EVALUATION OF THE SUSCEPTIBILITY OF TOBACCO BUDWORM (*HELIOTHIS VIRESCENS*) AND COTTON BOLLWORM (*HELICOVERPA ZEA*) POPULATIONS IN GEORGIA TO VARIOUS INSECTICIDES

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Abstract

Samples of tobacco budworm (*Heliothis virescens*; TBW) and cotton bollworm (*Helicoverpa zea*; CBW) populations were collected from cotton fields throughout the south/southwestern corner of Georgia. Larvae from those field-collected samples were assayed for susceptibility to a variety of insecticides using a treated diet bioassay. Several TBW strains demonstrated significant tolerance to MVP II® as compared to the most susceptible field strain and an insecticide-susceptible lab strain (OPS). MVP II® was less effective against CBW larvae. Several TBW strains were slightly tolerant (4-8-fold) to cypermethrin as compared to the most susceptible field strain. The activities of Fipronil, Pirate®, and Spinosad were good against all strains tested. In general, the 96 h activity spectrum for the compounds tested were: Spinosad > Pirate® > Fipronil > Cypermethrin > MVP II®.

Introduction

Currently, there are more than 500 insect and mite species that are resistant to one or more classes of insecticides (Sparks et al., 1993). Prominent among this list, as far as agricultural pest insects of the southern United States and Central America are concerned, is the tobacco budworm, *Heliothis virescens* (F.). The tobacco budworm is one of the more economically important pests of cotton, and without proper control methods, populations can easily reach damaging levels and severely reduce crop yields. In 1995, agricultural losses attributed to the destructive activities and costs to control this insect were more than 226 million dollars (Williams 1996). In Georgia alone, the tobacco budworm cost the cotton industry more than 16 million dollars (Williams 1996). Because the tobacco budworm has developed resistance to practically every major class of insecticide used for its control, it is critical that research efforts and agricultural practices be devoted to the preservation of those control strategies that are still effective and to the development of new replacement compounds and technologies. In addition to those efforts, equal consideration must be given to the design and implementation of effective resistance monitoring and management strategies.

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The primary objective of this research project was to initiate and develop a series of bioassays to effectively monitor insecticide susceptibilities and to provide supporting data for the characterization of insecticide resistance mechanisms in field-collected populations of TBW and CBW in the state of Georgia. This report summarizes our initial efforts to monitor the susceptibilities of TBW and CBW field-collected populations to *Bacillus thuringiensis* (Bt) insecticidal proteins (MVP II®) and a variety of other insecticides that are currently being used or developed for TBW and/or CBW control on cotton (e.g., Cypermethrin, Fipronil, Pirate®, and Spinosad).

Materials and Methods

Insects

Multiple samples of tobacco budworm and cotton bollworm larvae and adults were collected from designated field sites throughout the cottonbelt counties of Georgia during the 1996 growing season. Efforts were concentrated in those counties located in the south-southwestern corner of Georgia (because multiple control failures have often been reported in those areas). Larvae and adults (collected from both Bt cotton and non-Bt cotton) were transported back to our facilities at the State University of West Georgia. Larvae were then transferred to a pinto bean/wheat germ-based artificial diet and adults were placed in mating cages to produce adeqequate 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 following strains were established:

Tobacco Budworm Strains

HRV --a laboratory-maintained reference strain;
OPS --a laboratory-maintained, insecticide-susceptible strain;
OPR --a laboratory-maintained, organophosphate-resistant strain;
BLA --a 1996 field strain collected as larvae from non-Bt cotton located 5 miles south of Blakely, GA, Early County;
ERA --a 1996 field strain collected as adults from Bt cotton located 5 miles north of Blakely, GA, Early County;
MIL --a 1996 field strain collected as adults from a border plot surrounding a Bt cotton field located ca. 6 miles northwest of Colquitt, GA, Miller County;
TIF --a 1995 field strain collected as larvae from a border plot surrounding a Bt cotton field near Tifton GA, Tift County;
TOB --a 1996 field strain collected as larvae from tobacco near Tifton, GA, Tift County.

