SUSCEPTIBILITY OF FIELD-COLLECTED POPULATIONS OF TOBACCO BUDWORM AND COTTON BOLLWORM TO VARIOUS INSECTICIDES; 1995-1998

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Abstract

During the past four years, samples of tobacco budworm (TBW) and cotton bollworm (CBW) populations were collected from cotton fields throughout the south/southwestern corner of Georgia and South Carolina. Larvae from those field-collected samples were assayed for susceptibility to a variety of insecticides using a cotton leaf dip bioassay and an insecticide-treated diet bioassay. Throughout the evaluation period, TBW populations have demonstrated various levels of resistance to MVPII® as compared to the most susceptible field-collected population and two insecticide-susceptible, laboratory-maintained reference strains (HRV and OPS). MVPII® was less effective against CBW larvae. Decreased susceptibility of several TBW populations and a CBW population to cypermethrin were noted. Furthermore, an evaluation of LC_{95} values for cypermethrin against TBW larvae indicated an annual and sharp increase during the 1997 and 1998 seasons. In general,foliar leaf dip and treated diet 96h activity spectra for the compounds tested were: Spinosad > Pirate® ≥ Karate® >Fipronil ≥ Cypermethrin ≥ MVPII®.

Introduction

The tobacco budworm (TBW) and cotton bollworm (CBW) are two of the more economically important pests of cotton, and without proper control methods, populations of these pest insects can easily reach damaging levels and severely reduce crop yields. Because the tobacco budworm and cotton bollworm have developed resistance to most of the insecticides used for there control, 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 these pest insects are critical to the development of effective management strategies.

Over the past several years, the State University of West Georgia has attempted to establish and maintain a successful, multi-year tobacco budworm and cotton bollworm monitoring program in the state of Georgia. To date, insecticide susceptibility baseline data for TBW and CBW populations from the cottonbelt counties of southwestern Georgia have been collected over a four year period. These data will serve as reference points for a comparison of data collected from these locations in future surveys. This report summarizes the data that have been collected.

Materials and Methods

Tobacco budworm and cotton bollworm larvae and adults were collected from designated field sites throughout the cottonbelt counties of Georgia. TBW larvae (SC^{98}) and CBW larvae (Clm^{98}) were collected from locations in South Carolina also. The collections were transported or shipped to our facilities at the State University of West Georgia. Larvae were transferred to a pinto bean/wheat germ, agar-based artificial diet and adults were placed in mating cages to produce adequate numbers of larvae for testing. Larvae and adults were maintained in an environmental chamber at 27°C, LD 14:10, and 20-40 % relative humidity. The locations of the 1995 and 1996 field cultures have been presented in an earlier publication (Hasty et al. 1997). Following is a list of the reference strains and the 1997 and 1998 strains that were established and used in the insecticide susceptibility screens.

Tobacco Budworm Strains

HRV--a laboratory-maintained, insecticide susceptible strain; OPS--a laboratory-maintained insecticide-susceptible strain from South Carolina; OPR--a laboratory-maintained, organophosphate-resistant strain from South Carolina; PYR--a laboratory-maintained, organophosphate/pyrethroid-resistant strain selected for resistance to permethrin; Cam^{97}--a 1997 field strain collected as adults from non-Bt cotton located ca. 4 miles east of Camilla, GA, Mitchell County; Dec^{97}--a 1997 field strain collected as larvae from non-Bt cotton located ca. 15 miles west of Bainbridge, GA, Decatur County; Ear^{97}--a 1997 field strain collected as larvae from non-Bt cotton ca. 3 miles southwest of Blakely, GA, Early County; Mil^{97}--a 1997 field strain collected as larvae from non-Bt cotton located ca. 8 mi. west of Colquitt, GA, Miller County; Mol^{97}--collected as larvae from tobacco located ca. 12 miles northeast of Moultrie, GA, Colquitt County; Tif^{97}--a 1997 field strain collected as larvae from tobacco at the UGA Experiment Station, Tifton, GA, Tift County; Dec^{98}--a field strain collected as adults from non-Bt cotton ca. 18 miles west of Bainbridge, GA, Decatur County; Ear^{98}--a field strain collected as adults from non-Bt cotton ca. 5 miles east of Blakely, GA, Early County; and SC^{98}--a field strain collected as larvae from non-Bt cotton near Dalzell, SC.

