U.S. COTTONBELT SURVEY: TESTING THE SUSCEPTIBILITY OF THE BOLLWORM, *HELICOVERPA ZEA* (BODDIE), TO PYRETHROID INSECTICIDES—2001 UPDATE

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Abstract

In 2001, more than 15,000 bollworm moths and 4000 budworm moths were bioassayed. Survival values for bollworm moths at the 5 µg/vial concentration and 10 µg/vial concentration of cypermethrin were similar to survival values recorded during the 2000 season. Six sites had greater than 10% survival during at least one month at the 10 µg/vial concentration and 11 sites had greater than 5 % survival. This represented a 1.2-fold increase compared to data collected during the 2000 season, a 2-fold increase compared to data collected during the 1999 season and a 6-fold increase compared to data collected during the 1998 season. Survival at the 10 µg/vial concentration of cypermethrin ranged from a low of 0% to a high of 50%. As expected, survival values for tobacco budworm adults at the 10 µg/vial concentration of cypermethrin were high throughout the mid-south/southeastern test areas. All eighteen test sites had greater than 10% survival; eight of eighteen sites had greater than 50% survival; and two of eighteen test sites had greater than 70% survival. Survival at the 10 µg/vial concentration of cypermethrin ranged from a low of 12% to a high of 76%. Survival of bollworm adults at a 15 µg/vial concentration of

spinosad ranged from a low of 0% to a high of 57%. Eight of 33 sites evaluated had greater than 20% survival; three of 33 sites evaluated had greater than 50% survival. Tobacco budworm adults were less tolerant of spinosad treatment. Survival of tobacco budworm adults at a 15 µg/vial concentration of spinosad ranged from a low of 0% to a high of 24.4%. Only five of 19 sites evaluated had greater than 10% survival, and only one of 19 sites evaluated had greater than 20% survival. These data suggest that bollworm populations throughout the mid-south/southeastern U.S. are developing resistance to the pyrethroid insecticides; resistance to pyrethroids in tobacco budworm populations is widespread; and efforts to monitor pyrethroid resistance and the effects of spinosad in bollworm populations should remain a priority for the development and implementation of future resistance management strategies.

Introduction

The bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), are two of the more economically important pests of cotton in the United States, and without proper control methods, populations of these pest insects could reach damaging levels and severely reduce crop yields. Because the bollworm and tobacco budworm have developed resistance to many of the insecticides used for their control (Sparks 1981, Elzen et al. 1992), 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. Also, with the increased utilization of transgenic varieties and increased pressures by "secondary pests", programs to monitor insecticide susceptibilities in field-collected populations of heliothine pest insects are critical to the development of those management strategies.

Over the past decade, numerous studies have reported that bollworm and tobacco budworm populations in the U.S. and the Central and South Americas have developed resistance to pyrethroid insecticides (Graves et al. 1989, 1991, Ernst and Dittrich 1992, Abd-Elghafar et al. 1993, Graves et al. 1993, 1994, Bagwell et al. 1995, 1996, Kanga et al. 1996, Bagwell et al. 1997, 1998, 1999, 2000, 2001, Brown et al. 1998, Sorenson et al. 1998, Walker et al. 1998, Martin et al. 1999, 2000, Williams 1999, Payne et al. 1999, 2001). In at least one case, neurophysiological studies indicated that pyrethroid resistance in a bollworm population was related (in part) to decreased target site sensitivity (Ottea and Holloway 1998).

More recently, increased survivals of bollworm and tobacco budworm moths to spinosad have been observed. Laboratory tests *via* topical application of 1 µl aliquots of serial dilutions of technical grade spinosad to the thoracic dorsa of tobacco budworm larvae collected from areas where suspected control failures have occurred generated significantly higher LD_{50} values compared to the LD_{50} values generated from larvae of pre-season and laboratory reference colonies (Cook et al. 2001, Cook et al. 2002).

