U.S. COTTONBELT SURVEY: TESTING THE SUSCEPTIBILITY OF THE BOLLWORM, *HELICOVERPA ZEA* (BODDIE) TO PYRETHROID INSECTICIDES

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Abstract

In 2000, Cotton Incorporated sponsored a bollworm monitoring program to assess the extent of pyrethroid resistance in bollworm (*Helicoverpa zea*) populations throughout the Mid-South/Southeastern U.S. Cotton Belt. Bollworm moths were collected and tested in Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, Texas, and Virginia. Over 14,000 moths were bioassayed from May through October, 2000. Survival at the 5 µg/vial rate of cypermethrin ranged from a low of 0% to a high of 45.3% in Alabama during July. Thirteen states had greater than 10% survival during at least one month. This represents a 2.6-fold increase compared to data collected during the 1999 season and a 4.3-fold increase compared to data collected during the 1998 season. Survival at the 10 µg/vial rate of cypermethrin ranged from a low of 0% to a high of 34.2%. Five collections had greater than 10% survival during at least one month. This represents a 1.6-fold increase compared to data collected during the 1999 season and a 5-fold increase compared to 1998 data. These data suggest that bollworm populations throughout the mid-south/southeastern U.S. are developing resistance to the pyrethroid insecticides. Efforts to monitor pyrethroid resistance in bollworm populations should remain a priority for the development and implementation of future resistance management strategies.

Introduction

The cotton bollworm, *Helicoverpa zea* (Boddie), is one of the more economically important pests of cotton in the United States, and without proper control methods, populations of this pest insect could potentially reach damaging levels and severely reduce crop yields. Because the cotton bollworm has developed resistance to many of the insecticides used for its control (Sparks 1981), it is critical that research efforts and agricultural practices be devoted to the preservation of those insecticides that are still effective and to the development of new replacement compounds and technologies. Programs to monitor insecticide susceptibilities in field-collected populations of cotton bollworm are critical to the development of effective management strategies.

In recent years, several studies have reported that cotton bollworm populations in the U.S. and the Central and South Americas have developed resistance to pyrethroid insecticides (Ernst and Dittrich 1992, Abd-Elghafar et al. 1993, Bagwell et al. 1996, Kenga et al. 1996, Bagwell et al. 1998, Brown et al. 1998, Walker et al. 1998, Bagwell et al. 1999, Martin et al. 1999, Williams 1999). In at least one case, neurophysiological studies indicated that pyrethroid resistance in a cotton bollworm population was related (in part) to decreased target site sensitivity (Holloway et al. 1997). In 1998, the Insecticide Resistance Action Committee-United States (IRAC-US) funded a regional project to monitor the susceptibility of cotton bollworm populations to pyrethroid insecticides in order to determine the magnitude of the reported widespread changes in the susceptibility of bollworm populations to pyrethroid insecticides throughout the mid-south/southeastern United States. The original proposal called for a three-year study to begin in 1998 and to end in 2000. Results from the first two years of this proposed project were presented at the 1999 and 2000 Beltwide Cotton Conferences (Martin et al. 1999, Martin et al. 2000). In short, bollworm adults were collected and tested from eleven states including Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, Tennessee and Texas. In 1998, eleven of the twenty-three locations tested had survival at the 10 µg cypermethrin/vial dose, and 1999 data were comparable (thirteen of twenty-eight locations tested had survival at the 10 µg cypermethrin/vial dose). However, the percentages of cotton bollworm adults that survived the 10 µg cypermethrin/vial dose were higher in 1999 as compared to percent survival values recorded in 1998. In 1998, the highest percent survival recorded for the 10 µg rate was 4.9%. In 1999, six locations exceeded 5% survival at 10 µg cypermethrin, and three locations had survival that exceeded 10%. In 2000, 10 sites exceeded 5% survival at the 10 µg rate, and five sites exceeded 10% survival at the 10 µg rate. These data suggested that cotton bollworm populations throughout the mid-south/southeastern U.S. cottonbelt states have become more tolerant to the effects of pyrethroid insecticides. Furthermore, those studies have helped to identify areas within those states where pyrethroid insecticides may be the least effective strategy implemented for the control of cotton bollworm populations.

