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 2008, continuing a program initiated more than 20 years ago.

Bollworm cultures were established from larvae collected in corn in Sumter and Tift Counties. Tobacco budworm cultures were established from larvae collected in tobacco in Coffee and Tift Counties. Third-instar F_1 or F_2 (and in once case, F_3) progeny were treated with 89.9% technical grade 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 the larval bioassays, susceptibility of all the various populations of bollworms and tobacco budworms for both cyhalothrin and cypermethrin fluctuated in comparison with historical levels, although the overall levels did not appear to change significantly relative to results obtained in 2007. These results indicate that tolerance to pyrethroids in the bollworm and tobacco budworm has not changed in recent years, but has remained at serious high levels for the tobacco budworm, and at threatening levels for the corn earworm. Further, we obtained additional data supporting previous observations that pyrethroid tolerance in the corn earworm may be physiologically costly to maintain in the absence of insecticide pressure. If this is the case in the field, then reduced use of pyrethroids would contribute to some reduction in pyrethroid tolerance in this species. There continues to be a great need for growers to utilize insecticide resistance management practices to steward these products, and the growing availability of effective tools with alternative modes of action provides a valuable toolbox for resistance management.

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 the introduction of pyrethroid insecticides, Georgia has historically had few pyrethroid resistance problems with major caterpillar pests, whereas other states did, most notably in the Midsouth. The prolonged susceptibility in Georgia was verified beginning in 1979, when bioassays of Georgia populations of major lepidopteran cotton pests were initiated to monitor insecticide resistance. However, in 2001, we detected increased levels of tolerance to pyrethroids in the tobacco budworm, and by 2004 this tolerance was widespread and had increased significantly (12-15-fold) over historical levels. In addition, in 2005 we detected elevated levels of pyrethroid tolerance in populations of the corn earworm in Georgia (3-5 times higher than historical levels of susceptibility), and have since documented this upward trend to be widespread in the state, although the levels of tolerance have increased very little since 2005. Because of concerns about the possible intensification of resistance, monitoring of larvae and adults of the bollworm, *Helicoverpa zea*, and the tobacco budworm, *Heliothis virescens*, has continued for resistance to certain pyrethroids. Clearly, the potential for serious problems exists and our findings indicate pyrethroid resistance in certain caterpillar pests is a Georgia problem, as well as in other states.

Materials and Methods

Bollworm cultures were established from larvae collected in corn in Sumter and Tift Counties. 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 tobacco in Coffee and Tift Counties. 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. In addition, F_3 larvae of Tift County corn earworms were tested to evaluate the persistence of tolerance across generations. 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 2008.

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 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 (50 and 90 represent the dosage at which 50 and 90% of the individuals in the population would be impaired (ED) or killed (LD), respectively). In many instances, larvae treated with pyrethroids linger on several days beyond the 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.

Results and Discussion

The ED_{50}, ED_{90}, LD_{50}, and LD_{90} values for the 2008 Tift Co. bollworm larval bioassays are presented in tables 1, 2, 3, and 4, respectively. The cypermethrin ED_{50} for the Tift County population nearly doubled since 2007, but the ED_{50} for cyhalothrin declined by half, indicating that there is variability in response to types of pyrethroids (Table 1). The Sumter County populations exhibited a lower ED_{50} for cypermethrin than the Tift County population, but was comparable for cyhalothrin (Table 1). All ED_{50} and ED_{90} values for cypermethrin and cyhalothrin increased in comparison with the Tift County long-term average since testing began in 1983 (Tables 1 and 2). The LD_{50}, and LD_{90} values were more variable relative to the historical values (Table 3 and 4).

There was a decline in pyrethroid tolerance in Tift County corn earworms from the first (F_1) to the second generation (F_2) in the laboratory (Tables 1-4), where the insects were not exposed to insecticides except during the bioassays. This suggests that there are physiological costs associated with the elevated pyrethroid tolerance, which is degraded across at least one generation. These results confirm previous findings with Georgia corn earworms that pyrethroid tolerance declines significantly after one generation in the laboratory (in the absence of selection). However, the values for the third generation (F_3) were somewhat elevated relative to the second generation, although the values were still lower than those observed for the first generation. These results suggest that reduced pyrethroid use, reducing selection pressure on corn earworms, can effectively delay or prevent resistance from intensifying.

