

TOXICITY OF PYRETHROIDS AND OTHER INSECTICIDES
AGAINST SUSCEPTIBLE AND RESISTANT TOBACCO BUDWORM
LARVAE AND SYNERGISM BY CHLORDIMEFORM

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Abstract

The development of resistance to pyrethroid insecticides in the tobacco budworm, *Heliothis virescens* (F.), represents a problem to many cotton production areas in Texas and, potentially, to other areas in the United States. In an attempt to determine strategies to deal with the resistant populations, resistant and susceptible first instar larvae were exposed in glass vials to insecticides alone, or in combination with chlordimeform. Cross resistance was observed to all the pyrethroids tested. Resistance did not extend to avermectin or to the S-alkyl organophosphates profenofos and acephate, but did extend to the carbamates methomyl and thiodicarb. Chlordimeform synergized all insecticides, and synergism was higher against susceptible than against resistant larvae with organophosphate insecticides. However, no consistent pattern of synergism could be established for the different strains with synthetic pyrethroids or carbamates. The S-alkyl organophosphates seem to be a good alternative for the early season control of tobacco budworm resistant populations.

Introduction

Resistance of the tobacco budworm (TBW) to synthetic pyrethroid insecticides is becoming a serious threat to cotton production in the southwestern U.S. Tests with first instar TBW larvae from Glascock Co., TX. in 1985, indicate an approximately 16-fold resistance to permethrin in these insects (Plapp and Campanhola 1986). In the 1986 season, problems of TBW control with synthetic pyrethroids occurred in several cotton production areas in Texas.

There are many studies in the literature in which synergism of insecticides was observed against susceptible or resistant insects. These studies involved the combination of insecticides or addition of non-toxic compounds to the insecticides as synergists.

Chlordimeform (CDF), a formamidine, is an ovicide and an adult behavior modifier which has been proven a good synergist when combined with organophosphate and pyrethroid insecticides against the TBW (Plapp 1976, Plapp 1979). However, the mechanism by which CDF acts as synergist has not yet been elucidated. It may involve a competition between CDF and insecticides for insecticide-metabolizing enzymes or an increase of the insecticide binding to the target site (Plapp 1976, Chang and Plapp 1983).

The objectives of the present study were to evaluate resistance of pyrethroid-resistant TBW to different classes of insecticides, as well as the effect of different combinations of insecticides on susceptible and resistant strains of this insect. The synergistic action of chlordimeform to all the insecticides studied was also determined for both strains.

Materials and Methods

Insects

The susceptible and resistant populations for the bioassays were obtained from lab colonies maintained on artificial diet (Vanderzant et al. 1962).

The susceptible population has been reared in lab for several years and the resistant population was established in 1986 in the Department of Entomology, Texas A&M University, with insects provided by ICI, Goldsboro, NC.

In 1986, pyrethroid resistant budworms were collected from different locations for bioassays in the lab. However, data are not available at the present time.

Chemicals

The insecticides tested included the pyrethroids cypermethrin, permethrin, fenvalerate, cyhalothrin, bifenthrin, tralomethrin, and cyfluthrin; the organophosphates sulprofos, profenofos, and acephate; the carbamates methomyl and thiodicarb; and the alternate chemicals avermectin and rotenone. Also, combinations of cypermethrin with profenofos, methomyl, or thiodicarb were tested.

All the insecticides or combinations were evaluated for CDF synergism. Therefore, all bioassays were conducted both in the presence and absence of CDF.

The chemicals were supplied by commercial sources as technical grade materials.

Additional insecticides are being tested. These include the synthetic pyrethroids fluvalinate, decamethrin, and Asana; the organophosphates methyl parathion and monocrotophos; and combinations of cypermethrin with insecticides of other classes, such as methyl parathion, monocrotophos, sulprofos, avermectin, and rotenone. All these are also being evaluated for CDF synergism.

Test Procedure

TBW larvae were exposed to films of chemicals on the inner surfaces of 20-ml. glass liquid scintillation vials (Plapp 1971). A piece of artificial diet and five first instar larvae were placed in each vial. At least 20 larvae were tested per concentration of insecticide, and all the insecticides + CDF were tested for susceptible (S) and resistant (R) populations of TBW. Insecticide(s) + CDF were used at 1:10 (wt/wt) ratio and combinations of insecticides, at 1:1 (wt/wt) ratio.

