CHANGES IN TOLERANCE TO INSECTICIDES IN TOBACCO BUDWORM POPULATIONS, 1995

G. W. Elzen,
Research Entomologist, USDA-ARS
Southern Insect Management Laboratory
Stoneville, MS

Abstract

Strains of the tobacco budworm, *Heliothis virescens* (F.), collected in Mississippi were evaluated in bioassays to four classes of insecticides. High levels of resistance were found to cypermethrin, methomyl, and thiodicarb. Significant resistance to *Bacillus thuringiensis* Berliner was not observed during the season. Multiple resistance is still apparent and resistance to pyrethroids appears to be increasing.

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, 1994; Martin et al. 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).

Currently, there are few alternatives to these classes of insecticides for control of *H. virescens*. 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 generational changes in insecticide tolerance may aid in formulating resistance management plans. The present study evaluates cur-rent levels of resistance to four classes of insecticides in *H. virescens* in Mis-sissippi. An additional strain of *H. virescens* was also examined in bioassays and an ovicide bioassay was performed on susceptible and resistant strains.

Materials and Methods

Insects

The *H. virescens* strains evaluated and their collection dates are shown in Table 1. The Stoneville laboratory reference strain (STV-LAB) has 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 strain is annually infused with wild males captured in pheromone traps placed adjacent to cotton fields near Stoneville.

H. virescens were collected from the same areas or cotton fields around Stoneville (Washington County) throughout the season in 1995. Generation one, G1, was collected from geranium, *Geranium dissectum* L.; generations two, G2, three, G3, and four, G4, were collected from cotton, *Gossypium hirsutum* L. One additional strain (CAL) was collected from the hill area of Mississippi, Calhoun County. In addition, *H. virescens* moths captured in pheromone traps in Washington County in 1995 were bioassayed for the development of resistance using the adult vial test (Plapp et al. 1987; Kanga et al. 1995).

All *H. virescens* 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), cyfluthrin (Baythroid 2 [EC]; Bayer, Inc., Kansas City, MO), amitraz (Ovasyn 1.5 [EC]; AgrEvo USA Co., Wilmington, DE), thiodicarb (Larvin 3.2 flowable [F]; Rhone-Poulenc Agric. Co., Research Triangle Park, NC), methomyl (Lannate 2.4 liquid [LV]; E. I. Dupont de Nemours & Co., Wilmington, DE), acephate (Orthene 90 soluble [S]; Valent U.S.A. Corporation, Walnut Creek, CA), imidacloprid (Provado 1.6 [F]; Bayer, Inc., Kansas City, MO) and *Bacillus thuringiensis* Berliner (Dipel ES; Abbott Laboratories, North Chicago, IL).

Technical-grade cypermethrin, profenofos, and methomyl (Chem Service, Inc., West Chester, PA) were used in the adult vial test.

Adult Vial Test

Wire cone traps (Hartstack et al. 1979) baited with artificial sex pheromone lures (Hendricks et al. 1987) were used to collect adult male *H. virescens*. Twenty traps were placed on cotton field borders in Washington County, MS, near Stoneville. Traps were monitored weekly except when

moths were collected for bioassays, where they were collected from one-night's capture.

The adult vial test procedure was described in detail in Plapp et al. (1987)and Kanga et al. (1995). Male moths from a one-night capture were removed and taken to the laboratory. One moth was placed in a 20-ml glass scintillation vial previously coated with a residual film of cypermethrin at a dose of 5 and 10 μg per vial; methomyl at 1 and 2.5 μg; and profenofos at 5 and 10 μg. Vials treated with acetone were used as controls. Vials containing moths were stored on their sides at room temperature and mortality was recorded after 24 h of exposure. Adults that were unable to fly a short distance (less than 1 m) were considered dead. Control mortality was never greater than 5%; data were corrected with Abbott's (1925) formula.

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]).