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Cotton Bollworm Strains

Dez[97]--a field strain collected as larvae from non-Bt cotton ca. 15 miles west of Bainbridge, GA, Decatur County; and Miz[97]--a field strain collected as larvae from non-Bt cotton located ca. 8 mi. west of Colquitt, GA, Miller County; and Clm[98]--a field strain collected as larvae from non-Bt cotton at the Edisto Research and Education Center, Blackville, SC.

Larvae were evaluated for susceptibilities to a variety of technical grade insecticides and insecticidal formulations including MVPII® (28% A.I., Mycogen Corporation, San Diego, CA), cypermethrin (99+% pure, FMC Corporation, Princeton, NJ), fipronil (99.9% pure, Rhone-Poulenc, RTP, NC), lambda-cyhalothrin (98% pure, Chem Service, West Chester, PA), chlorfenapyr (99.9% pure, American Cyanamid, Princeton, NJ), and spinosad (91.3% pure, Dow AgroSciences, Indianapolis, IN).

Larvae were tested using a standardized leaf dip bioassay (IRAC Method No. 7, 1990), a modified insecticide-treated diet bioassay (Ross and Brown 1982), or by topical application. Following is a brief description of each treatment method.

In the leaf dip method, test solutions were prepared by adding 100 µl of an appropriate insecticide stock solution into 50 ml of water plus 1 drop of Triton X-100 as a wetting agent. Control solutions were prepared by adding 100µl of the carrier solvent into 50 ml of water plus1 drop of Triton X-100. A 4.5 cm cotton leaf disk was excised from an untreated cotton leaf and dipped for 5 s with gentle agitation in an appropriate treatment solution. The leaf disks were treated individually and allowed to surface-dry on paper toweling. Two replicates of ten leaves each were treated at a minimum of five rates plus a control. The treated leaf disks were placed into a 50 x 9 mm Falcon® tight-fitting petri dish on moistened filter paper. Two first-instar larvae were placed in each dish. Mortality was monitored over a 5 day period. Mortality was defined as the inability to translocate across the leaf surface when probed. Forty larvae were used for each concentration.

In the treated diet method, 100 µl of an appropriate insecticide test solution were added to 50 ml of liquefied pinto bean/wheat germ, agar-based diet at 57°C while mixing with a variable speed-controlled laboratory stirrer at a Variac® setting of "60-70" for 10-15 s. The insecticide-treated diet was distributed in 2.5 ml aliquots to 20 separate 1 oz. clear plastic medicine cups. The treated diets were allowed to gel and cool. One late second-instar larva was added to each cup, and mortality was monitored over a 5 day period. Two replicates of twenty cups each at a minimum of five rates plus a control served as a test. Mortality was defined as the inability to translocate across the diet surface when probed. Forty larvae were used for each concentration.

Topical applications were performed according to modified standard procedures for the detection of insecticide resistance in TBW as recommended by the Entomological Society of America (Anonymous 1970). Fourth-instar OPS and SC[98] larvae (35 ± 5 mg) were treated at each of five serial doses of technical grade cypermethrinin in 1.0 μl of acetone. Control larvae were treated with acetone alone. Mortality, the inability to translocate when probed, was assessed at 48 h. A total of 20 larvae were used for each concentration.

In all bioassay protocols, treated larvae were held at 27°C, LD 14:10, and 20-40 % relative humidity. Percent mortality was corrected for control mortality (Abbott 1925). Median lethal concentrations with 95% confidence intervals and regression slopes were estimated by computerized probit analysis (SAS Institute 1989).

Results and Discussion

Although foliar LC_{50} values were generally lower than treated diet LC_{50} values, trends in susceptibilities of the TBW strains to the various insecticides between the two tests were similar (Tables 1 and 2). As reported for 1995 and 1996 (Hasty et al. 1997), 1997 and 1998 LC_{50} values for most of the TBW field-collected populations were comparable to the laboratory-maintained, insecticide-susceptible HRV and OPS strains. However, various levels of resistance to MVPII® and cypermethrin have been noted throughout the study period.