Five different concentrations were used for each insecticide or combination of insecticides.

Percent response was determined at 24 hours for the R strain and at 48 hours for the S strain, since the R larvae responded faster to the insecticides than the S larvae. For avermectin the results were collected at 48 hours for both strains, because of the slow response of the larvae to this chemical. LC50s, in ug toxicant/vial, and slopes of the response curves were calculated by probit analysis.

The resistance level was determined as the quotient of the LC50 of each toxicant for the R strain and the LC50 for the S strain.

The synergism levels due to CDF were calculated dividing the LC50 for the insecticide only by the LC50 for the insecticide + CDF. The synergistic effects of the insecticide combinations were determined with cotoxicity coefficients (Sun and Johnson 1960).

Results and Discussion

Table 1 shows toxicity data for seven synthetic pyrethroids only and in combination with CDF at 1:10 (pyrethroid:CDF) ratio against S and R first instar TBW larvae. Resistance extended to all the pyrethroids studied, with the resistance level varying from 8.2-fold (bifenthrin) to 33.9-fold (permethrin).

CDF synergized all the synthetic pyrethroids against both strains. The lowest level of synergism was observed with tralomethrin (2.3-fold) and the highest occurred with cypermethrin (18.3-fold), both on the R strain. The CDF synergism was higher against the S as compared to the R strain for fenvalerate, cyhalothrin, and tralomethrin. The opposite was observed for cypermethrin, permethrin, bifenthrin, and cyfluthrin. Therefore, no pattern of CDF synergism could be established for the different strains and different pyrethroids.

There were no marked differences in the slopes of the pyrethroid response curves when CDF was added. Also, CDF did not affect the slopes more in one strain than the other.

CDF did not block resistance completely to any of the pyrethroids studied. Furthermore, there were cases in

which the level of resistance was even increased with the addition of CDF, such as cyhalothrin, fenvalerate, and tralomethrin. Despite that, in most cases CDF increased the toxicity of the pyrethroids to a level where the LC50 for the combination of insecticide + CDF for the R strain became nearly equal to the LC50 for the insecticide only, for the S strain. Therefore, combinations of pyrethroids + CDF seem to be an option for the management of resistant TBW populations when a low resistance level to pyrethroids is present. Also in favor of CDF use there is a previous study which demonstrated that after 10 generations of selection with permethrin:CDF, at the LD80 level, the susceptibility did not change (Crowder et al. 1984).

Early in the season, alternate classes of insecticides could be used for TBW control. Such use might delay the onset of pyrethroid resistance by removing early season selection pressure.

Results obtained for the organophosphate insecticides are in Table 2. All these toxicants are S-alkyl phosphates, having four different substituents attached to the central phosphorus atom. As earlier reported, insects have difficulty in dealing with such insecticides (Plapp 1986).

In the present study, no resistance was observed to profenofos or acephate; that is, these chemicals were more toxic to R than to S larvae. However, some resistance was present to sulprofos. Therefore, profenofos and acephate are good alternate insecticides to the synthetic pyrethroids early in the season. They will select less for resistance, making the pyrethroids more effective later in the season.

CDF synergized all of the organophosphates against both strains. CDF synergism was greater against the S than against the R strain for acephate and sulprofos; as much as 119-fold synergism was obtained with acephate + CDF against the S strain. For profenofos, the synergistic ratio was approximately the same for both strains (4-fold). Consequently, resistance to profenofos + CDF was maintained at the same level as compared with profenofos only. However, the use of CDF enhanced the resistance level to sulprofos and acephate. Nevertheless, CDF increased the toxicity of these compounds to R larvae, making them equally or more toxic to R larvae than the insecticide only to S larvae.

The only apparent disadvantage of the use of CDF with the S-alkyl organophosphates is the decrease in the slope of the response curve for the R strain, leading to greater variability of response to these insecticides. For the S strain, the addition of CDF did not significantly change the slope for profenofos, but decreased the slope for sulprofos and acephate.

