INSECTICIDE RESISTANCE MONITORING IN LEPIDOPTERAN COTTON PESTS

Russell J. Ottens, John R. Ruberson, and Phillip M. Roberts
Department of Entomology, University of Georgia, Tifton

Abstract

In 2004, larvae and adults of the bollworm, *Helicoverpa zea*, and adults of the tobacco budworm, *Heliothis virescens*, were bioassayed for resistance to selected carbamate, organophosphate, and pyrethroid insecticides.

Bollworm cultures were established from larvae collected in Tift County corn in June 2004. Tobacco budworm cultures were established from larvae collected in tobacco in July 2004 in Tift County and from adults collected with sugar lines in Mitchell and Terrell Co. cotton in June and July 2004. Newly-hatched larvae obtained from eggs of the collected larvae or moths were used in the laboratory tests. Larvae were treated with 99.2% technical grade acephate, 89.9% technical grade cyhalothrin, 92.4% technical grade cypermethrin, 98% technical grade methomyl, or 95% technical grade permethrin. Stock solutions in acetone were prepared and serially diluted to obtain the desired concentrations. Larvae were checked 72 hr post-treatment for death.

For adult tobacco budworm and bollworm bioassays, moths were collected in the summer of 2004 from pheromone traps placed near cotton fields at two locations in Tift Co. There were four tobacco budworm traps and four bollworm traps. Three additional tobacco budworm traps were placed at separate locations in Colquitt Co. Tests were performed using 20 ml scintillation vials coated with an acetone solution of 92.4% technical grade cypermethrin with dosages ranging from 1 to 10 µg/vial and an acetone check. One moth was placed in each vial and survival was checked after 24 hours.

No changes in susceptibility of corn earworm larvae to acephate or cypermethrin were detected, in comparison with historical levels. Similarly, in the adult tests of corn earworms from Tift County, we observed no change in pyrethroid tolerance compared to previous years.

In the larval bioassays, susceptibility of the various populations of tobacco budworms for acephate and methomyl was comparable to historical levels. However, there were significant changes in the susceptibility of tobacco budworm larvae to cypermethrin. The populations from Terrell and Tift Counties were 13-14 times more tolerant of cypermethrin than historical averages of the susceptible Tift County populations, suggesting a significant level of tolerance for pyrethroids. Further, the adult assays indicated that the budworm moths from Colquitt County were approximately 8 times as tolerant of cypermethrin as the historically susceptible populations. These results indicate that tolerance to pyrethroids in tobacco budworm may be increasingly widespread in Georgia, and that there is a great need for growers to utilize insecticide resistance management practices to steward these products.

Introduction

Insecticides remain the method of choice for control of lepidopteran pests in Georgia cotton, though great strides have been made during the past two decades in reducing chemical use. The successful eradication of the boll weevil combined with the planting of transgenic cotton, effective scouting, and careful crop management have served to significantly lessen reliance on insecticides. Nevertheless, the older insecticides, particularly pyrethroids, continue to play a key role in management of pests in cotton due to their general effectiveness and their low costs. Newer insecticides have become available, but their specificity tends to impose limits on their general utility, and they are more expensive to use. It is, therefore, important that we understand the susceptibility of target pests to insecticides so that we can continue to use them effectively and so we can make appropriate management decisions to prolong the life of effective insecticides.

Though Georgia has not experienced any widespread resistance problems during this time, other states have and the potential exists. Since 1979, we have performed bioassays on major lepidopteran cotton pests to monitor development of insecticide resistance. In 2004, larvae and adults of the bollworm, *Helicoverpa zea*, and the tobacco budworm, *Heliothis virescens*, were bioassayed for resistance to certain carbamate, organophosphate, and pyrethroid insecticides.

