INSECTICIDE RESISTANCE MONITORING IN LEPIDOPTERAN COTTON PESTS

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Abstract

Larvae of the bollworm, *Helicoverpa zea*, and the tobacco budworm, *Heliothis virescens*, were bioassayed for resistance to selected pyrethroid insecticides in 2007, continuing a program initiated more than 20 years ago.

Bollworm cultures were established from larvae collected in corn, cotton, millet and tobacco in Burke, Coffee, Screven, Sumter, Tift, and Union Counties. Tobacco budworm cultures were established from larvae collected in tobacco in Tift County. Third-instar F_1 or F_2 progeny were treated with 89.9% technical grade λ-λ-cyhalothrin and 92.4% technical grade cypermethrin. Stock solutions in acetone were prepared and serially diluted to obtain the desired concentrations. Larvae were observed 72 hr post-treatment for mortality. In addition, adult bollworm moths were collected in pheromone traps in Sumter County (1 mile east of Plains)

In the larval bioassays, susceptibility of all the various populations of bollworms and tobacco budworms for both λ-λ-cyhalothrin and cypermethrin was elevated in comparison with historical levels, although the overall levels did not change relative to the 2006 results. Similarly, susceptibility of adults males in the adult vial tests did not change significantly from 2006 to 2007, although there were a few higher responses in early July. These results indicate that tolerance to pyrethroids in the bollworm and 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. However, there appears to have been no significant change in susceptibility of the bollworm to pyrethroids since 2006, which is a promising development. Our results also suggest that the developing resistance may not be

stable, as we observed again in 2007 a tendency for reduced tolerance after only a single generation in the laboratory in the Sumter County population.

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 all 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 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 make appropriate management decisions to prolong their effectiveness.

Since 1979, we have performed bioassays on major lepidopteran cotton pests to monitor development of insecticide resistance. In 2004, we began to see elevated levels of tolerance to pyrethroids. Our monitoring has continued with larvae and adults of the bollworm, *Helicoverpa zea*, and the tobacco budworm, *Heliothis virescens*, bioassayed for resistance to certain pyrethroid. Throughout most of the past 28 years, Georgia did not experience any widespread resistance problems, while other states did. Clearly, the potential exists and our findings indicate pyrethroid resistance is now our problem as well as that of other states.

Materials and Methods

Bollworm cultures were established from larvae collected in corn, cotton, or millet in Burke, Screven, Sumter, Tift, and Union Counties. Eggs and larvae collected on tobacco in Coffee Co., though expected to be tobacco budworms, were found to be almost entirely bollworms. These were included in our bollworm bioassays. Two collections were made in Tift Co. corn, the first in June and the second in September. Tobacco budworm cultures were established from eggs and larvae collected in Tift Co. tobacco. 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. Both F_1 and F_2 larvae were used for the bioassays. 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. No adult bioassays were performed in 2007.

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 89.9% technical grade λ-λ-cyhalothrin or 92.4% technical grade cypermethrin. 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 vial tests were conducted on adult males collected in pheromone traps in Crisp, Macon, Mitchell, Sumter, and Tift Counties during the summer of 2007. Traps were monitored periodically and when adequate CEW captures were attained, moths (captured the previous night) were assayed using the Adult Vial Test (AVT) procedure. AVTs were performed using 20 ml scintillation vials coated with an acetone solution of technical grade cypermethrin with dosages of 5 or 10 µg/vial and an acetone treated check. Vials were obtained from two sources, Russ Ottens at the University of Georgia and Greg Payne at West Georgia College. Individual moths were placed in treated and untreated vials and survival was checked after 24 hours. Only moths that were able to fly in a normal manner were considered alive. Percent mortality in the treated vials was corrected for mortality in the untreated. If survivorship in the untreated vials was below 80 percent, the test was discarded.