Ovicidal Activity Bioassay

Treatments were applied to cotton grown in small plots at Stoneville, MS. Plot size was 9 m x 4 rows arranged in a randomized complete block with 4 replicates/treatment. Treatments were applied on 12 July (Test 1) and 18 August (Test 2) by a high-clearance spray machine equipped with a compressed air spray system. Total spray volume was 6 gal/acre at 5 mph and 35 psi using two TX10 nozzled/row. *H. virescens* eggs were obtained from cultures maintained in the laboratory. In Test 1, eggs from the STV-LAB strain were used; Test 2 used eggs from the G2 strain. All eggs used were laid the previous night on tulle cloth material. Eggs were not washed or chemically treated before use and were easily removed by crumpling the material. Eggs treated in the field were placed on the upper surface of the last 3-4 expanded leaves in the terminal of 4 plants in each replication by brushing a weak solution of xanthan gum and water on the leaves and sprinkling eggs on the surface. Plots were sprayed 30 min after application of the eggs. Eggs remained in the field for 2 h after treatment and were then taken to the laboratory to determine mortality. The number of viable eggs was counted on each leaf in the laboratory 24 h after being sprayed. The number of eggs averaged 15.0 ± 5.5 (S.D.). Leaves were held in petri dishes on water-moistened filter paper at 82° F. The number of unhatched eggs was determined at 48 and 72 h after eggs were applied. Eggs were considered dead when black and shriveled. Cumulative egg mortality (EM) 72 h after treatment is reported. Hatched larval mortality (LM) was observed at 48 and 72 h after treatment and is reported as cumulative larval mortality at 72 h after treatment. Larval mortality was used to calculate total mortality (TM) at 72 h.

Results and Discussion

The number of moths captured during 1995 in pheromone traps placed in Washington County is shown in Figure 1. Results of the adult vial tests with cypermethrin on *H. virescens* moths obtained from the traps showed an increase in resistance from May to June (as the cotton growing season commenced) followed by a relatively stable level of resistance during the remaining test period (Figure 2). Since 1987 (when extensive monitoring for resistance began using this technique) levels of resistance to pyrethroids have generally increased. In general, the pattern observed was increasing resistance as the cotton growing season progressed and a correlation with increased usage of insecticides for control of *H. virescens* (Graves et al. 1991, 1992; Mullins et al. 1991; Clower et al. 1992). Resistance to methomyl (Figure 3) and profenofos (Figure 4) likewise increased from May to June. However, resistance levels dropped from June to July and then again increased from July to August. These patterns could be a result of changes in insecticide use.

A decrease in efficacy of all classes of insecticides tested except the *B. thuringiensis* based material was detected initially in strains collected from Washington County using the spray chamber bioassay in comparison with mortality of the STV-LAB strain using the discriminating doses.

The efficacy of cypermethrin remained low from May through generation 4 in August (Figure 5). The efficacy of thiodicarb was significantly lower in August than in May and June (Figure 6). Percent mortality with thiodicarb was lower in collected strains than for the STV-LAB strain throughout the season, but efficacy was greater than with cypermethrin. The efficacy of profenofos increased following generation 1 and was not different from the STV-Lab strain through generation 4 in the Washington County strains (Figure 7). Profenofos was more effective than other classes of insecticides. *B. thuringiensis* was more effective on the July generation 3 compared with the May generation 1. The efficacy of methomyl was significantly reduced initially and through the remaining generations (Figure 9). There was some evidence that this pattern was similar to the pattern of efficacy of thiodicarb, as might be predicted, given that these are both carbamates. The

patterns observed may indicate multiple resistance in carbamates (Elzen 1994).

The CAL strain showed a high level of resistance to cypermethrin, an intermediate level of resistance to thiodicarb and methomyl, and no resistance to the organophosphorus insecticides profenofos and acephate, nor resistance to *B. thuringiensis* in comparison with the STV-LAB strain (Table 2). The cotton field from which this strain was collected had received six applications of a pyrethroid insecticide prior to collection of the larvae.