Results for the carbamates methomyl and thiodicarb are in Table 2. Resistance was observed to both insecticides, but a much higher level occurred to thiodicarb (120-fold). With thiodicarb, CDF synergized more against the S strain as compared to the R strain, but the opposite was observed with methomyl. In either case, CDF did not block resistance. The highest synergism level for either carbamate was observed with thiodicarb against the S strain (34.7-fold). In combination with CDF, the two carbamates were nearly equal in toxicity to the S strain, but thiodicarb + CDF was less effective against the R strain.

CDF in combination with methomyl decreased the slope of response curve as compared to methomyl only. With thiodicarb, CDF increased the slope of response curve for the R strain, which is an advantage for using the thiodicarb + CDF combination.

Avermectin and rotenone were tested as alternate compounds for control of R TBW. Rotenone was tested because it was recommended in the northeastern U.S. for the control of pyrethroid-resistant Colorado potato beetles. The bioassay results for these two toxicants are also shown in Table 2. No resistance was observed to avermectin or avermectin + CDF. Rotenone was practically nontoxic to both strains. When CDF was combined with rotenone, high levels of synergism were observed against both strains.

CDF in combination with avermectin caused no significant change in the slope of the response curves in

relation to avermectin only. For rotenone, no comments are possible since accurate response curves for this chemical only were not determined.

More data are being obtained for these chemicals, but avermectin + CDF seems to be an option for the control of R TBW. Furthermore, the high level of CDF synergism of CDF synergism with rotenone suggests this combination may be useful as another option for TBW control.

In the field, the incidence of pyrethroid-R TBWs is more frequent late in the season. In an attempt to improve the control of R TBW at this time, combinations of cypermethrin with insecticides of other classes are being tested.

Some results for the combinations cypermethrin + other insecticides are presented in Table 3. No one of the combinations blocked resistance completely, and in the case of cypermethrin + thiodicarb a resistance level as high as 575-fold was observed. However, only 2.4-fold resistance was present for cypermethrin + profenofos and 4.7-fold to cypermethrin + methomyl.

CDF tended to synergize the combinations more against the S strain. Thus, there seems to be no advantage in adding CDF to these mixtures for R populations, except in the case of cypermethrin + thiodicarb, where 92-fold synergism was obtained with CDF. Furthermore, for the R strain the addition of CDF to this combination did not change the slope of the response curve in comparison with the combination only.

Table 4 shows the cotoxicity coefficients for the insecticide combinations. The only highly synergistic combination was cypermethrin + thiodicarb, but it was synergistic only with the S strain. This combination was antagonistic with the R strain (cotoxicity coeff. < 1). Therefore, it seems that there is no advantage in combining cypermethrin with profenofos, methomyl or thiodicarb for control of R TBW. However, when CDF is added to cypermethrin + thiodicarb, high synergism is observed.

In summary, based on the conditions under which the present results were obtained, the following insecticides, or combinations, can be recommended for field testing for control of R TBW: all of the pyrethroids (cypermethrin, permethrin, fenvalerate, cyhalothrin, bifenthrin, tralomethrin, cyfluthrin) + CDF; profenofos or acephate + CDF; sulprofos + CDF; avermectin + CDF; and cypermethrin + thiodicarb + CDF.

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Table 1. LC50s (ug/vial) and slopes of response curves of synthetic pyrethroids + chlordimeform (CDF) against susceptible (S) and resistant (R) tobacco budworm 1st. instar larvae.

Insecticide	S strain		R strain	
	LC50 1	Slope	LC50 2	Slope
Cypermethrin	0.19	1.38	2.93	1.24
Cyperm. + CDF	0.019	1.16	0.16	0.86
Permethrin	0.51	1.45	17.31	1.23
Perm. + CDF	0.060	1.38	1.30	2.21
Fenvalerate	0.38	0.83	9.91	1.38
Fenval. + CDF	0.024	1.22	0.96	1.32
Bifenthrin	0.068	1.06	0.56	2.05
Biphen. + CDF	0.010	1.02	0.040	1.01
Cyhalothrin	0.045	1.50	0.40	1.38
Cyhalot. + CDF	0.0040	1.50	0.040	0.92
Tralomethrin	0.025	1.13	0.21	1.53
Tralom. + CDF	0.0050	2.18	0.090	1.90
Cyfluthrin	0.078	0.91	1.08	1.12
Cyflut. + CDF	0.027	1.39	0.19	1.40

Resistance = column 2+1, for each pyrethroid or pyrethroid-CDF combination.

