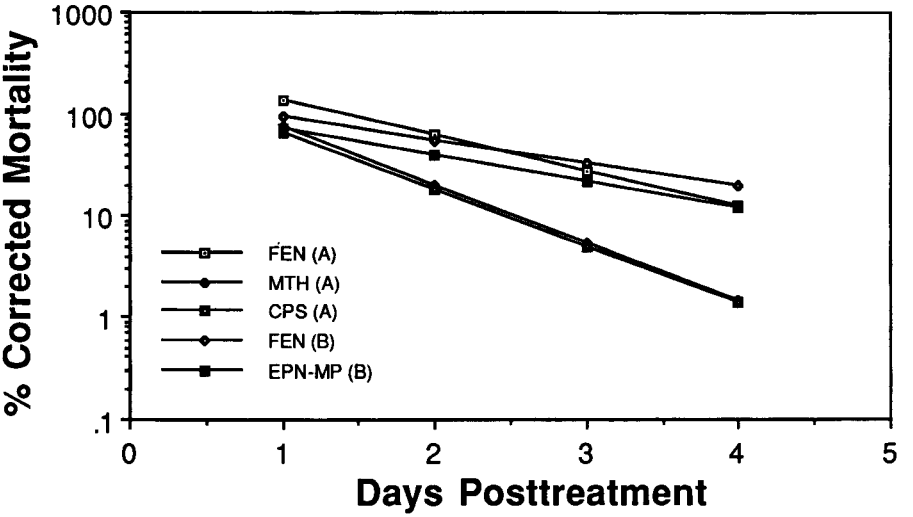

FIG. 4. Persistence of fenvalerate (FEN), methomyl (MTH), chlorpyrifos (CPS), and EPN-methyl parathion (EPN-MP) on cotton terminals in two separate studies (A and B).

instars from the susceptible strain, 1X rates of fenvalerate 0.112 kg ai/ha) caused 95% mortality. Mortality of third instars from the resistant strain was only 22%. Significant differences ($P \leq 0.05$) in mortality of eggs exposed to pyrethroid treatments were not observed between the pyrethroid susceptible and resistant strains. Differences among treatments directed at the susceptible strain were similar to those described in the residual activity study (Table 4). Against third instar larvae from the resistant strain, the 0.5X rate of fenvalerate caused 10% mortality and chlordimeform only caused 5% mortality. When fenvalerate and chlordimeform were included in a mixture, the resulting mortality was 50%. Mixtures of fenvalerate with methomyl and chlorpyrifos also gave higher levels of mortality than those observed with fenvalerate alone. However, the resistant strain did not exhibit resistance to methomyl or chlorpyrifos and the levels of mortality measured with the compounds applied alone were relatively high. Ovicidal rates of methomyl caused rather high mortality of larvae (90%) and adults (86%).

From a resistance management perspective, it is important to note that the mortality of third instars from the resistant colony exposed to the mixture of chlordimeform and fenvalerate (50%) was less than that observed for the susceptible colony exposed to fenvalerate alone (87%). It is also important to note that while mixtures of fenvalerate with methomyl or chlorpyrifos were not synergistic, the levels of mortality observed against pyrethroid resistant larvae and adults were higher than those observed with pyrethroid alone treatments.

The increased activity of pyrethroids in combination with chlordimeform supports the findings of Plapp (1979), Jensen et al. (1984), Campanhola and Plapp (1989a, and 1989b). Selection for resistance to pyrethroids appeared to be inhibited by chlordimeform - pyrethroid mixtures (Bohman et al. 1988). Because chlordimeform is no longer available for use in cotton production, research should be intensified with other formamdines as possible components of mixtures with pyrethroids. Although mixtures of pyrethroids with carbamate and organophosphorous insecticides generally showed less of an advantage for mixtures than chlordimeform, the low levels of mortality observed with pyrethroids against pyrethroid-resistant insects illustrates the potential importance of these compounds in mixtures.

Behavior of bollworm and tobacco budworm adults as a result of interactions with insecticides is poorly understood. These studies indicate that mortality of tobacco budworm adults exposed to insecticide on cotton plants may be significant. It is important with *Heliothis armigera* (Forrester and Cahill 1987). Pyrethroid resistance exhibited in *H. virescens* adults has been a major factor in the development of region-wide resistance monitoring (Plapp et al. 1990). Mortality of adults may also be a critical component of population control and development of resistant populations. The high mortality of adults, as well as larvae, exposed to methomyl warrants further consideration of carbamate insecticides in the development of management strategies.

Persistence or residual activity is a critical component of models designed to study resistance management strategies (Roush 1989). It is likely that residual activity of insecticide would be higher at lower positions in the cotton canopy that are not experiencing rapid expansion of plant tissue. However, most insecticides are directed at insects present in the terminal bud region of cotton. Residual activity of mixtures is complex and the effects from a mixture will change with time. For example, these data suggest that methomyl - fenvalerate mixtures are highly active on the date of application but no more active than fenvalerate alone at 1 or 2 days posttreatment. After this period, remaining insects would be exposed only to the residual activity of fenvalerate. Thus, although methomyl - fenvalerate mixtures are highly effective, they would not delay resistance as effectively as two compounds with more similar persistence (Roush 1989). In this regard, a compound with persistence of EPN-methyl parathion would seem promising (Fig. 4), although EPN-methyl parathion itself may not be desirable because of the possibility that resistance genes to it are still common from past resistance problems (Sparks 1981).

The present study suggests that certain insecticide mixtures can be used to provide effective control of tobacco budworm in cotton. It is difficult to measure the relative value of mixtures in field experiments against low population densities of the target pest. Estimating insect mortality in assays with treated plants provides valuable and timely information, but the behavior of insects in field environments and the relationships of insect behavior to contact with insecticides on plant tissue are relatively unknown. In insecticide resistance studies, this is further complicated by the effects of laboratory rearing on the genetic make-up of colonized insects. The use of insecticide mixtures as a strategy to delay the development of resistance deserves additional theoretical examination. Use of mixtures in production agriculture seems to have immediate value strictly from the perspective of maintaining acceptable levels of insect control, especially where resistance already limits efficacy.

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