EVALUATION OF TOLERANCE TO INSECTICIDES IN TOBACCO BUDWORM AND BOLLWORM POPULATIONS, 1996

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Abstract

Strains of the tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie), collected in Mississippi were evaluated in bioassays to four classes of insecticides, and mixtures of insecticides and a synergist. High and intermediate levels of resistance were found to cypermethrin and thiodicarb, respectively, in *H. virescens*. No increased tolerance was seen to profenofos in *H. virescens* and no increased tolerance to any insecticide tested was detected in *H. zea*. Significant resistance to *Bacillus thuringiensis* Berliner was not observed in either species during the season. No synergism was detected in a bioassay using insecticide mixtures and piperonyl butoxide on a resistant strain of *H. virescens*. Multiple resistance in *H. virescens* is still evident and resistance to pyrethroids appears to be stabilized.

Introduction

Populations of the tobacco budworm, *Heliothis virescens* (F.), in the mid-South have developed resistance to pyrethroid, carbamate, organophosphorus, and cyclodiene insecticides (Elzen et al. 1990, 1992, 1993, 1994a,b; Martin et al. 1994, 1995). Initial resistance to pyrethroid insecticides in U. S. populations of *H. virescens* has been shown to be due to a kdr-like nerve insensitivity (McCaffery et al. 1989). Evidence for metabolic resistance also exists (Ottea et al. 1993; Clower et al. 1992; Graves et al. 1991; McCaffery et al. 1991; Nicholson and Miller 1985). However, target site insensitivity is probably the major mechanism of resistance to organophosphorus and carbamate insecticides (Kanga et al. 1994).

While *H. zea* has been found to be resistant in the past (Sparks 1981), only recently has evidence for current resistance been demonstrated (Abd-Elghafar et al. 1993; Kanga et al. 1996).

Currently, there are few alternatives to the available classes of insecticides for control of *H. virescens* and *H. zea*. Recently, resistance management strategies have placed greater emphasis on the use of biologicals (i.e. *B. thuringiensis*) and further partitioning of chemical classes into "windows" (Baldwin and Graves 1991).

An investigation of the frequency of insecticide resistance may aid in formulating resistance management plans. The present study evaluates current levels of resistance to four classes of insecticides in *H. virescens* and *H. zea* in Mississippi. An additional bioassay examined possible synergism of insecticide mixtures and piperonyl butoxide (PBO) on a resistant strain of *H. virescens*.

Materials and Methods

Insects

The *H. virescens* and *H. zea* strains evaluated and their collection dates are shown in Table 1. The Stoneville laboratory reference strains (STV-LAB) have been in culture at the Southern Insect Management Laboratory, Stoneville, MS, since collection from a Mississippi cotton field in 1984 (Elzen et al. 1992). The STV-LAB strains are annually infused with wild males captured in pheromone traps placed adjacent to cotton fields near Stoneville.

Heliothis virescens and *H. zea* were collected from the same areas or cotton fields around Stoneville (Washington County) throughout the season in 1996. Generation one (G1) was collected from geranium, *Geranium dissectum* L.; generations two (G2), and three (G3) were collected from cotton, *Gossypium hirsutum* L.

All strains were reared in a similar manner. Adults were maintained in 3.8-liter cardboard cartons covered with cotton gauze as an ovipositional substrate and were fed a 5% sugar-water solution. Eggs were collected at least every other day and allowed to hatch at room temperature. Larvae were reared on a soybean flour-wheat germ diet (King and Hartley 1985).

Insecticides

Formulated insecticides tested were profenofos (Curacron 8 emulsifiable concentrate [EC]; CIBA-GEIGY, Greensboro, NC), cypermethrin (Cymbush 3 [EC]; ZENECA, Mountain View, CA), thiodicarb (Larvin 3.2 flowable [F]; Rhone-Poulenc Agric. Co., Research Triangle Park, NC), *lambda*-cyhalothrin (Karate 1 [EC]; ZENECA, Mountain View, CA), progargite (Comite 6.55 [EC]; Uniroyal, Middlebury, CT), phosmet (Imidan 70 wettable powder [WP]; Gowan, Yuma, AZ), *Bacillus thuringiensis* Berliner (Dipel ES; Abbott Laboratories, North Chicago, IL), and (MVPII; Mycogen Corp., San Diego, CA), and the synergist piperonyl butoxide (Butacide 8 [EC]; ZENECA, Mountain View, CA).

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Spray Chamber Bioassays

The methods and materials used in this bioassay were previously described in detail (Elzen et al. 1992). Cotton terminals clipped from greenhouse grown plants were placed in floral piks (Dakota Plastics, Watertown, SD). Each treatment consisted of three replicates of 20 terminals each. Controls were treated with water only. Formulated insecticides were applied with a calibrated laboratory spray chamber (Elzen et al. 1992). A single third instar (20 ± 3 mg) was placed on each terminal 30 min after spraying and each plant was covered with a ventillated paper cup. Treatment efficacy was determined after 72 h and numbers of dead or moribund larvae were used to calculate total mortality. Control mortality was never greater than 5%; data were corrected with Abbott's (1925) formula. Percent mortalities were transformed by arcsin and analyzed by analysis of variance; means were separated by least significant difference ($P = 0.05$ [SAS Institute 1988]).

Results and Discussion

Few changes in tolerance to the insecticides tested were found in *H. zea* strains collected in 1996, with the exception of an increase in the effect of MVPII on the G2 strain (Table 2). Thus, these tests did not detect increased tolerance in the *H. zea* strains tested (Table 2). In contrast, tolerance to cypermethrin and endosulfan has recently been demonstrated in *H. zea* field populations from Texas (Kanga et al. 1996).