Since 1998, Cotton Incorporated and the Insecticide Resistance Action Committee (IRAC-US) have sponsored a monitoring program to assess the extent of pyrethroid resistance in bollworm (*Helicoverpa zea*) populations throughout the mid-south/ southeastern U.S. Cotton Belt. The original proposal called for a three-year study to begin in 1998 and to end in 2000. During the 2001 assessment season, the focus of the project was expanded to include evaluations of both bollworm and tobacco budworm moths against cypermethrin and spinosad (the active ingredient found in Tracer®). As a result of those monitoring efforts, valuable baseline data for pyrethroid- and spinosad-susceptibilites in bollworm and tobacco budworm populations have been generated. This report summarizes the results obtained during this four-year monitoring project.

Materials and Methods

An adult vial test (AVT) similar to that described by Plapp et al. (1987, 1990) was used to monitor the susceptibilities of bollworm and tobacco budworm moths to cypermethrin and spinosad. Male moths were collected and tested from twelve states (including Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, Missouri, North Carolina, South Carolina, Tennessee, Texas and Virginia) using wire cone traps (Hartstack et al. 1979; Davis Tool and Die (226 CR 235, Abbeville, MS 38601) baited with *Helicoverpa zea* or *Heliothis virescens* pheromone lures (Hendricks et al. 1987). In general, the sampling season extended from May through August/early September. Traps were placed in open areas, upwind of the likely source of moths, near the edges of cotton fields and monitored on a regular basis. Multiple collection locations were sampled in nine of the twelve participating states.

Clean, borosilicate glass scintillation vials (20 ml) were used for the assay. The test vials were prepared at a central location (State University of West Georgia) by coating the inside of the vial with an acetone solution of technical grade cypermethrin (94.4% pure, FMC Corp., Princeton, NJ) or spinosad (88% pure, Dow AgroSciences, Indianapolis, IN). The insecticide concentrations used in this study were 5 µg cypermethrin/vial, 10 µg cypermethrin/vial and 15 µg spinosad/vial. 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 15 μg spinosad/vial treatment was used based on tests that indicated it to be the LC_{90} against laboratory reference colonies of tobacco budworm moths (Thompson, personal communication) and the existence of historical data from Louisiana (Cook et al. 2001). The vials were treated by pipetting 0.5 ml of the appropriate treatment solution into a glass scintillation vial. Control vials were treated with 0.5 ml 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 evaporated completely (15-30 min.). Vials were removed from the roller and capped loosely. Treated vials were stored in a cool, dry and dark area until they were used.

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 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 ≥ 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 only once. If individual cooperators prepared their own vials, a set of standard vials prepared at the central location (State University of West Georgia) were incorporated into their tests for comparison.

Results

From 1998 to 2001, > 52,000 male bollworm moths were evaluated from twelve states for pyrethroid resistance at a concentration of 5 or 10 μg/vial of cypermethrin. In 1998, bollworm survival at the 5 μg/vial concentration of cypermethrin ranged from 0-21%, 0-13%, 1-15% and 4-14% in June, July, August and September, respectively (Payne et al. 2001). Louisiana (June and July), Alabama (July) and North Carolina (September) were the only collection locations to exceed 10% survival at the 5 μg/vial concentration. Survival at the 10 μg/vial concentration of cypermethrin ranged from 0-2%, 0-5%, 0-1% and 0-3% in June, July, August and September, respectively (Table 1). Alabama (July), Louisiana (July) and North Carolina (September) were the only three collection locations to exceeded 2% survival at the 10 μg/vial concentration.

In 1999, bollworm survival at the 5 μg/vial concentration 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 (Payne et al. 2001). 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 concentration. Survival at the 10 μg/vial concentration 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 2). Louisiana (July), Missouri (June) and South Carolina (June) were the only three collection locations to exceeded 15% survival at the 10 μg/vial concentration.