Provado, an insecticide targeted primarily against aphids and plant bugs, gave control comparable to other materials in the ovicide bioassays (Table 3). Significantly lower larval mortality resulted from Ovasyn treatment in most cases. Lower egg mortality of Baythroid on the G2 strain is an indication of possible resistance in eggs.

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. 1994; Martin et al. 1995). The data presented herein document the continued presence of resistance to all classes of insecticides in *H. virescens*. However, the data do not yet indicate resistance to *B. thuringiensis*. 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*. 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.

Acknowledgments

Larry Adams (Southern Insect Management Laboratory, Stoneville, MS) reared insects, made field collections, and performed bioassays. The Calhoun County strain was provided by Blake Layton (Mississippi Cooperative Extension Service, Mississippi State, MS).

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Table 1. Location and collection date for 1995 field strains of tobacco budworm tested in bioassays. See text for additional notes.

Strain	Location	Date
STV-LAB	Laboratory Reference	
G1	Washington County, MS	8-17 May
G2	Washington County	13-16 June
G3	Washington County	18-24 July
G4	Washington County	17-22 August
CAL	Calhoun County, MS	11 August

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

Insecticide	Rate (lb[AI]/a)	% Mortality 72 h after treatment	
		STV-LAB	CAL
Cypermethrin	0.08	95.5bA	15.5aB
Thiodicarb	0.90	95.5bA	75.6cB
Profenofos	1.00	95.5bA	97.8dA
B. thuringiensis	2.00 pt	40.0aA	42.2bA
Methomyl	0.45	95.5bA	64.4bcB
Acephate	1.00	----	93.3d

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]).

Table 3. Efficacy of selected insecticides against a laboratory-susceptible and field-collected strain of tobacco budworm in an ovicide bioassay.

		H. virescens (susceptible STV-LAB)			G2 (Resistant *H. virescens*)		
Insecticide	Rate (lb [AI]/a)	% EM	% LM	% TM	% EM	% LM	% TM
Baythroid 2 EC	0.03	43.2b	85.8c	65.5b	17.5ab	75.5c	60.8d
Baythroid 2EC + Provado 1.6F	0.03 0.022	31.5ab	63.5bc	52.5ab	23.1b	51.3bc	49.0cd
Provado 1.6F	0.044	36.7ab	36.5b	47.0ab	30.0c	21.6ab	37.1bc
Provado 1.6F	0.022	24.7a	45.3b	44.7a	20.5b	7.1a	23.8b
Provado 1.6F	0.0125	----	----	----	5.6ab	0.0a	4.2a
Ovasyn 1.5E	0.25	44.5b	8.8a	46.2a	63.8d	9.7a	64.1d
Larvin 3.2F	0.25	31.2ab	82.0c	62.2b	56.4d	35.7b	62.9d

EM = egg mortality; LM = larval mortality; TM = total mortality. Means within a column, analyzed separately for each strain, followed by the same letter are not significantly different
(P = 0.05; LSD).

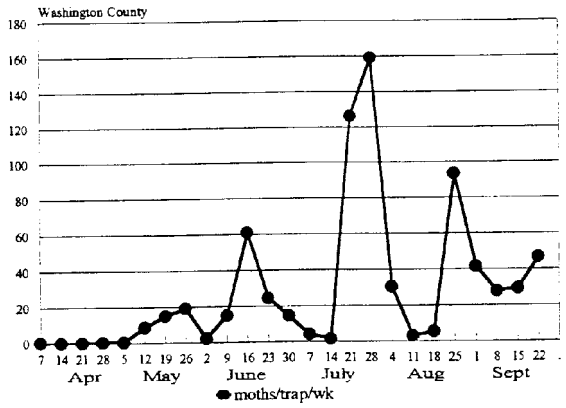

Figure 1. Number of tobacco budworm moths captured in pheromone traps in Washington County, MS in 1995 (moths/trap.wk).