The ED_{50}, ED_{90}, LD_{50}, and LD_{90} values for the 2008 tobacco budworm larval bioassays are presented in tables 5, 6, 7, and 8, respectively. The values for cyhalothrin and cypermethrin tolerance varied relative to the Tift Co. value for 2007, with a very large drop in the cyhalothrin LD_{90} for the Tift County population. Nevertheless, nearly all values continue to be higher than the long-term average of bioassays performed on Tift Co. larvae since 1985 for cyhalothrin and since 1983 for cypermethrin (Tables 5-8), indicating that resistance continues to be a serious issue with the tobacco budworm.

The results of adult vial testing yielded results similar to those obtained with the larval testing (Fig. 1) – no significant change from recent years (Fig. 2). Overall levels of pyrethroid tolerance did not increase relative to those observed in 2006 and 2007, and may have even declined somewhat. This is encouraging, because resistance mechanisms can vary between adults and caterpillars. Concentration on only one life stage can lead to deceptive results.

Elevated pyrethroid tolerance in tobacco budworms and bollworms appears to have persisted in 2008, although it has not intensified in bollworms. It will be critical that current insecticide resistance management schemes continue to be emphasized and utilized by growers to preserve these important management tools.

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Table 1. ED_{50}'s for various insecticides against larval *Helicoverpa zea* (CEW) at 72 hr post-treatment (2008).

Chemical	Gen.	No. Reps	ED_{50} (□g/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2007	Change (+/-) from Tift Co. avg	Slope ± SE
Cyhalothrin							
Tift Co.	F_1	4	0.24	0.16 - 0.32	-0.33	+0.10	1.76 ± 0.27
Sumter Co.	F_1	4	0.20	0.16 - 0.27	-0.37	+0.06	2.10 ± 0.36
Cypermethrin							
Tift Co.	F_1	4	1.16	0.92 - 1.52	+0.51	+0.72	2.01 ± 0.26
Tift Co.	F_2	4	0.48	0.36 - 0.61	-0.17	+0.04	2.06 ± 0.30
Tift Co.	F_3	4	0.80	0.65 - 0.98	+0.15	+0.36	2.51 ± 0.30
Sumter Co.	F_1	4	0.69	0.50 – 0.90	+0.04	+0.26	2.01 ± 0.31

Table 2. ED_{90}'s for various insecticides against larval *Helicoverpa zea* (CEW) at 72 hr post-treatment (2008).

Chemical	Gen.	No. Reps	ED_{90} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2007	Change (+/-) from Tift Co. avg	Slope ± SE
Cyhalothrin							
Tift Co.	F_1	4	1.28	0.90 - 2.25	-1.08	+0.64	1.76 ± 0.27
Sumter Co.	F_1	4	0.82	0.52 - 1.95	-1.54	+0.18	2.10 ± 0.36
Cypermethrin							
Tift Co.	F_1	4	5.04	3.35 - 9.62	+1.36	+3.21	2.01 ± 0.26
Tift Co.	F_2	4	2.01	1.41 - 3.56	-1.67	+0.18	2.06 ± 0.30
Tift Co.	F_3	4	2.61	1.96 - 3.96	-1.07	+0.78	2.51 ± 0.30
Sumter Co.	F_1	4	3.01	2.12 – 5.33	-0.67	+1.18	2.01 ± 0.31

Table 3. LD_{50}'s for various insecticides against larval *Helicoverpa zea* (CEW) at 72 hr post-treatment (2008).