Leaf dip bioassays indicated that the Mil[97] and Mol[97] strains were ca. 17-fold resistant to MVPII® as compared to the HRV strain (Table 1), and treated diet tests indicated that the Mol[97] strain was ca. 41-fold more resistant to MVPII® than the OPS strain (Table 2). As anticipated, MVPII® was less effective against CBW larvae (Table 3). These data suggest the presence of low levels of Bt resistant individuals within the populations sampled and emphasize the importance of maintaining a widespread Bt resistance monitoring program in Georgia.

In 1997, the highest levels of resistance to cypermethrin were recorded in the Cam[97] and Mol[97] strains. Leaf dip LC_{50} values for cypermethrin were 14-fold higher than the HRV LC_{50} for cypermethrin (Table 1). The treated diet LC_{50} value for cypermethrin against the MOL[97] strain was 8-fold higher than the comparable HRV LC_{50} (Table 2). In 1998, higher levels of resistance were noted. The Ear[98] and SC[98] strains were greater than 48-fold resistant to cypermethrin when evaluated using the leaf dip bioassay (Table 1) and 8-16-fold resistant when evaluated using the treated diet bioassay (Table 2). Also, cypermethrin LC_{95} mean values increased in 1997 and 1998 (Figure 1), and the slopes of the concentration/mortality lines have decreased throughout the evaluation period. In general, those strains that exhibited higher LC_{50} values for cypermethrin also exhibited higher LC50 values for cyhalothrin (Tables 1 and 2). Furthermore,

topical application of cypermethrin to fourth-instar SC^{98} larvae indicated that the SC^{98} strain was 26-fold more resistant than the OPS laboratory-susceptible strain. The LD_{50} values for cypermethrin against OPS and SC^{98} larvae were 0.49 µg/g larva and 12.6 µg/g larva, respectively. These data suggest an increased heterogeneity in response to cypermethrin exposure and the existence of low levels of pyrethroid-resistant individuals in certain TBW populations in Georgia.

In addition to pyrethroid resistance being detected in Georgia and South Carolina TBW populations, increased resistance to pyrethroid insecticides in field-collected CBW populations was observed (Table 3). Clm^{98} CBW larvae were 11-fold resistant to cypermethrin when compared to the most susceptible field strain tested. Resistance to cyhalothrin had already been demonstrated in CBW populations collected from the Estill/Edisto River Valley region of South Carolina (Brown et al. 1998) and similar reports have been have been published from other locations in the US (Abd-Elghafar et al. 1993, Kenga et al. 1996, Bagwell et al. 1996, 1998) and Central and South America (Ernst and Dittrich 1992).

Responses of TBW and CBW strains to fipronil, chlorfenapyr and spinosad are presented (Tables 1-3). When evaluated using the leaf dip bioassay (Table 1), the activities of chlofenapyr, fipronil and cypermethrin against TBW larvae were comparable. Cyhalothrin was more effective than either chlorfenapyr, fipronil or cypermethrin. The most active compound evaluated was spinosad. These same trends were noted in tests to evaluate the activities of those compounds against CBW larvae (Table 3). When TBW larvae were evaluated using the treated diet bioassay (Table 2), the activities of fipronil and cypermethrin were comparable. Cyhalothrin and chlorfenapyr were more effective than fipronil and cypermethrin, and spinosad was the most active compound tested.