In general, it is recognized that pyrethroids are critical components in the current strategies adopted to effectively manage the bollworm/budworm complex on cotton. Therefore, it is necessary that we "get a handle" on this issue as quickly as possible so that we may be in a better position to circumvent the development of widespread pyrethroid resistance in bollworm populations, or in the event that widespread resistance does occur, the agrochemical and agricultural communities would be better prepared to deal with the issue. To date, the Cotton Incorporated/IRAC-US sponsored pyrethroid-resistance monitoring project, has generated valuable pyrethroid-susceptibility baseline data for cotton bollworm populations. Also, these studies have helped researchers to identify/locate those areas where pyrethroid susceptibilities in cotton bollworm populations are decreasing.

Materials and Methods

Sampling Methodology

Pheromone Trap. Wire cone Hartstack traps (Harstack et al. 1979) or equivalent were utilized. Hartstack Lepidoptera moth traps can be obtained from Davis Tool and Die (226 CR 235, Abbeville, MS 38601, Tel. 601-234-4007).

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Pheromone Blend and Source. The traps were baited with bollworm (*Helicoverpa zea*) pheromone lures (Hendricks et al. 1987) to collect male moths throughout the sampling season. In general, the sampling season extended from June through August/early September. The pheromone lures may be obtained directly from the manufacturer (717-764-1192). Each lure will only be used for a period not to exceed 2-3 weeks.

Trap Placement. Traps were placed in open areas near the edge of cotton fields upwind of the likely source of moths. If necessary, traps may be moved to optimize moth collection.

Vial Preparation Methodology

Clean, borosilicate glass scintillation vials (20 ml) were used for the assay. The test vials were prepared by coating the inside of the vial with an acetone solution of technical grade cypermethrin (94.4% pure, FMC Corp., Princeton, NJ). Rates of 5 µg cypermethrin/vial and 10 µg cypermethrin/vial were used. The 5 µg cypermethrin/vial treatment was chosen as a low concentration for this monitoring program because historical data exists for this concentration against cotton bollworm moths (Bagwell et al. 1998). The 10 µg cypermethrin/vial treatment was chosen as the high concentration for this monitoring program because it was considered lethal to homozygous, pyrethroid-susceptible tobacco budworm, *Heliothis virescens*, moths, as well as moths heterozygous for pyrethroid resistance (Plapp et al. 1987).

The vials were treated by pipetting 500 µl of the appropriate treatment solution into a glass scintillation vial. Control vials were treated with 500 µl of acetone only. The treated vials were quickly placed on a hot dog roller (with the heating elements disconnected) in a fume hood and rotated until the acetone evaporates completely (15-30 min.). Vials were removed from the roller and capped loosely. Treated vials were stored in a cool, dry place until they were used.

The vials were stored and shipped to each of the collaborating researchers in units of 100 vials/flat with six flats/box (200 control vials, 200 5 µg/vial vials, and 200 10 µg/vial vials). Each flat was be clearly labeled with the treatment and cypermethrin rate. For each cypermethrin test solution prepared from the stock solution, two vials from each treatment were randomly selected for archive storage in a freezer at -4° C.

Adult Vial Test Methodology

Moth Collection. Male moths were collected from the traps in the morning. Only fresh/healthy moths were used in the assays. The collected moths were maintained overnight and fed a 10% sucrose solution. The wings of the moths were examined and a general assessment of health was made. The wings of healthy moths should have scales over almost the entire surfaces of the wings. Moths whose wings have lost most of the scales or whose wings were damaged were not used for the adult vial test. One moth was placed in each vial and capped loosely. The vials containing moths were placed back into the shipping flats and held on an angle (tilted) for 24 hours at room temperature (ca. 24° C).

Mortality counts were recorded 24 h after the test was initiated. The moths were evaluated as either alive, dead, or knocked-down. Knocked-down moths were those moths that were alive but unable to fly in a normal manner. The treatment vials were turned "upside down". Moths able to fly $\geq$ 3 meters were considered alive. Moths not able to fly > 3 meters were recorded as "knocked down". "Knocked down" moths were evaluated further by tossing them into the air. All data was corrected for control mortality using Abbott's (1925) formula. Treated vials were used once only. If individual cooperators prepared their own vials, a set of standard vials prepared by Dr. Greg Payne (State University of West Georgia) were incorporated into their tests for comparison.

Data Recording

Data were recorded for each assay on a data record sheet. One copy of the data record was kept by the cooperating researcher, and one copy was sent to Dr. Greg Payne (Department of Biology, State University of West Georgia, Carrollton Georgia 30118, Tel. 770-836-4542, Fax. 770-836-6633, E-Mail: gpayne@westga.edu).