The continued presence of resistance to pyrethroid and carbamate insecticides was seen in *H. virescens* strains collected in 1996 (Table 3). Significant levels of resistance to cypermethrin and thiodicarb were found in the G1, G2, and G3 strains. Percentage mortalities in all strains were significantly lower than the mortality of the STV-LAB strain in response to treatment with MVPII; however, it may be premature to suggest that resistance to *B. thuringiensis* exists because there was no evidence of resistance to Dipel. Bioassays using *B. thuringiensis* should be intensified in the future.

Synergism was not demonstrated when PBO, phosmet, or propargite were mixed with *lambda*-cyhalothrin. Either metabolic resistance is not present or the bioassay method could not detect synergism. Synergism of pyrethroids by PBO on *H. virescens* has previously been demonstrated (Elzen et al. 1993, Martin et al. 1994). Phosmet and propargite were determined to be promising synergists for pyrethroids used against *H. armigera* in Australia (Forrester et al. 1993). Propargite is currently being used in Australia as a mixture with pyrethroids to control both mites and resistant *H. armigera* on cotton (R. Moore, pers. comm.).

Until recently, following the initial documentation of pyrethroid resistance (Plapp and Campanhola 1986; Leonard et al. 1987; Luttrell et al. 1987), major concerns had been primarily with pyrethroid resistance in *H. virescens*. Resistance to non-pyrethroids was documented in Mississippi and Louisiana in 1990 field populations (Leonard et al. 1991; Elzen et al.1992). Resistance to all classes of insecticides was again found in Louisiana and Mississippi, and also Texas in 1991 (Elzen et al. 1994a; Martin et al. 1995). The data presented herein document the continued presence of resistance to pyrethroid and carbamate insecticides in *H. virescens*. However, the data do not indicate resistance to the organophosphorus insecticide tested. Furthermore, the possible increased tolerance to *B. thuringiensis* needs confirmation through additional testing. In other cases, considerable variation in susceptibility of *H. virescens* to *B. thuringiensis* has been shown (Stone and Sims 1993).

Multiple and cross-resistance in *H. virescens*, as well as continued development of resistance in other pests, could seriously jeopardize the cotton industry in the U. S. Resistance management plans should strongly emphasize strategies that involve conservation of all insecticides used against *H. virescens* and *H. zea*. Increased emphasis on the use of biological insecticides, insect growth regulators, and the discovery and registration of new classes of insecticide chemistry for control of all pests should be emphasized.

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Table 1. Location and collection date for 1996 field strains of *H. virescens* and *H. zea* tested in bioassays.

STRAIN	LOCATION	DATE
STV-LAB	Laboratory Reference	
G1	Washington County, MS	7-31 May
G2	Washington County	6-28 June
G3	Washington County	11-31 August

Table 2. Efficacy of selected insecticides against laboratory-susceptible and field-collected strains of *H. zea* in a spray chamber bioassay.

Treatment	lb[AI]/a	% Mortality 72 h after treatment			
		STV-LAB	G1	G2	G3
Cypermethrin	0.08	93.3c A	88.9c A	95.5b A	95.5b A
Thiodicarb	0.90	93.3c AB	88.9c A	95.5b B	95.5b AB
Profenofos	1.00	93.3c A	88.9c A	95.5b A	95.5b A
B. thuringiensis	2.00[a]	60.0b A	53.4b A	---	---
B. thuringiensis	3.00[b]	37.7a A	37.8a A	62.2a B	44.4a A

Means within a column followed by the same lowercase letter or means within a row followed by the same uppercase letter are not significantly different ($P = 0.05$; least significant difference [SAS Institute 1988]).
[a]Dipel ES; pts/acre.
[b]MVPII; pts/acre.

Table 3. Efficacy of selected insecticides against laboratory-susceptible and field-collected strains of *H. virescens* in a spray chamber bioassay

Treatment	lb[AI]/a	% Mortality 72 h after treatment			
		STV-LAB	G1	G2	G3
Cypermethrin	0.08	91.1c C	62.2b B	48.9a bAB	37.8a A
Thiodicarb	0.90	93.3c B	71.1b A	68.9b cA	75.5b A
Profenofos	1.00	93.3c A	93.3c A	82.2c A	100.0 cB
B. thuringiensis	2.00[a]	42.2a A	64.4b A	---	---
B. thuringiensis	3.00[b]	62.2b B	40.0a A	42.2a A	44.4a A

Means within a column followed by the same lowercase letter or means within a row followed by the same uppercase letter are not significantly different ($P = 0.05$; least significant difference [SAS Institute 1988]).
[a]Dipel ES; pts/acre.
[b]MVPII; pts/acre.

Table 4. Efficacy of selected insecticides against a resistant field strain of *H. virescens* in a spray chamber bioassay.

Treatment	lb[AI]/a	% Mortality 72 h after treatment
Cyhalothrin	0.025	66.6d
Cyhalothrin	0.015	46.6c
PBO	0.25	4.4ab
Cyhalothrin + PBO	0.015 + 0.25	60.0cd
Propargite	0.60	8.8b
Cyhalothrin + Propargite	0.015 + 0.60	53.3cd
Phosmet	0.125	0.0a
Cyhalothrin + Phosmet	0.015 + 0.125	51.1cd

Means within a column followed by the same letter are not significantly different ($P = 0.05$; least significant difference [SAS Institute 1988]).