Chemical	Gen.	No. Reps	LD_{50} (μg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2007	Change (+/-) from Tift Co. avg	Slope ± SE
Cyhalothrin							
Tift Co.	*F_1*	4	0.52	0.38 - 0.70	-0.28	+0.25	1.54 ± 0.23
Sumter Co.	*F_1*	4	0.22	0.17 - 0.31	-0.58	-0.05	1.96 ± 0.35
Cypermethrin							
Tift Co.	*F_1*	4	1.92	1.52 - 2.62	+0.72	+0.85	2.22 ± 0.31
Tift Co.	*F_2*	4	0.66	0.51 - 0.84	-0.54	-0.41	2.08 ± 0.29
Tift Co.	*F_3*	4	1.19	0.98 - 1.46	-0.01	+0.12	2.60 ± 0.32
Sumter Co.	*F_1*	4	0.95	0.68 – 1.27	-0.25	-0.12	1.66 ± 0.27

Table 4. LD_{90}'s for various insecticides against larval *Helicoverpa zea* (CEW) at 72 hr post-treatment (2008).

Chemical	Gen.	No. Reps	LD_{90} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2007	Change (+/-) from Tift Co. avg	Slope ± SE
Cyhalothrin							
Tift Co.	F_1	4	3.55	2.18 - 8.23	-2.86	+1.17	1.54 ± 0.23
Sumter Co.	F_1	4	1.00	0.59 - 2.79	-5.41	-1.38	1.96 ± 0.35
Cypermethrin							
Tift Co.	F_1	4	7.27	4.72 - 14.92	-23.86	-0.39	2.22 ± 0.31
Tift Co.	F_2	4	2.71	1.87 - 4.90	-28.42	-4.95	2.08 ± 0.29
Tift Co.	F_3	4	3.70	2.75 - 5.77	-27.43	-3.96	2.60 ± 0.32
Sumter Co.	F_1	4	5.58	3.54 – 12.45	-25.55	-2.08	1.66 ± 0.27

Table 5. ED_{50}'s for cyhalothrin and cypermethrin against larval *Heliothis virescens* (TBW) at 72 hr post-treatment (2008).

	Gen.	No. Reps	ED_{50} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2007	Change (+/-) from Tift Co. avg	Slope ± SE
Cyhalothrin							
Tift Co.	F_1	4	1.38	2.26 - 1.00	-1.94	+1.00	1.62 ± 0.34
Cypermethrin							
Coffee Co.	F_1	4	6.86	4.65 – 10.68	+3.48	+5.65	1.29 ± 0.25
Tift Co.	F_1	4	4.80	3.86 – 6.32	+1.42	+3.59	2.72 ± 0.47

Table 6. ED_{90}'s for cyhalothrin and cypermethrin against larval *Heliothis virescens* (TBW) at 72 hr post-treatment (2008).

Chemical	Gen.	No. Reps	ED_{90} (µg/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2007	Change (+/-) from Tift Co. avg	Slope ± SE
Cyhalothrin							
Tift Co.	F_1	4	8.54	4.21 - 43.84	-38.35	+4.69	1.62 ± 0.34
Cypermethrin							
Coffee Co.	F_1	4	67.77	31.94 – 333.44	+52.90	+61.73	1.29 ± 0.25
Tift Co.	F_1	4	14.22	9.73 – 29.09	-0.65	+8.18	2.72 ± 0.47

Table 7. LD_{50}'s for cyhalothrin and cypermethrin against larval *Heliothis virescens* (TBW) at 72 hr post-treatment (2008).

	Gen.	No. Reps	LD_{50} (□g/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2007	Change (+/-) from Tift Co. avg	Slope + SE
Cyhalothrin							
Tift Co.	*F_1*	4	2.50	1.67 - 6.02	-9.39	+1.30	1.64 + 0.39
Cypermethrin							
Coffee Co.	*F_1*	4	16.89	10.56 – 40.25	+11.19	+12.02	1.13 + 0.23
Tift Co.	*F_1*	4	7.49	5.60 – 11.99	+1.79	+2.62	2.16 + 0.38

Table 8. LD_{90}'s for cyhalothrin and cypermethrin against larval *Heliothis virescens* (TBW) at 72 hr post-treatment (2008).