Cotton Bollworm Strains

BLB --a 1996 field strain collected as larvae from non-Bt cotton located 5 miles south of Blakely, GA, Early County;

ERB --a 1996 field strain collected as adults from Bt cotton located 5 miles north of Blakely, GA, Early County;

MIT --a 1996 field strain collected as adults from late-season, non-Bt cotton near Camilla, GA, Mitchell County.

Bioassays

Larvae were tested with a variety of insecticides (e.g., MVP II®, cypermethrin, Fipronil, Pirate®, and Spinosad) using a modified insecticide-treated diet bioassay (Ross and Brown 1984). Second instar larvae were placed on treated diet and mortality was monitored over a seven day period. Insecticide concentration-mortality regressions were generated for each strain using a SAS-Probit Analysis program.

Technical grade insecticides and insecticidal formulations were provided by Mycogen Corp. (MVP II; 28 % A.I.), FMC Corp. (Cypermethrin; 99+ % pure), Rhone Poulenc (Fipronil; 99.9% pure), American Cyanamid (4-bromo-2(*p*-chlorophenyl)-1-(ethoxymethyl)-5-trifluoromethyl-pyrrole-3-carbonitrile (active ingredient of Pirate®); 99.9% pure), and DowElanco (Spinosad; 88% pure).

Results and Discussion

Our data indicated that there was considerable variability among TBW field populations in their response to MVP II® following 96 h and 144h exposure periods (Table 1). The MVP II® LC_{50} values for most of the field-collected TBW field strains were comparable to the laboratory-maintained, insecticide-susceptible OPS strain; however, the ERA and MIL TBW strains were 91-foldand 40-fold more tolerant to MVP II® following 96 h exposure periods, respectively. Furthermore, the slopes of the concentration-mortality regressions were relatively flat suggesting a heterogeneous response of those strains to MVP II® exposure. Although generally more tolerant of MVP II® (29-fold-69-fold), cotton bollworm strains from the sampling areas were more consistent in their response.

Table 2 shows the responses of TBW and CBW strains to cypermethrin, Fipronil, Pirate®, and Spinosad. Several TBW strains (i.e., OPR, BLA, and MIL) showed a slight tolerance to cypermethrin as compared to the most susceptible field strain (TIF) and the lab reference strain (HRV). The activities of Fipronil and cypermethrin were comparable. The most effective compounds in this study were Pirate® and Spinosad with LC_{50} values typically less than 1 ppm. In general, CBW larvae were more susceptible to cypermethrin, Fipronil, Pirate®, and Spinosad as compared to TBW larvae (Table 2). Spinosad was the most effective compound tested while MVP II® (a much slower acting compound) was generally the least effective (Tables 1 and 2).

One of the newer classes of insecticide used to control TBW larvae is agroup of microbial d-endotoxins derived from the soil bacterium, *Bacillus thuringiensis*. The development and use of these microbial insecticides has been especially promising because these compounds are effective, environmentally friendly, and highly selective; however, two major factors have limited the use of Bt endotoxin proteins in the control of tobacco budworm field populations. First, because these proteins are heat and photo labile, they have a short half-life under field conditions (Gelernter 1990). Second, the mode of action of these insecticidal proteins (resulting in the lysis of the epithelial cells lining the insect's midgut) only allows them to be effective if they are ingested (Gelernter 1990; Gill et al., 1992; Slaney et al., 1992).