Results and Discussion

The ED_{50}, ED_{90}, LD_{50}, and LD_{90} values for the 2007 Tift Co. bollworm larval bioassays are presented in tables 1, 2, 3, and 4, respectively. With the exception of Screven Co., all ED_{50} values for λ-λ-cyhalothrin increased in comparison with those of Tift Co. in 2006 and all were higher than the Tift Co. long-term average since testing began in 1985 (Table 1). Though all ED_{50} values for cypermethrin, with the exception of Coffee Co. bollworms, decreased in comparison with those of 2006, all were higher than the Tift Co. long-term average since testing began in 1983 (Table 1).

Comparing the 2007 bollworm results with those from which collections in the same counties were evaluated in 2006 (Tift and Union counties) indicates that the levels of pyrethroid tolerance changed little, and may have even declined slightly. In 2006, the F_1 studies revealed an ED_{50} of 1.25 for the Tift County population, compared with ED_{50}s of 0.65 and 0.47 in 2007 for the June and September populations, respectively (Table 1).

Similarly, the ED_{50} of the Union County population declined from 1.68 in 2006 to 0.45 in 2007. It is unclear why these changes occurred; however, if they are real, then it is possible that the development of resistance in bollworm populations may be slowing. We would need to compare more geographic populations year to year to be able to determine more clearly if this trend is real. It is possible that there are significant fitness costs associated with maintaining pyrethroid resistance in bollworms. In the laboratory we have found that ED_{50}s consistently decline as bollworms are reared for successive generations in the laboratory, suggesting that the resistance declines in the absence of section pressure.

The ED_{50}, ED_{90}, LD_{50}, and LD_{90} values for the 2007 tobacco budworm larval bioassays are presented in tables 5, 6, 7, and 8, respectively. All values for λ-cyhalothrin and cypermethrin were higher than the Tift Co. value for 2005 (tobacco budworm bioassays were not performed in 2006), and higher than the long-term average of bioassays performed on Tift Co. larvae since 1985 for λ-cyhalothrin and since 1983 for cypermethrin (Tables 5-8).

Adult vial tests indicated that in early July a number of sampled populations exhibited elevated pyrethroid tolerance relative to that observed in 2006 (Fig. 1). However, the remainder of the season, tolerance levels returned to rates similar to those observed in 2006. This indicates that although overall pyrethroid tolerance levels in the corn earworm have not intensified since 2006, they also have not improved, and there was at least one period when tolerance was higher. These results suggest that the problem persists, and may have the potential to worsen.

The trend toward increased pyrethroid tolerance in bollworms and tobacco budworms appears to have continued in 2007, although there appears to be evidence for slowing in bollworms relative to the upward trend since 2003. It will be critical that current insecticide resistance management schemes continue to be emphasized and utilized by growers

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Table 1. ED_{50}'s for various insecticides against larval *Helicoverpa zea* (CEW) at 72 hr post-treatment. 2007.

Chemical	Gen.	No. Reps	ED_{50} (μg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2006	Change (+/-) from Tift Co. avg	Slope ± SE
Λ-cyhalothrin							
Coffee Co.	F_1	4	0.48	0.33 – 0.65	+0.22	+0.35	1.61 ± 0.27
Screven Co.	F_1	4	0.21	0.11 – 0.31	-0.05	+0.08	1.22 ± 0.22
Tift Co.	F_1	5	0.57	0.38 – 0.75	+0.31	+0.44	2.07 ± 0.36
Union Co.	F_1	4	0.29	0.22 - 0.40	+0.03	+0.16	1.56 ± 0.24
Cypermethrin							
Burke Co.	F_1	4	0.42	0.33 - 0.51	-0.37	+0.04	2.53 ± 0.34
Coffee Co.	F_1	5	0.80	0.59 – 1.02	+0.01	+0.42	2.22 ± 0.28
Screven Co.	F_1	5	0.56	0.41 – 0.70	-0.23	+0.18	2.02 ± 0.27
Sumter Co.	F_2	4	0.44	0.35 – 0.53	-0.35	+0.06	3.15 ± 0.44
Tift Co. (Jun)	F_1	4	0.65	0.34 - 0.96	-0.14	+0.27	1.71 ± 0.30
Tift Co. (Sep)	F_1	3	0.47	0.31 – 0.62	-0.32	+0.09	2.92 ± 0.58
Union Co.	F_1	4	0.45	0.30 - 0.60	-0.34	+0.07	1.83 ± 0.28

Table 2. ED_{90}'s for various insecticides against larval *Helicoverpa zea* (CEW) at 72 hr post-treatment. 2007.