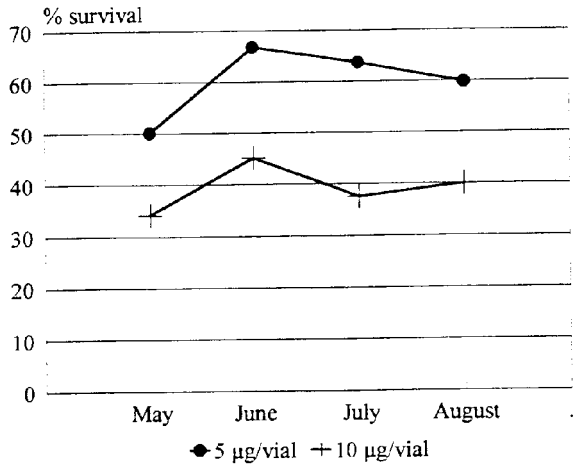

Figure 2. Results of the adult vial tests with cypermethrin on male tobacco budworms captured in pheromone traps in Washington County, MS in 1995.

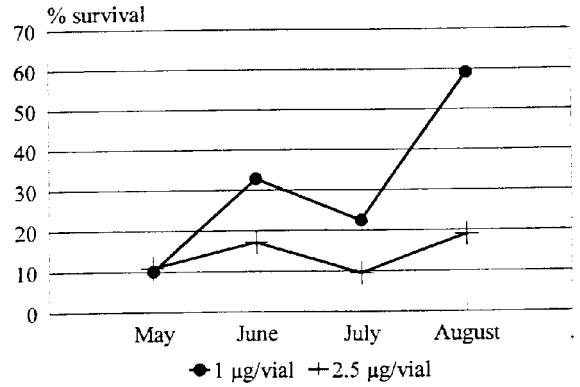

Figure 3. Results of the adult vial tests with methomyl on male tobacco budworms captured in pheromone traps in Washington County, MS in 1995.

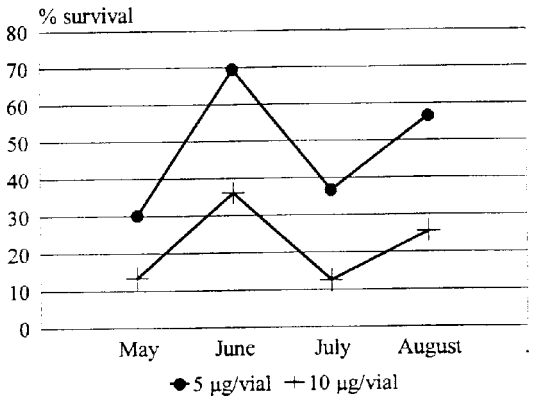

Figure 4. Results of the adult vial tests with profenofos on male tobacco budworms captured in pheromone traps in Washington County, MS in 1995.

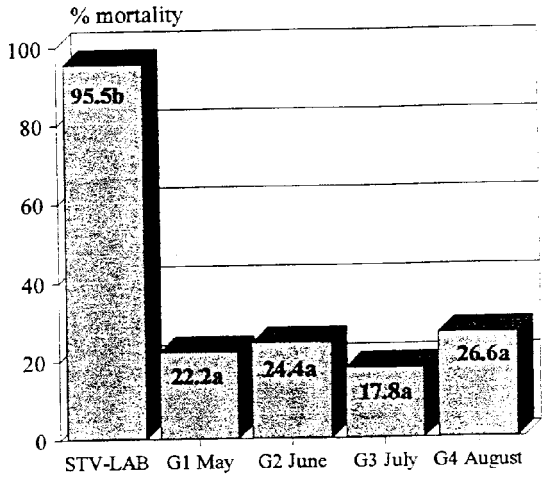

Figure 5. Responses by a laboratory reference strain and field strains of the tobacco budworms collected in Washington County, MS to cypermethrin (0.08 lb[AI]/a) in a spray chamber bioassay. Bars carrying the same letter are not significantly different ($P \geq 0.05$; least significant difference [SAS Institute 1988]).