Materials and Methods

Larval Bioassays. Bollworm cultures were established from larvae collected in Tift County corn in June 2004. Tobacco budworm cultures were established from larvae collected in tobacco in July 2004 in Tift County and from adults collected with sugar lines in Mitchell and Terrell Co. cotton in June and July 2004. Sugar lines consisted of field-edge rows of cotton that were sprayed with a 5% sugar solution shortly before sunset. Moths coming to the sprayed plants to feed on the sugar-water were then captured as they arrived. Field-collected larvae were reared to adulthood and eggs were collected from the moths confined in 1 gal plastic containers with cheesecloth lids serving as oviposition sites. Upon hatching, neonate larvae were placed on pinto bean meal synthetic diet in 30 ml plastic cups. For the bollworm bioassays, F1 larvae were used exclusively. For the tobacco budworm, F2 larvae were used in all cases except F3 larvae from Terrell Co. bioassayed with cyhalothrin. All life stages of the insects were held in an incubator at 27 $\pm$ 2^{o}C, ca 60% RH and a 14:10 hr light: dark cycle.

Evaluation of larval susceptibility of *H. zea* basically followed protocol outlined in the ESA Standard Test Method for detection of resistance in *Heliothis* spp. (Anon. 1970). Larvae were treated with 99.2% technical grade acephate, 89.9% technical grade cyhalothrin, 92.4% technical grade cypermethrin, 98% technical grade methomyl, or 95% technical grade permethrin. Stock solutions in acetone were prepared and serially diluted to obtain the desired concentrations. Microgram equivalents were calculated, adjusting for the percent active ingredient in the technical materials. One microliter of solution was applied to the dorsal thoracic region of each larva using a Microliter no.

705 (Hamilton Company, Reno, NV) hand-held applicator. Three to five replications were used in each bioassay with ten third instar, 30-40 mg larvae per dosage and an acetone check.

Observations were made 72 hr post-treatment and a larva was considered dead if it made no movement when prodded with a pencil point. Larvae were considered moribund if they moved when prodded, yet appeared black and as small or smaller than their size at treatment. These were considered alive when determining LD (lethal dosage) values, but considered dead when calculating ED (effective dosage) values. In many instances, larvae treated with pyrethroids linger on several days beyond observation time as moribund larvae that eventually die. For this reason we present ED values as well as LD values to present a more complete picture of dosage-response. Data were analyzed using Daum's (1970) probit analysis computer program.

Adult Bioassays. For adult tobacco budworm and bollworm bioassays, moths were collected in the summer of 2004 from pheromone traps placed near cotton fields at two locations in Tift Co. There were four tobacco budworm traps and four bollworm traps. Three additional tobacco budworm traps were placed at separate locations in Colquitt Co. Pheromone lures attached to each trap were replaced every two weeks. The morning of each test, the trap tops were brought to the laboratory and the moths were removed for the bioassays. The empty trap tops were then returned to the field. In all instances, bioassayed moths were trapped the previous night and never confined during the heat of the day.

Tests were performed using 20 ml scintillation vials coated with an acetone solution of 92.4% technical grade cypermethrin with dosages ranging from 1 to 10 µg/vial and an acetone check. A total volume of 0.5 ml of acetone/insecticide mixture was placed in each vial and rolled horizontally on a modified hot dog roller until the acetone had evaporated. The vials were then stored in a freezer until used. As in the larval bioassays, the amount of technical compound weighed out for the stock solution was corrected for purity. One moth was placed in each vial with the cap screwed on loosely. Percent survival was checked after 24 hours. Counts were taken for live, knocked-down (moribund), and dead moths. Only moths able to fly in a normal manner were counted as alive. Numbers assayed varied with the number of moths available and the percentage data were transformed (arcsin($\sqrt{\%}$)) prior to analysis with the General Linear Models procedure of SAS (SAS Institute 1988).

Results and Discussion

Larval Bioassays. The ED_{50}, ED_{90}, LD_{50}, and LD_{90} values for the 2004 Tift Co. bollworm larval bioassays are presented in tables 1, 2, 3, and 4, respectively. All values for cypermethrin and methomyl were lower than the Tift Co. value for 2003, and lower than the average of bioassays performed on Tift Co. larvae since 1983. ED_{50} and ED_{90} values for acephate were slightly higher.