Chemical	Gen.	No. Reps	ED_{90} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2006	Change (+/-) from Tift Co. avg	Slope $\pm$ SE
Λ-cyhalothrin							
Coffee Co.	F_1	4	3.01	1.86 – 7.21	+1.39	+2.45	1.61 $\pm$ 0.27
Screven Co.	F_1	4	2.30	1.34 – 6.30	+0.68	+1.74	1.22 $\pm$ 0.22
Tift Co.	F_1	5	2.36	1.67 – 4.34	+0.74	+1.80	2.07 $\pm$ 0.36
Union Co.	F_1	4	1.94	1.16 - 4.83	+0.68	+1.38	1.56 $\pm$ 0.24
Cypermethrin							
Burke Co.	F_1	4	1.34	1.01 - 2.05	-3.07	-0.26	2.53 $\pm$ 0.34
Coffee Co.	F_1	5	3.03	2.33 – 4.39	-1.38	+1.43	2.22 $\pm$ 0.28
Screven Co.	F_1	5	2.40	1.80 – 3.69	-2.01	+0.80	2.02 $\pm$ 0.27
Sumter Co.	F_2	4	1.12	0.90 – 1.58	-3.29	-048	3.15 $\pm$ 0.44
Tift Co. (Jun)	F_1	4	3.68	2.58 - 6.50	-0.73	+2.08	1.71 $\pm$ 0.30
Tift Co. (Sep)	F_1	3	1.30	0.98 – 2.09	-3.11	-0.30	2.92 $\pm$ 0.58
Union Co.	F_1	4	2.26	1.60 - 3.92	-2.15	+0.66	1.83 $\pm$ 0.28

Table 3. LD_{50}'s for various insecticides against larval *Helicoverpa zea* (CEW) at 72 hr post-treatment. 2007.

Chemical	Gen.	No. Reps	LD_{50} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2006	Change (+/-) from Tift Co. avg	Slope ± SE
Λ-cyhalothrin							
Coffee Co.	F_1	4	0.68	0.48 – 0.96	-0.04	+0.41	1.54 ± 0.28
Screven Co.	F_1	4	0.26	0.14 – 0.39	-0.46	-0.01	1.17 ± 0.21
Tift Co.	F_1	5	0.80	0.50 – 1.15	+0.08	+0.53	1.42 ± 0.29
Union Co.	F_1	4	0.51	0.39 - 0.71	-0.21	+0.24	1.78 ± 0.27
Cypermethrin							
Burke Co.	F_1	4	0.58	0.46 - 0.73	-1.19	-0.43	2.35 ± 0.33
Coffee Co.	F_1	5	1.18	0.89 – 1.50	-0.59	+0.17	1.86 ± 0.21
Screven Co.	F_1	5	0.81	0.55 – 1.10	-0.96	-0.20	1.33 ± 0.22
Sumter Co.	F_2	4	0.65	0.28 – 1.15	-1.12	-0.36	1.97 ± 0.45
Tift Co. (Jun)	F_1	4	1.20	0.0004 - 3.38	-0.57	+0.19	0.91 ± 0.31
Tift Co. (Sep)	F_1	3	0.74	0.53 – 0.94	-1.03	-0.27	2.92 ± 0.52
Union Co.	F_1	4	0.66	0.45 - 0.89	-1.11	-0.35	1.61 ± 0.25

Table 4. LD_{90}'s for various insecticides against larval *Helicoverpa zea* (CEW) at 72 hr post-treatment. 2007.