Synergism = each insecticide:each + CDF.

Table 2. LC50s (ug/vial) and slopes of response curves of non-pyrethroid insecticides + CDF against S and R 1st. instar larvae.

Insecticide	S strain		R strain	
	LC50	Slope	LC50	Slope
Sulprofos	0.16	1.58	0.73	3.73
Sulprofos + CDF	0.028	1.06	0.17	0.95
Profenofos	0.19	1.46	0.15	3.81
Profen. + CDF	0.047	1.58	0.039	1.87
Acephate	8.93	1.57	3.34	2.17
Acephate + CDF	0.075	0.89	0.37	1.38
Methomyl	0.023	1.02	0.11	1.19
Methomyl + CDF	0.0058	0.66	0.016	0.74
Thiodicarb	0.25	1.57	30	1.36
Thiod. + CDF	0.0072	1.27	2	2.50
Avermectin	1.22	1.03	1.40	0.89
Averm. + CDF	0.043	1.21	0.043	0.98
Rotenone	> 25	?	> 25	?
Rotenone + CDF	0.008	0.79	2.77	1.72

Table 3. LC50s (ug/vial) and slopes of response curves of combinations of cypermethrin + other insecticides + CDF against S and R 1st. instar larvae.

Insecticides	S strain		R strain	
	LC50 1	Slope	LC50 1	Slope
Cyp. + profenofos	0.11	2.06	0.26	3.37
Cyp. + prof. + CDF 2	0.014	1.21	0.12	2.54
Cyp. + methomyl	0.030	1.81	0.14	1.16
Cyp. + meth. + CDF	0.011	1.40	0.068	1.06
Cyp. + thiodicarb	0.016	0.44	9.20	1.08
Cyp. + thiod. + CDF	0.014	1.34	0.10	1.08

1 Each value composed of 50% of each insecticide.

2 Ratio insecticide:insecticide:CDF = 1:1:10.

Table 4. Cotoxicity coefficients for the combinations cypermethrin + other insecticides against S and R 1st. instar larvae.

Combination	S strain	R strain
Cyp. + profenofos	1.8	1.1
Cyp. + methomyl	1.4	1.5
Cyp. + thiodicarb	13.5	0.6

BELTWIDE COTTON HELIOTHIS MONITORING 1980-1986:
STATUS OF FIELD POPULATIONS TO CYPERMETHRIN

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Key Words: Ammo, Cypermethrin, *Heliothis virescens*, Insecticides, Insect resistance, Insect susceptibility, Insect monitoring, Tobacco budworm.

Abstract

FMC Corporation initiated a pyrethroid susceptibility monitoring program in 1979 to monitor tobacco budworm, *Heliothis virescens*, larval susceptibility to permethrin. Cypermethrin was added to the program in 1980. Annual collections of *H. virescens* have been made in GA, MS, LA, TX, AZ and CA to determine if any changes in susceptibility have occurred. Overall, no consistent or generalized decrease in *H. virescens* susceptibility has been detected. However, considerable site-to-site and year-to-year variation in susceptibility has been observed with higher LD values typically being followed by lower values in subsequent years.

Introduction

A major difficulty in past episodes with insect resistance has been the lack of adequate baseline susceptibility data. All too often very susceptible laboratory strains or extremely limited data from field strains have been used to determine susceptibility levels of field strains after the insect population has been exposed to numerous treatments of a particular insecticide or insecticide class.

FMC was aware of the problems created by the lack of adequate baseline data. Therefore, a pyrethroid monitoring program was initiated in 1979 (Staetz, 1984) to survey several populations of *Heliothis virescens* to determine susceptibility levels for permethrin and establish baseline data for *H. virescens* larvae. Standard collection and test procedures were developed, annual collections of adults was initiated, and a joint project involving many cotton insect researchers was undertaken. In 1980 cypermethrin was introduced into the testing program.