Tables 5, 6, 7, and 8 present the ED_{50}, ED_{90}, LD_{50}, and LD_{90} values, respectively, for the 2004 tobacco budworm larval bioassays. For cypermethrin, an ED_{50} of 6.33 µg/g larval wt. was found in Terrell Co. larvae and 5.81 µg/g larval wt. In Tift Co. larvae. These values are ca 8x and ca 7x higher, respectively, than the Tift Co. long term average of 0.78 µg/g larval wt. since testing began in 1983. Prior to 2004, our highest Tift Co. ED_{50} was 0.79 µg/g larval wt. in 1988. If the 2004 bioassay is not included in the long-term Tift Co. average, Terrell Co. larvae are ca 14x higher and Tift Co. larvae are ca 13x higher. There may also be resistance to cyhalothrin (another pyrethroid) by the same populations. An ED_{50} of 0.60 µg/g larval wt. was found in Terrell Co. larvae and 0.28 µg/g larval wt. in Tift Co. larvae, ca 10x higher and 4x higher, respectively than the Tift Co. long term average (including 2004) of 0.06 µg/g larval wt. since testing began in 1985. Prior to 2004, our highest Tift Co. ED_{50} was 0.13 µg/g larval wt. in 1988. These findings should be of special concern as it appears there is pyrethroid resistance in both Terrell and Tift Co. tobacco budworms.

Adult Bioassays. Tables 9 and 10 present the results of our adult tobacco budworm and bollworm glass vial bioassays. A diagnostic dose of cypermethrin is considered to be 10 µg/vial for *H. virescens* and 5 µg/vial for *H. zea*. At 10 µg/vial, there was 6.7% survival for Tift Co. *H. virescens*, about twice the 2003 value of 3.2%. There was even higher survival (28.6%) for Colquitt Co. *H. virescens*. At 5 µg/vial, there was 3.8% survival for *Helicoverpa zea* from Tift County compared to 30.0% from the same location last year. Though cypermethrin appears even more effective on *H. zea* than last year, *H. virescens* seems ca twice as tolerant as was the case in 2003 and Colquitt Co. populations seem ca 9x more tolerant.

Apparent pyrethroid resistance in larval and adult tobacco budworm bioassays should be viewed with great concern. The 2004 results were the most widespread incidence of pyrethroid tolerance in tobacco budworm of year to date. Interestingly, there was not concomitant elevation of tolerance for organophosphate (acephate) or carbamate (methomyl). This contrasts with the findings of Zhao et al. (1996) who observed cross-resistance among these classes in a budworm population from Louisiana. This difference suggests that other resistance mechanisms may be at play in our populations than is the case for at least some populations in the Midsouth. Future monitoring in Georgia is essential.

References Cited

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Table 1. ED_{50}'s for various insecticides against Tift Co. larval *Helicoverpa zea* (CEW) at 72 hr post-treatment. 2004.

Chemical	Gen.	No. Reps	ED_{50} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2003	Change (+/-) from Tift Co. avg	Slope $\pm$ SE
Acephate	F_1	4	21.90	17.36 - 27.72	+3.30	+4.97	1.90 $\pm$ 0.20
Cypermethrin	F_1	4	0.24	0.18 - 0.30	-0.30	-0.02	2.23 $\pm$ 0.28
Methomyl	F_1	4	1.68	1.23 - 2.20	-19.07	-3.84	1.68 $\pm$ 0.21

Table 2. ED_{90}'s for various insecticides against Tift Co. larval *Helicoverpa zea* (CEW) at 72 hr post-treatment. 2004.

Chemical	Gen.	No. Reps	ED_{90} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2003	Change (+/-) from Tift Co. avg	Slope $\pm$ SE
Acephate	F_1	4	103.82	73.64 - 168.44	+26.37	+38.70	1.90 $\pm$ 0.20
Cypermethrin	F_1	4	0.91	0.69 - 1.37	-0.56	-0.05	2.23 $\pm$ 0.28
Methomyl	F_1	4	9.70	6.71 - 16.86	-55.99	-29.44	1.68 $\pm$ 0.21

Table 3. LD_{50}'s for various insecticides against Tift Co. larval *Helicoverpa zea* (CEW) at 72 hr post-treatment. 2004.

Chemical	Gen.	No. Reps	LD_{50} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2003	Change (+/-) from Tift Co. avg	Slope $\pm$ SE
Acephate	F_1	4	27.88	21.70 - 36.22	-13.41	-18.22	1.69 $\pm$ 0.18
Cypermethrin	F_1	4	0.31	0.11 - 0.58	-0.54	-0.57	1.75 $\pm$ 0.40
Methomyl	F_1	4	2.12	1.01 - 3.71	-86.96	-31.76	1.24 $\pm$ 0.23

Table 4. LD_{90}'s for various insecticides against Tift Co. larval *Helicoverpa zea* (CEW) at 72 hr post-treatment. 2004.