Chemical	Gen.	No. Reps	LD_{90} (□g/g larval wt.)	95% C.I.	Change (+/-) from Tift Co. 2007	Change (+/-) from Tift Co. avg	Slope + SE
Cyhalothrin							
Tift Co.	*F_1*	4	15.12	6.20 - 160.73	-495.74	-25.76	1.64 + 0.39
Cypermethrin							
Coffee Co.	*F_1*	4	231.86	76.69 – 2981.11	+191.51	+144.46	1.13 + 0.23
Tift Co.	*F_1*	4	29.35	16.64 – 89.30	-11.00	-58.05	2.16 + 0.38

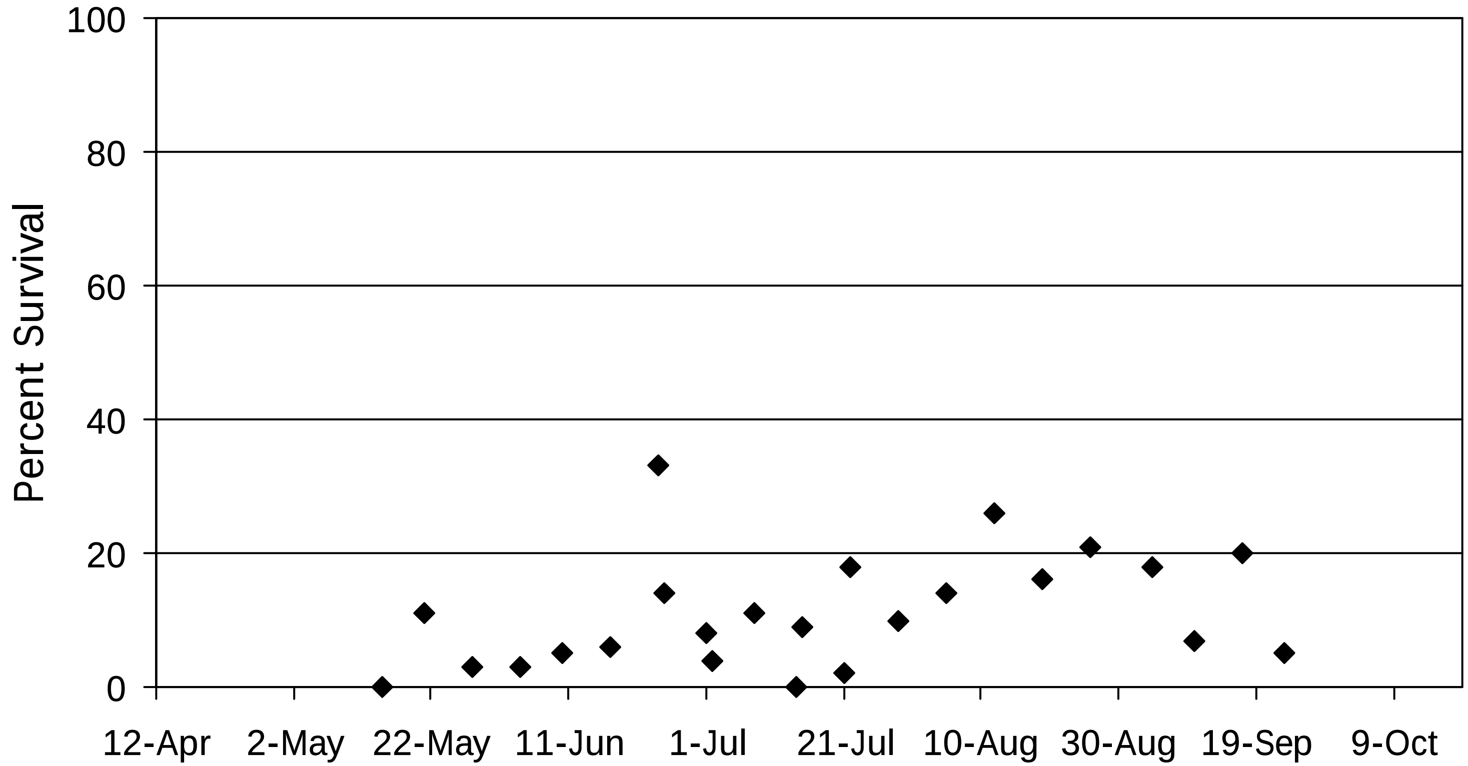

Fig. 1. Survival of adult corn earworm moths after 24 hours of exposure to 5 µg of cypermethrin in a glass vial, 2008.