Materials and Methods

A generally accepted test method was developed and adopted for the FMC monitoring program (Staetz, 1985). Briefly, adults (occasionally larvae) are collected from the field, eggs are obtained from the field-collections, and the resulting larvae are used for testing. The larvae (20-25 mg) are topically treated and mortality is recorded 24-72 hours after treatment with the 48 hour data used for all comparisons. The results are reported in ug of insecticide per gram of insect body weight to compensate for variations in the size of the larvae used. Generally, 5-7 rates with 2 or 3 replicates were run for each test with larvae from each collection location; two to four tests being conducted annually with larvae from each site. The data were subjected to probit analysis to generate LD values and fiducial limits.

Baseline Data

Baseline data for permethrin were determined in 1979 and are reported elsewhere (Staetz, 1985); similar data for cypermethrin were established for six states in 1980 and 1981 (Table 1). There was a considerable amount of variation in susceptibility of *H. virescens*

larvae originating in Georgia, Mississippi, Louisiana, Texas, Arizona and California. Larvae from the East were more susceptible than those collected in the West.

These data are important because it is against these values that data generated later can be compare in order to determine if susceptibility levels in a particular area are increasing or decreasing. It is readily apparent the baseline values from field collected populations are more of a range of values than a single number. It is also important to examine entire dose-response (D-R) lines rather than single values, e.g., LD₅₀'s, because shifts at the upper portion represent changes in susceptibility which may translate into changes in field efficacy. In addition, the first indications of changing susceptibility will occur in this portion of the D-R line.

Results and Discussion

FMC has persisted with its monitoring program. However, in recent years efforts have been focused more in the West than the East because of greater *H. virescens* population pressure in the West. A summary of the test data is shown in Table 2. Data for *H. virescens* larvae from each state are presented below. The data shown in each figure shows the highest LD values obtained thus far (AZ - 1982), the base year for the state and values for each subsequent when data were obtained.

Data by State

Georgia: Populations were sampled and tested in 1980-1982 and in 1986. The 1986 population was somewhat more susceptible than the 1980 population (Figure 1) indicating no change in tobacco budworm susceptibility to cypermethrin has occurred.

Mississippi: Good data were generated on larvae from this area in 1980, 1981, 1983 and 1986 (Figure 2). Two collections were made in 1986; one from a cypermethrin performance complaint field (Site #1) and one from a neighboring field with excellent *Heliothis* control (Site #2). While susceptibility was somewhat less than that observed in 1981 or 1983 there was essentially no difference in susceptibility between the two 1986 samples and the original base sample collected and tested in 1980. All larvae collected in this area have been significantly more susceptible than the Arizona strains collected in 1980 or 1982. No control problems were noted in AZ, therefore, the susceptibility level indicated by this strain would not cause any control problems.

Louisiana: Only two good collections have been made in this area, 1981 and 1986 (Figure 3). Toxicity data indicate the susceptibility of the populations sampled in these two years to be more similar to the susceptibility of populations in Texas, Arizona and California than those in Georgia. Susceptibility has decreased from the 1981 baseline but is still considerably greater than that observed in 1980 and 1982 in AZ.

Texas: Collections have been made from two locations, the Rio Grande Valley and the Uvalde area (Figure 4). Uvalde populations have tended to be less susceptible than the Rio Grande Valley populations; LD values, while higher in 1985 and 1986 than the 1983 values, remain substantially lower than those obtained in 1980 or for Arizona larvae tested in 1980 and 1982. No change was observed in the 1985 and 1986 susceptibility levels.

Arizona: Collections have been made from the Phoenix area since 1980 (Figure 5). The original collections (Site #1) were made from fields which had received a number of insecticide applications with different classes of insecticides. Site #2 were fields which had received a number of pyrethroid treatments prior to collection. The highest LD values generated for the monitoring program were with larvae collected from this state (1980 and 1982). However, no field control problems or consistent trend has been observed state. In fact, high LD values tend to be followed by lower values in succeeding years. The 1986 values were lower than the 1984 values and significantly lower than the baseline values.

California: Data were generated with larvae from the Imperial Valley every year except 1984. The highest