Chemical	Gen.	No. Reps	LD_{90} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2003	Change (+/-) from Tift Co. avg	Slope $\pm$ SE
Acephate	F_1	4	159.75	107.29 - 283.17	-122.45	-278.58	1.69 $\pm$ 0.18
Cypermethrin	F_1	4	1.7	0.85 - 11.06	-2.14	-3.66	1.75 $\pm$ 0.40
Methomyl	F_1	4	22.67	10.70 - 111.52	-753.41	-1765.53	1.24 $\pm$ 0.23

Table 5. ED_{50}'s for various insecticides against larval *Heliothis virescens* (TBW) at 72 hr post-treatment. 2004.

Chemical	Gen.	No. Reps	ED_{50} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2002	Change (+/-) from Tift Co. avg	Slope ± SE
Acephate							
Mitchell Co.	F_2	3	43.41	32.14 - 58.37	nt	+20.60	1.97 ± 0.34
Terrell Co.	F_2	3	50.34	31.78 - 71.18	nt	+27.53	1.65 ± 0.32
Tift Co.	F_2	5	43.96	19.34 - 66.01	nt	+21.15	1.38 ± 0.37
Cyhalothrin							
Terrell Co.	F_3	4	0.60	0.44 - 0.84	+0.54	+0.54	1.50 ± 0.22
Tift Co.	F_2	4	0.28	0.20 - 0.39	+0.22	+0.21	1.60 ± 0.23
Cypermethrin							
Mitchell Co.	F_2	4	1.10	0.84 - 1.44	+0.38	+0.32	1.70 ± 0.20
Terrell Co.	F_2	4	6.33	4.64 - 8.96	+5.61	+5.55	1.40 ± 0.23
Tift Co.	F_2	4	5.81	3.68 - 13.13	+5.09	+5.03	1.48 ± 0.32
Methomyl							
Terrell Co.	F_2	3	2.84	1.29 - 4.72	nt	-5.06	1.07 ± 0.27
Tift Co.	F_2	4	12.92	8.91 - 21.24	nt	+5.01	1.52 ± 0.29

nt indicates that this insecticide was not tested in 2002

Table 6. ED_{90}'s for various insecticides against larval *Heliothis virescens* (TBW) at 72 hr post-treatment. 2004.

Chemical	Gen.	No. Reps	ED_{90} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2002	Change (+/-) from Tift Co. avg	Slope ± SE
Acephate							
Mitchell Co.	F_2	3	193.61	123.77 - 443.45	nt	+82.48	1.97 ± 0.34
Terrell Co.	F_2	3	301.76	182.50 - 820.92	nt	+190.63	1.65 ± 0.32
Tift Co.	F_2	5	369.62	197.25 -2185.57	nt	+258.49	1.38 ± 0.37
Cyhalothrin							
Terrell Co.	F_3	4	4.31	2.51 - 10.87	+4.20	+4.05	1.50 ± 0.22
Tift Co.	F_2	4	1.79	0.20 - 0.39	+1.68	+1.53	1.60 ± 0.23
Cypermethrin							
Mitchell Co.	F_2	4	6.24	4.18 - 11.30	+2.61	+1.80	1.70 ± 0.20
Terrell Co.	F_2	4	52.20	28.32 - 160.24	+48.57	+47.76	1.40 ± 0.23
Tift Co.	F_2	4	42.54	17.09 - 355.64	+38.91	+38.10	1.48 ± 0.32
Methomyl							
Terrell Co.	F_2	3	44.56	18.61 - 464.49	nt	-50.27	1.07 ± 0.27
Tift Co.	F_2	4	89.51	44.22 - 374.75	nt	-5.32	1.52 ± 0.29

nt indicates that this insecticide was not tested in 2002

Table 7. LD_{50}'s for various insecticides against larval *Heliothis virescens* (TBW) at 72 hr post-treatment. 2004.