Cooperating Researchers/Subcontracted Research Personnel

Fifteen of the sixteen cooperating researchers have been actively involved in the project during the 1998 and 1999 seasons. Dr. Ames Herbert (Virginia Tech) was added to the list for the year 2000 as a result of previously collected data that suggested that the susceptibility of cotton bollworm populations in the neighboring counties of North Carolina were decreasing.

Results and Discussion

From 1998 to 2000, 37,269 male bollworm moths were evaluated from twelve states for pyrethroid resistance using the adult vial test at a dose of 5 or 10 µg/vial of cypermethrin. In 1998, survival at the 5 µg/vial dose of cypermethrin by state ranged from 0-21.2%, 0-12.9, 0.7-15.1 and 4.4-13.7 in June, July, August and September, respectively (Table 1). Louisiana (June and July), Alabama (July) and North Carolina (September) were the only collection locations to exceed 10% survival at the 5 µg/vial dose. Survival at the 10 µg/vial dose of cypermethrin ranged from 0-1.8%, 0-4.9, 0-1.2 and 0-3.0 in June, July, August and September, respectively (Table 2). Alabama (July), Louisiana (July) and North Carolina (September) were the only three collection locations to exceeded 2% survival at the 10 µg/vial dose.

In 1999, survival at the 5 µg/vial dose of cypermethrin ranged from 0-18%, 0-34%, 0-17%, 0-14%, 0-6% and 0% in May, June, July, August, September and October, respectively (Table 3a and 3b). Alabama (July and August), Louisiana (May, June, July and August), Missouri (June), North Carolina (August) and South Carolina (June and July) were the collection locations that exceeded 10% survival at the 5 µg/vial dose. Survival at the 10 µg/vial dose of cypermethrin ranged from 6%, 0-25%, 0-22%, 0-7%, 0-2% and 0-3% in May, June, July, August, September and October, respectively (Table 4a and 4b). Louisiana (July), Missouri (June) and South Carolina (June) were the only three collection locations to exceeded 15% survival at the 10 µg/vial dose.

In 2000, survival at the 5 µg/vial dose of cypermethrin ranged from 0-28%, 0-45%, 0-20%, and 0-14% in June, July, August, and September, respectively (Table 5). Alabama (June, July and August), Georgia (July), Louisiana (June), Missouri (July and August), North Carolina (August and September) South Carolina (June and July), Texas (July) and Virginia (June, August and September) were the collection locations that exceeded 10% survival at the 5 µg/vial dose. Survival at the 10 µg/vial dose of cypermethrin ranged from 0-21%, 0-34%, 0-14% and 0-6% in June, July, August and September, respectively (Table 6). Alabama (July and August), Louisiana (June), Missouri (August) and South Carolina (June) were the only collection locations to exceeded 10% survival at the 10 µg/vial dose.

Summary

Since 1998, an increase in survival of bollworm adults to pyrethroid insecticides has been observed throughout the mid-south and southeastern U.S. In 1998, 5 sites were observed to have > 10% survival at the 5 µg rate. Nine sites were observed to have > 5% survival at the 5 µg rate. All sites had < 5% survival at the 10 µg rate. In 1999, 10 sites were observed to have > 10% survival at the 5 µg rate. Fourteen sites were observed to have > 5% survival at the 5 µg rate. Two sites had > 10% survival at the 10 µg rate, and 5 sites had > 5% survival at the 10 µg rate. In 2000, 13 sites were observed to have > 10% survival at the 5 µg rate. Eighteen sites were

observed to have > 5% survival at the 5 µg rate. Five sites had > 10% survival at the 10 µg rate, and 10 sites had > 5% survival at the 10 µg rate. In general, % survival decreased through the season from June to September; however, this pattern was not observed in North Carolina and Virginia. In North Carolina and Virginia, the % survivals increased through the season from June to September. This study has identified areas where the susceptibilities of bollworm populations to pyrethroid insecticides have decreased during the past several years. In addition, this study has provided valuable baseline data for future comparisons and stresses the importance of continued resistance monitoring efforts and the development and implementation of effective resistance management plans.

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Table 1. Percent survival of bollworm male moths at 5µg cyper-methrin per vial by state and month during 1998. Data taken from Martin et al. 1999.