In 2000, bollworm survival at the 5 μg/vial concentration of cypermethrin ranged from 0-28%, 0-45%, 0-20%, and 0-14% in June, July, August, and September, respectively (Payne et al. 2001). 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 concentration. Survival at the 10 μg/vial concentration of cypermethrin ranged from 0-21%, 0-34%, 0-14% and 0-6% in June, July, August and September, respectively (Table 3). 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 concentration.

In 2001, both bollworm and tobacco budworm adults were evaluated. Evaluations using the 5 μg/vial concentration of cypermethrin were limited. Bollworm survival at the 5 μg/vial concentration of cypermethrin ranged from 5-10%, 1-32%, and 2-17% in June, July, and August, respectively. Mean survival values by state and month were as follows: Florida (5%-June and July, 17% August); Louisiana (10% June, 32% July and 13% August); North Carolina (8% August); South Carolina (2% August); Tennessee (3% August); and Virginia (1% July and 4% August). Florida (August) and Louisiana (June, July and August) were the only collection locations that exceeded 10% survival at the 5 μg/vial concentration. Of the twelve sites evaluated, four exceeded 10% survival, and six of the twelve sites evaluated exceeded 5% survival. Bollworm survival at the 10 μg/vial concentration of cypermethrin ranged from 0-50%, 1-9%,

0-14% and 0-16% in June, July, August and September, respectively (Table 4). Georgia (August), Louisiana (September), Mississippi (August and September) and South Carolina (June) were the only collection locations to exceeded 10% survival at the 10 µg/vial concentration. Tobacco budworm survival at the 10 µg/vial concentration of cypermethrin ranged from 40-69%, 20-58%, 60-72%, 18-76% and 12-57% in May, June, July, August and September, respectively (Table 4). Sixteen of the eighteen sites evaluated exceeded 20% survival; eight sites exceeded 50% survival and two sites exceeded 70 % survival.

In addition to cypermethrin, bollworm and tobacco budworm moth survival was evaluated using a 15 µg/vial concentration of spinosad. Bollworm survival ranged from 0-4%, 0-50%, 0-60%, and 0-6% in May, June, July, and August, respectively (Table 5). Of the 33 sites evaluated, three exceeded 50% survival; eight exceeded 20% survival, and twelve exceeded 10% survival. Tobacco budworm survival ranged from 0-15%, 0-3%, 0-24%, 0-17% and 0-13% in May, June, July, August and September, respectively (Table 5). Only one site had survival values that exceeded 20%. The highest survival value (24%) occurred in Alabama during the month of July.

Discussion

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) (Tables 1 and 2). However, the percentages of 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 concentration was 4.9% (Table 1). In 1999, six locations exceeded 5% survival at 10 µg cypermethrin, and three locations had survival that exceeded 10% (Table 2). In 2000, ten sites exceeded 5% survival at the 10 µg concentration, and five sites exceeded 10% survival at the 10 µg concentration (Table 3). In 2001, 11 sites exceeded 5% survival at the 10 µg concentration and six sites exceeded 10% survival at the 10 µg concentration (Table 4). These data suggest that bollworm populations throughout the mid-south/southeastern U.S. have become more resistant to the effects of pyrethroid insecticides.

In 2001, survival values for tobacco budworm adults at the 10 µg/vial concentration of cypermethrin were high throughout the mid-south/southeastern test areas. Survival at the 10 µg/vial concentration of cypermethrin ranged from a low of 12% to a high of 76%. These data suggest that pyrethroid resistance in tobacco budworm populations remains widespread and stable despite the introduction of new chemistries and transgenic cotton varieties. These data support observations by Graves et al. (1988, 1993) and Bagwell et al. (2001) suggesting that pyrethroid resistance in tobacco bubworm populations has steadily increased since the mid-1980's.

In general, spinosad was less effective against bollworm moths as compared to tobacco budworm moths. Mean monthly bollworm survival values throughout the test area were comparable to those obtained in Louisiana (Cook et al. 2002). In areas where survival values exceeded 20% (especially those sites where Tracer® has never been applied—Missouri (57% survival) and Tennessee (24% survival)) efforts to monitor the effects of spinosad should remain a priority.