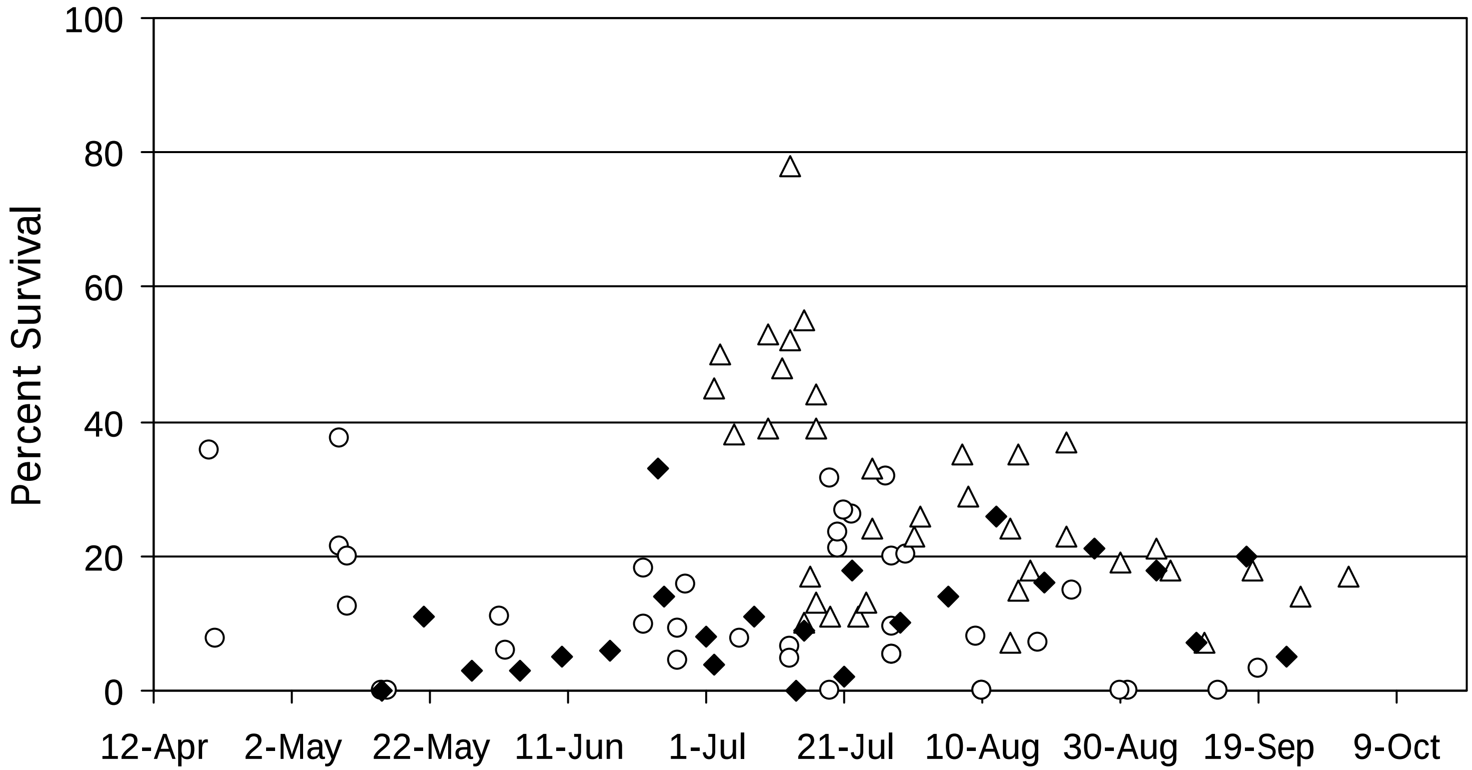

Fig. 2. Survival of adult corn earworm moths after 24 hours of exposure to 5 µg of cypermethrin in a glass vial, 2006 (open circles), 2007 (open triangles), and 2008 (closed diamonds).