Chemical	Gen.	No. Reps	LD_{90} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2006	Change (+/-) from Tift Co. avg	Slope ± SE
Λ-cyhalothrin							
Coffee Co.	F_1	4	4.64	2.65 – 13.82	-4.57	+2.39	1.54 ± 0.28
Screven Co.	F_1	4	3.25	1.79 – 10.21	-5.96	+1.00	1.17 ± 0.21
Tift Co.	F_1	5	6.41	3.52 – 23.11	-2.80	+4.16	1.42 ± 0.29
Union Co.	F_1	4	2.68	1.59 - 6.71	-6.53	+0.43	1.78 ± 0.27
Cypermethrin							
Burke Co.	F_1	4	2.04	1.48 - 3.41	-9.10	-5.65	2.35 ± 0.33
Coffee Co.	F_1	5	5.75	4.29 – 8.64	-5.39	-1.94	1.86 ± 0.21
Screven Co.	F_1	5	7.43	4.44 – 18.88	-3.71	-0.26	1.33 ± 0.22
Sumter Co.	F_2	4	2.90	1.52 – 20.69	-8.24	-4.79	1.97 ± 0.45
Tift Co. (Jun)	F_1	4	31.13	7.96 – inf.	+19.99	+23.44	0.91 ± 0.31
Tift Co. (Sep)	F_1	3	2.02	1.53 – 3.22	-9.12	-5.67	2.92 ± 0.52
Union Co.	F_1	4	4.10	2.68 - 8.34	-7.04	-3.59	1.61 ± 0.25

Table 5. ED_{50}'s for λ-cyhalothrin and cypermethrin against larval *Heliothis virescens* (TBW) at 72 hr post-treatment. 2007.

	Gen.	No. Reps	ED_{50} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2005	Change (+/-) from Tift Co. avg	Slope ± SE
Λ-cyhalothrin							
Tift Co.	F_1	5	3.32	2.22 – 5.63	+2.76	+3.01	1.11 ± 0.18
Cypermethrin							
Tift Co.	F_1	5	3.38	2.65 – 4.21	+0.94	+2.37	1.99 ± 0.26

Table 6. ED_{90}'s for λ-cyhalothrin and cypermethrin against larval *Heliothis virescens* (TBW) at 72 hr post-treatment. 2007.

Chemical	Gen.	No. Reps	ED_{90} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2005	Change (+/-) from Tift Co. avg	Slope ± SE
Λ-cyhalothrin							
Tift Co.	F_1	5	46.89	20.23 – 215.59	+44.13	+43.36	1.11 ± 0.18
Cypermethrin							
Tift Co.	F_1	5	14.87	10.63 – 24.95	+0.21	+9.28	1.99 ± 0.26

Table 7. LD_{50}'s for λ-cyhalothrin and cypermethrin against larval *Heliothis virescens* (TBW) at 72 hr post-treatment. 2007.

Chemical	Gen.	No. Reps	LD_{50} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2005	Change (+/-) from Tift Co. avg	Slope ± SE
Λ-cyhalothrin							
Tift Co.	F_1	5	11.89	5.82 – 50.56	+10.70	+10.78	0.78 ± 0.17
Cypermethrin							
Tift Co.	F_1	5	5.70	4.32 – 7.72	+1.38	+0.99	1.51 ± 0.22

Table 8. LD_{90}'s for λ-cyhalothrin and cypermethrin against larval *Heliothis virescens* (TBW) at 72 hr post-treatment. 2007.