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Table 1. Percent survival of bollworm male moths at 10 µg cypermethrin per vial by state and month during 1998. Data taken from Martin etal. 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	0
North Carolina	--	0	0.5	3.0
South Carolina	0.4	1.4	1.2	0.8
Texas	0	0	0.4	--

Table 2. Percent survival of bollworm male moths at 10 µg cypermethrin per vial by state and month during 1999. Data taken from Martin etal. 2000.

	% Survival					
State	**May**	**June**	**July**	**August**	**September**	**October**
Alabama	--	--	0	2.0	--	--
Arkansas	--	--	0	0	--	--
Florida	--	--	--	0	--	--
Georgia	--	--	--	--	--	--
Louisiana	--	0	22	0	--	--
Mississippi	--	--	0	0	--	--
Missouri	--	16	0	7.0	0	--
North Carolina	--	--	0	3.0	2.0	--
South Carolina	--	25	6.0	0	0	0
Tennessee	--	--	--	1.0	--	--
Texas	6.0	2.0	1.0	--	0	3.0

Table 3. Percent survival of bollworm male moths at 10 µg cypermethrin per vial by state and month during 2000. Data taken from Payne etal. 2001.

	% Survival			
State	**June**	**July**	**August**	**September**
Alabama	6.2	34	11	--
Arkansas	--	0	0	--
Florida	--	--	--	--
Georgia	--	2.8	4.2	--
Louisiana	11	5.4	0	--
Mississippi	--	0	0	--
Missouri	--	7.9	14	--
North Carolina	0	0	4.0	5.8
South Carolina	21	4.4	1.5	--
Tennessee	--	0	0	--
Texas	0	2.6	0	0
Virginia	0	3.9	9.4	0

Table 4. Percent survival of bollworm/tobacco budworm male moths at 10 µg cypermethrin per vial by state and month during 2001.

	% Survival				
State	**May**	**June**	**July**	**August**	**September**
Alabama	--/--	0/--	8.9/--	7.5/22	--/20
Arkansas	--/40	2.8/20	2.5/60	0/60	--/--
Florida	--/--	--/--	--/--	--/--	--/--
Georgia	--/--	--/--	0.6/--	11/--	1.8/12
Louisiana	--/69	--/47	8.8/73	3.4/76	16/57
Mississippi	--/40	10/58	7.4/60	14/--	13/--
Missouri	--/--	--/--	--/--	8.4/--	--/--
North Carolina	--/--	--/--	--/--	3.0/18	3.0/--
South Carolina	--/--	50/33	2.8/--	0.5/--	--/--
Tennessee	--/--	--/--	--/--	0/--	--/--
Texas	2.6/--	0/--	1.2/--	--/--	0/--
Virginia	--/--	--/--	0.9/--	0/--	0/--

Table 5. Percent survival of bollworm/tobacco budworm male moths at 15 µg spinosad per vial by state and month during 2001.

	% Survival				
State	**May**	**June**	**July**	**August**	**September**
Alabama	--/--	27/--	28/24	14/8.1	--/13
Arkansas	--/--	8.3/0	7.5/0	60/6.7	--/--
Florida	--/--	--/--	9.7/--	4.0/--	--/--
Georgia	--/--	--/--	6.0/--	12/--	6.0/7.4
Louisiana	0/15	--/15	28/19	3.0/17	0/0
Mississippi	--/--	--/--	1.8/0	0/0	--/0
Missouri	--/--	--/--	--/--	57/--	--/--
North Carolina	--/--	--/--	--/--	13/0	0/--
South Carolina	--/--	50/--	11/--	27/--	--/--
Tennessee	--/--	--/--	--/--	24/--	--/--
Texas	3.8/--	0/--	4.8/0	--/--	1.7/--
Virginia	--/--	--/--	0.9/--	5.1/--	5.3/--