	% Survival			
State	**June**	**July**	**August**	**September**
Alabama	---	10.8	3.9	---
Arkansas	8.2	5.6	---	---
Georgia	---	4.6	1.1	---
Louisiana	21.2	12.9	2.7	---
Mississippi	0	0	0.7	---
Missouri	---	---	15.1	7.4
North Carolina	---	3.5	9.5	13.7
South Carolina	4.4	3.3	1.2	4.4
Texas	0	1.9	1.8	---

Table 2. Percent survival of bollworm male moths at 10µg cyper-methrin per vial by state and month during 1998. Data taken from Martin et al. 1999.

	% Survival			
State	**June**	**July**	**August**	**September**
Alabama	---	4.9	0	---
Arkansas	1.8	1.6	---	---
Georgia	---	1.0	0	---
Louisiana	---	3.8	0	---
Mississippi	0	0	0	---
Missouri	---	---	0	0
North Carolina	---	0	0.5	3.0
South Carolina	0.4	1.4	1.2	0.8
Texas	0	0	0.4	---

Table 3a. Percent survival of bollworm male moths at 5µg cyper-methrin per vial by state and month during 1999. Data taken from Martin et al. 2000.

	% Survival		
State	May	June	July
Alabama	---	0	11
Arkansas	---	---	2
Florida	---	---	0
Georgia	---	---	2
Louisiana	18	11	14
Mississippi	---	---	0
Missouri	---	34	0
North Carolina	---	---	3
South Carolina	---	25	17
Tennessee	---	---	---
Texas	0	4	0

Table 3b. Percent survival of bollworm male moths at 5µg cyper-methrin per vial by state and month during 1999. Data taken from Martin et al. 2000.

	% Survival (Number Tested)		
State	**August**	**September**	**October**
Alabama	12	---	---
Arkansas	0	---	---
Florida	0	---	---
Georgia	2	---	---
Louisiana	14	0	---
Mississippi	0	---	---
Missouri	6	0	---
North Carolina	13	6	---
South Carolina	4	4	0
Tennessee	6	---	---
Texas	6	0	---

Table 4a. Percent survival of bollworm male moths at 10µg cyper-methrin per vial by state and month during 1999. Data taken from Martin et al. 2000.

	% Survival		
State	May	June	July
Alabama	---	---	0
Arkansas	---	---	0
Florida	---	---	---
Georgia	---	---	---
Louisiana	---	0	22
Mississippi	---	---	0
Missouri	---	16	0
North Carolina	---	---	0
South Carolina	---	25	6
Tennessee	---	---	---
Texas	6	2	1

Table 4b. Percent survival of bollworm male moths at 10 µg cyper-methrin per vial by state and month during 1999. Data taken from Martin et al. 2000.

	% Survival		
State	August	September	October
Alabama	2	---	---
Arkansas	0	---	---
Florida	0	---	---
Georgia	---	---	---
Louisiana	0	---	---
Mississippi	0	---	---
Missouri	7	0	---
North Carolina	3	2	---
South Carolina	0	0	0
Tennessee	1	---	---
Texas	---	0	3

Table 5. Percent survival of bollworm male moths at 5µg cyper-methrin per vial by state and month during 2000.

	% Survival			
State	**June**	**July**	**August**	**September**
Alabama	13.2	45.3	15.9	---
Arkansas	---	---	---	---
Florida	---	4.8	0	---
Georgia	---	22.1	8.3	---
Louisiana	11.1	8.1	2.8	---
Mississippi	---	0	1.8	---
Missouri	---	24.8	18.3	---
North Carolina	4.8	5.4	11.7	14.6
South Carolina	28.6	12.4	3.1	---
Tennessee	---	0	0	---
Texas	3.4	11.5	0	0
Virginia	16.3	9.5	20	1.2

Table 6. Percent survival of bollworm male moths at 10µg cyper-methrin per vial by state and month during 2000.

	% Survival			
State	June	July	August	September
Alabama	6.2	34.2	11.4	---
Arkansas	---	0	0	---
Florida	---	---	---	---
Georgia	---	2.8	4.2	---
Louisiana	11.1	5.4	0	---
Mississippi	---	0	0	---
Missouri	---	7.9	14.3	---
North Carolina	0	0	4	5.8
South Carolina	21.4	4.4	1.5	---
Tennessee	---	0	0	---
Texas	0	2.6	0	0
Virginia	0	3.9	9.4	0