Chemical	Gen.	No. Reps	LD_{90} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2005	Change (+/-) from Tift Co. avg	Slope ± SE
Λ-cyhalothrin							
Tift Co.	F_1	5	510.86	94.18 – 29,513	+500.08	+468.00	0.78 ± 0.17
Cypermethrin							
Tift Co.	F_1	5	40.35	23.93 – 97.78	+17.36	-50.68	1.51 ± 0.22

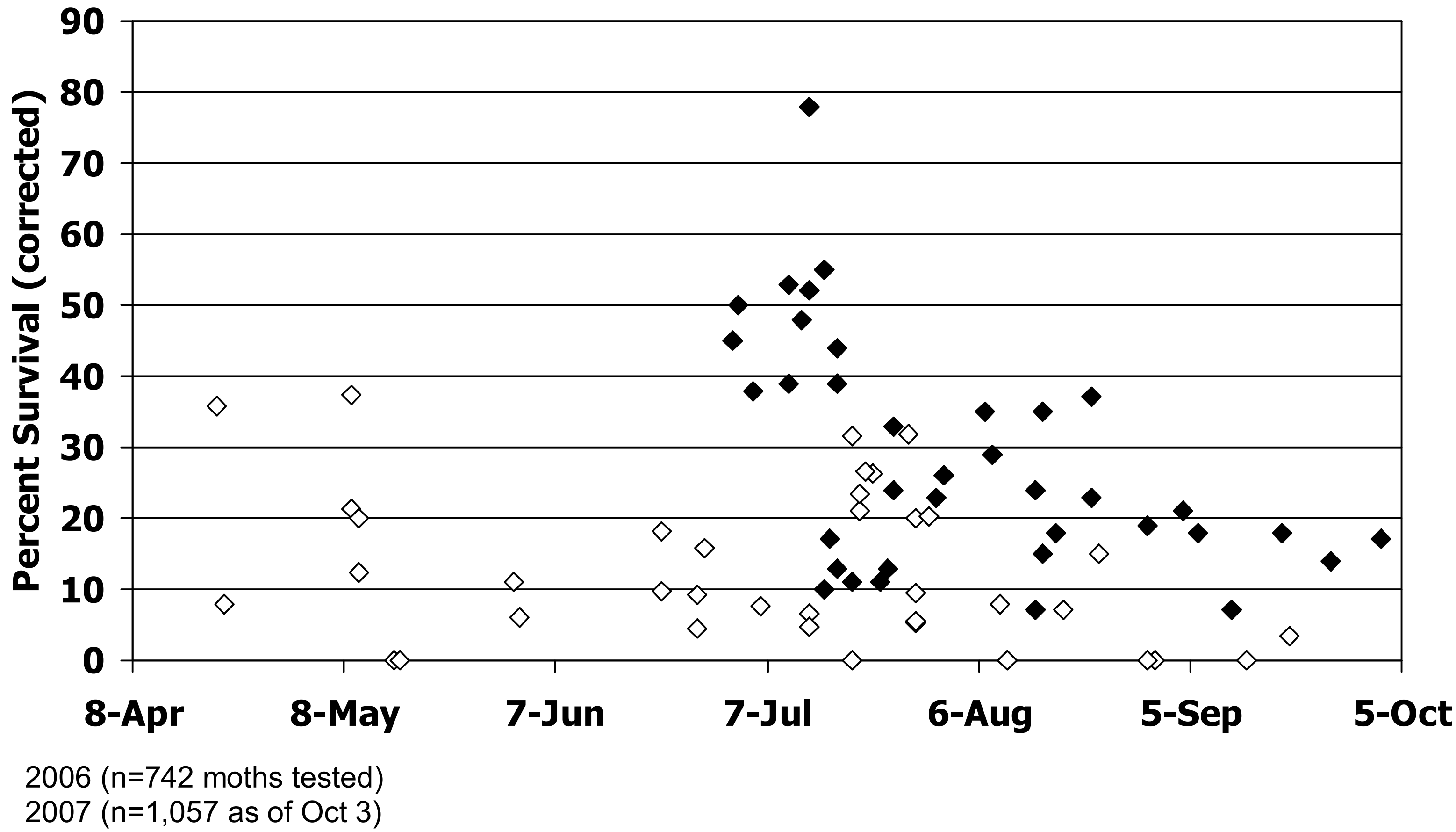

Fig. 1. Percent survival of adult male *Helicoverpa zea* in treated vials at 5 g cypermethrin per vial. Open diamonds are data from 2006 and closed diamonds are from 2007.