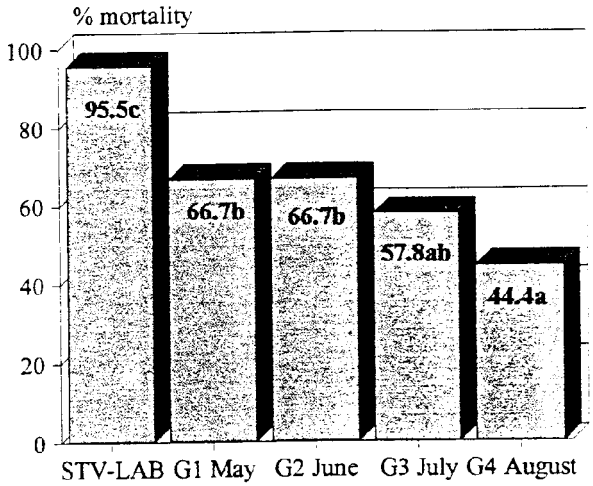

Figure 6. Responses by a laboratory reference strain and field strains of the tobacco budworm collected in Washington County, MS to thiodicarb (0.9 lb[AI]/a) in a spray chamber bioassay. Bars carrying the same letter are not significantly different ($P \geq 0.05$; least significant difference [SAS Institute 1988]).

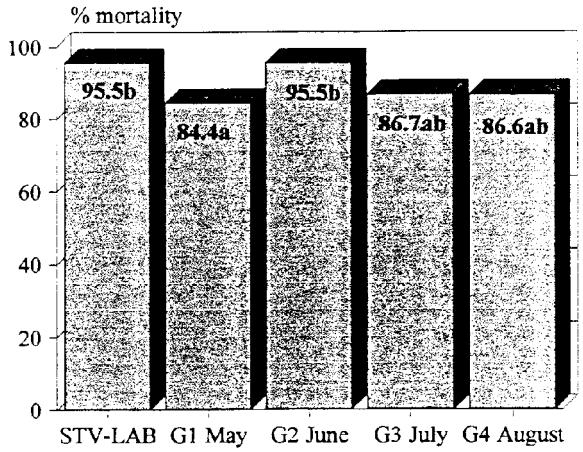

Figure 7. Responses by a laboratory reference strain and field strains of the tobacco budworms collected in Washington County, MS to profenofos (1.0 lb[AI]/a) in a spray chamber bioassay. Bars carrying the same letter are not significantly different ($P \geq 0.05$; least significant difference [SAS Institute 1988]).

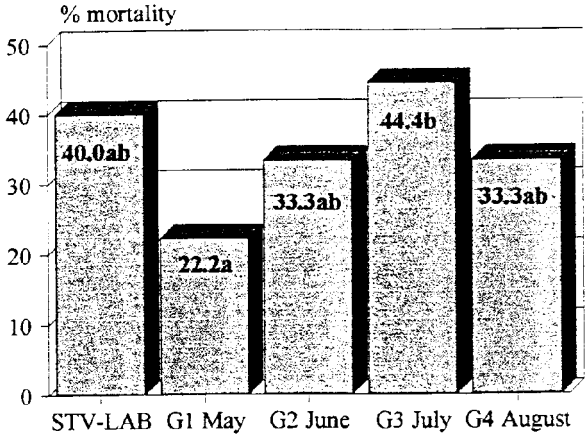

Figure 8. Responses by a laboratory reference strain and field strains of the tobacco budworms collected in Washington County, MS to *B. thuringensis* (2.0 pt/a) in a spray chamber bioassay. Bars carrying the same letter are not significantly different ($P \geq 0.05$; least significant difference [SAS Institute 1988]).