Chemical	Gen.	No. Reps	LD_{50} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2002	Change (+/-) from Tift Co. avg	Slope $\pm$ SE
Acephate							
Mitchell Co.	F_2	3	50.18	37.18 - 69.03	nt	+3.81	1.90 $\pm$ 0.33
Terrell Co.	F_2	3	54.89	33.53 - 79.98	nt	+8.52	1.49 $\pm$ 0.31
Tift Co.	F_2	5	93.28	57.39 - 161.83	nt	+46.91	1.25 $\pm$ 0.35
Cyhalothrin							
Terrell Co.	F_3	4	0.71	0.52 - 1.00	+0.56	+0.51	1.56 $\pm$ 0.23
Tift Co.	F_2	4	0.35	0.24 - 0.50	+0.20	+0.15	1.39 $\pm$ 0.21
Cypermethrin							
Mitchell Co.	F_2	4	1.50	1.17 - 1.95	-2.58	-3.17	1.85 $\pm$ 0.21
Terrell Co.	F_2	4	9.58	7.16 - 14.03	+5.50	+4.91	1.55 $\pm$ 0.24
Tift Co.	F_2	4	8.63	4.76 - 32.27	+4.55	+3.96	1.25 $\pm$ 0.30
Methomyl							
Terrell Co.	F_2	3	3.64	1.86 - 6.18	nt	-28.75	1.06 $\pm$ 0.27
Tift Co.	F_2	4	13.42	9.25 - 22.32	nt	-18.97	1.52 $\pm$ 0.29

nt indicates that this insecticide was not tested in 2002

Table 8. LD_{90}'s for various insecticides against larval *Heliothis virescens* (TBW) at 72 hr post-treatment. 2004.

Chemical	Gen.	No. Reps	LD_{90} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2002	Change (+/-) from Tift Co. avg	Slope ± SE
Acephate							
Mitchell Co.	F_2	3	236.60	145.58 - 592.96	nt	-36.29	1.90 ± 0.33
Terrell Co.	F_2	3	398.15	221.68 - 1400.76	nt	+125.25	1.49 ± 0.31
Tift Co.	F_2	5	992.88	388.79 - 22,471	nt	+719.99	1.25 ± 0.35
Cyhalothrin							
Terrell Co.	F_3	4	4.72	2.76 - 11.80	+3.90	+1.45	1.56 ± 0.23
Tift Co.	F_2	4	2.87	1.59 - 8.01	+2.05	-0.40	1.39 ± 0.21
Cypermethrin							
Mitchell Co.	F_2	4	7.43	5.03 - 13.20	-51.47	-92.65	1.85 ± 0.21
Terrell Co.	F_2	4	64.14	35.10 - 189.48	+5.24	-35.94	1.55 ± 0.24
Tift Co.	F_2	4	92.02	26.72 - 2592.36	+33.12	-8.06	1.25 ± 0.30
Methomyl							
Terrell Co.	F_2	3	57.93	22.73 - 735.54	nt	-701.54	1.06 ± 0.27
Tift Co.	F_2	4	93.12	45.58 - 401.02	nt	-666.34	1.52 ± 0.29

nt indicates that this insecticide was not tested in 2002

Table 9. Percent survival at 24 hr post-treatment of Tift Co. adult *Heliothis virescens* and *Helicoverpa zea* in glass vial cypermethrin bioassays. Tifton, GA, 2004.

Dosage (µg/vial)	*H. zea*			*H. virescens*		
	No. of trials	Total No. of moths	% Survival ± SD	No. of trials	Total No. of moths	% Survival ± SD
10	10	120	0.9 ± 2.3	11	133	6.7 ± 6.6
5	10	120	3.8 ± 4.7	11	133	17.5 ± 5.8
1	4	40	28.0 ± 12.8	10	113	49.6 ± 2.1
0	10	120	87.4 ± 0.1	11	133	79.6 ± 0.7

Table 10. Percent survival at 24 hr post-treatment of Colquitt Co. adult *Heliothis virescens* in glass vial cypermethrin bioassays. Tifton, GA, 2004.

Dosage (µg/vial)	*H. virescens*		
	No. of trials	Total No. of moths	% Survival ± SD
10	8	177	28.6 ± 3.3
5	8	162	44.9 ± 0.8
1	8	177	73.7 ± 1.4
0	8	177	88.8 ± 0.2