Studies of this nature are critical to the development of effective resistance management strategies. Although transgenic varieties of cotton have been effective in controlling TBW populations in the field, the development of resistance to Bt has been reported (Stone et al. 1989, Gould and Anderson 1991, Gould et al. 1992). In addition, CBW larvae are less susceptible to the effects of Bt cotton. With the widespread planting of Bt cotton throughout the southeast and the introduction of Bt corn, selection pressures on these two economically important pest insects will be significant. In these circumstances, conventional insecticides such as the pyrethroids, cyperemthrin and cyhalothrin, may be used to control outbreaks of TBW and CBW populations. Unfortunately, our data support previous findings and indicate that certain TBW and CBW populations in Georgia and South Carolina are becoming more resistant to the pyrethroid insecticides. Therefore, it is imperative that measures be taken to prolong the use of these insecticides as quickly as possible. One principal step toward this goal was initiated this past year. During the 1998 season, a mid-south and southeast regional monitoring program was initiated by the Insecticide Resistance Action Committee (IRAC) to determine and track the status of CBW susceptibility to pyrethroid insecticides (Scott et al., 1999 Beltwide Cotton Conferences Presentation, Orlando, FL). The information collected during the 1998 season has been used to fine-tune and focus the project for 1999, and it is anticipated that this concerted effort will continue into the year 2000.

In order to prolong the effective use of pyrethroid insecticides in the control of lepidopterous pests of cotton, the use and effectiveness of alternative insecticides with different modes of action, such as chlorfenapyr and spinosad, must be evaluated. Chlorfenapyr is a novel pyrrole proinsecticide that is oxidatively bioactivated to an insecticidal metabolite that disrupts cellular respiration (Treacy et al. 1994, Black et al. 1994). Spinosad is a mixture of two naturally occurring metabolites resulting from the fermentation of the actinomycete, *Saccharopolyspora spinosa* (Kirst et al. 1991). Spinosyn A, the major component, exerts its toxicity by the activation of nicotinic acetylcholine receptors (Salgado 1997). Both chlofenapyr (Treacy et al. 1991, Whitehead et al. 1993, Whitehead and Treacy 1995, Wiley et al. 1995) and spinosad (Bret et al. 1997) are highly effective against a wide spectrum of pest insects and effectively control pyrethroid-resistant TBW larvae (Hasty et al. 1997, Pimprale et al. 1997).

Based on these studies, chlorfenapyr and spinosad are viable alternative insecticides that may be used effectively control pest insects of cotton and reduce selection pressures for the expression of pyrethroid resistance in TBW and CBW field populations (Walker et al. 1998).

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Table 1. Susceptibility of first-instar tobacco budworm larvae to MVPII®, Cypermethrin (Cyp), fipronil (Fip), cyhalothrin (Cyh), chlofenapyr (Chl), and spinosad (Spin) following a 96 h exposure period using a cotton leaf-dip bioassay (IRAC No. 7).

Strain	LC_{50} (Slope)					
	MVPII®	Cyp	Fip	Cyh	Chl	Spin
0						
HRV	0.30 (1.0)	0.25 (2.4)	0.73 (2.0)	0.06 (4.1)	3.74 (3.0)	< 0.03
OPS	0.93 (3.0)	1.33 (2.3)	1.17 (2.1)	0.13 (2.1)	2.25 (3.6)	0.04 (1.3)
OPR	6.23 (0.7)	3.23 (2.3)	0.62 (1.2)	0.86 (1.9)	2.13 (3.6)	0.29 (0.7)
PYR	0.62 (1.0)	2.84 (1.6)	0.61 (2.6)	0.41 (1.1)	1.96 (4.2)	0.07 (1.5)
Cam[97]	0.84 (0.9)	3.56 (2.7)	4.98 (3.0)	0.42 (1.2)	0.46 (1.0)	0.06 (2.0)
Dec[97]	0.71 (1.2)	0.45 (1.2)	0.30 (2.2)	0.03 (0.8)	0.46 (1.4)	0.12 (2.1)
Ear[97]	0.44 (0.8)	0.78 (1.3)	0.27 (1.9)	< 0.1	< 0.1	0.02 (2.3)
Mil[97]	5.77 (1.8)	1.07 (2.2)	ND	ND	ND	ND
Mol[97]	4.88 (1.4)	3.61 (1.5)	ND	1.37 (2.7)	ND	ND
Tif[97]	1.62 (1.4)	0.96 (1.8)	ND	0.12 (1.4)	ND	0.02 (1.1)
Dec[98]	ND	ND	ND	ND	1.55 (4.4)	ND
Ear[98]	ND	12.1 (2.1)	ND	ND	0.82 (1.4)	ND
SC[98]	ND	13.1 (1.3)	ND	ND	1.36 (5.7)	ND

ND = Not Determined

Table 2. Susceptibility of second-instar tobacco budworm larvae to MVPII®, cypermethrin (Cyp), fipronil (Fip), cyhalothrin (Cyh), chlorfenapyr (Chl), and spinosad (Spin) following a 96 h exposure period using an insecticide-treated diet bioassay.

Strain	LC_{50} (Slope)					
	MVPII®	Cyp	Fip	Cyh	Chl	Spin
HRV	ND	1.42 (5.2)	1.64 (4.5)	ND	0.51 (6.2)	0.38 (1.4)
OPS	0.75 (0.7)	5.01 (3.2)	2.14 (3.1)	0.50 (3.6)	0.76 (3.2)	0.14 (3.3)
OPR	ND	5.48 (2.7)	2.36 (1.9)	0.18 (2.1)	0.81 (3.7)	0.37 (2.2)
PYR	1.23 (1.9)	36.5 (2.1)	2.14 (3.1)	3.04 (2.0)	1.13 (3.5)	0.40 (3.4)
Tif[95]	0.95 (1.0)	0.46 (1.1)	ND	ND	1.73 (6.9)	0.84 (1.7)
Bla[96]	1.87 (0.8)	7.05 (3.1)	2.47 (1.7)	ND	0.60 (3.0)	0.62 (1.5)
Ear[96]	68.4 (1.5)	0.82 (2.2)	1.59 (1.3)	ND	0.67 (2.8)	0.41 (2.6)
Mil[96]	30.4 (0.7)	3.44 (2.6)	8.99 (3.0)	ND	1.21 (2.6)	0.55 (6.3)
Tif[96]	0.95 (1.0)	5.96 (4.3)	5.08 (5.6)	ND	1.01 (3.3)	0.35 (1.8)
Cam[97]	0.56 (1.4)	ND	ND	ND	ND	ND
Dec[97]	0.30 (1.7)	ND	ND	ND	ND	0.35 (1.8)
Ear[97]	3.38 (1.0)	3.00 (2.5)	ND	ND	ND	ND
Mol[97]	30.5 (0.9)	12.1 (2.6)	ND	ND	ND	ND
Tif[97]	ND	1.39 (2.0)	ND	2.16 (2.3)	ND	ND
Ear[98]	ND	12.1 (1.7)	ND	ND	ND	ND
SC[98]	1.36 (1.7)	23.2 (1.5)	ND	ND	ND	0.68 (3.4)

ND = Not Determined

Table 3. Susceptibility of cotton bollworm larvae to MVPII®, Cypermethrin (Cyp), fipronil (Fip), cyhalothrin (Cyh) chlofenapyr (Chl), and spinosad (Spin) following a 96 h exposure period.

Strain	LC_{50} (Slope)					
	MVPII®	Cyp	Fip	Cyh	Chl	Spin
Bla[96*]	21.8 (1.2)	1.95 (1.8)	ND	ND	ND	ND
Ear[96*]	51.8 (1.6)	1.00 (1.3)	ND	ND	ND	ND
Mit[96*]	43.2 (2.3)	1.24 (3.1)	1.45 (5.2)	ND	0.55 (5.7)	0.30(1.6)
Dez[97**]	> 100	1.23 (2.1)	ND	ND	ND	ND
Miz[97**]	31.6 (2.6)	1.39 (4.0)	ND	0.28 (2.3)	ND	ND
Clm[98**]	ND	11.3 (3.7)	ND	ND	2.65 (2.4)	ND

*Evaluated using the treated diet protocol
**Evaluated using the leaf dip protocol
ND = Not Determined

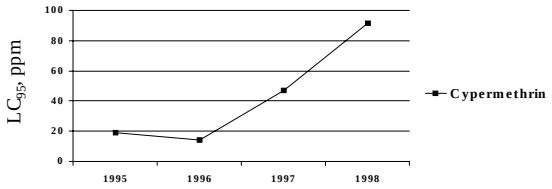

Figure 1. Susceptibility of tobacco budworm larvae to cypermethrin expressed as the LC_{95} following exposure to treated diet.