Recently, cotton plants have been genetically modified to contain the genes coding for the expression of d-endotoxins that are specifically toxic to many lepidopteran species including the tobacco budworm (Gelernter 1990; Benedict et al., 1992). These genetically-modified plants, referred to as transgenic plants, may be a solution to the problems presented above. However, in solving some problems, the use of transgenic plants may foster others. The potential for the rapid development of resistant tobacco budworm populations is high. Laboratory and field results have indicated that resistance to Bt is possible in a number of lepidopteran species including the tobacco budworm (Stone et al., 1989; Gould and Anderson 1991; Gould et al., 1992), and based on data obtained from resistance studies in field and laboratory strains of another lepidopteran pest insect, the diamondback moth, resistance to Bt from the use of transgenic plants appeared to be inevitable (Tabashnik et al., 1991). In addition, variations in susceptibility to Bt endotoxins have been documented in tobacco budworm populations (Stone et al., 1991), and resistance to one Bt toxin may confer cross-resistance to other Bt toxins (Gould etal., 1992).

This past year, 1996, was the first year that transgenic varieties of cotton plants expressing Bt toxins were commercially available. Because conventional control strategies were limited and the new technology was promising, the purchase and planting of transgenic cotton plants throughout the cottonbelt states was widespread, and in the near future, transgenic varieties of corn will be planted also. Although the transgenic varieties of cotton were effective in controlling tobacco budworm populations this past year, the protein toxins produced by these plants were less effective against other lepidopteran species such as the cotton bollworm. Furthermore, 1996 cotton bollworm populations in South Carolina and Louisiana were suspected of being resistant to Karate® (Brown 1996; Ottea 1996). Therefore, an effective monitoring program must survey the susceptibilities of tobacco budworm and cotton bollworm populations to the various insecticides used for their control at a minimum.

Summary

Last year, there were over 1.5 million acres of cotton harvested in the state of Georgia, and cotton production in the state of Georgia is expected to increase in 1997. Our goal was to establish a multi-year, insecticide-resistance monitoring program at the State University of West Georgia, in Carrollton, GA. The data obtained from this program will provide useful information pertaining to the current status of insecticide resistance in Georgia tobacco budworm and cotton bollworm field populations and provide critical baseline information for future comparisons and assessments. In addition, these studies may lead to the development of viable alternative strategies to control these insects, extend the lives of existing insecticides, and/or monitor the development of resistant populations.

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Table 1. Susceptibility of tobacco budworm and cotton bollworm larvae to MVP II®.

Strain	96 hour	144 hour
Slope	LC_{50}, ppm (C.I.); Slope	LC_{50}, ppm (C.I.);
OPS	0.75 (0.27-2.63); 0.7	0.20 (0.08-0.46); 1.0
OPR	9.30 (2.42-48.7); 0.9	1.53 (0.46-4.74); 1.2
BLA	1.87 (0.94-3.66); 0.8	0.71 (0.23-1.66); 1.5
ERA	68.4 (22.6-2456); 1.5	5.44 (1.84-15.0); 1.4
MIL	30.4 (6.90-1105); 0.7	6.54 (2.11-21.6); 1.2
TIF	0.95 (0.37-2.33); 1.0	0.34 (0.09-0.88); 0.9
TOB	0.90 (0.27-2.63); 0.9	0.71 (0.34-1.60); 1.2
BLB	21.8 (8.95-61.6); 1.2	26.6 (14.5-50.5); 2.6
ERB	51.8 (23.6-163); 1.6	20.0 (9.08-45.3); 1.7
MIT	43.2 (31.8-77.6); 2.3	31.6 (13.8-72.5); 2.6

Table 2. Susceptibility of tobacco budworm and cotton bollworm larvae to cypermethrin, Fipronil, Pirate®, and Spinosad insecticides following a 96 h exposure period.

Strain	LC_{50}, ppm (Slope)			
	Cypermethrin	Fipronil	Pirate®	Spinosad
HRV	1.4 (5.2)	1.6 (4.5) (1.4)	0.5 (6.2)	0.4
OPS	ND	ND	0.8 (3.2) (3.3)	0.1
OPR	5.5 (4.9)	2.4 (1.9)	0.8 (3.7) (2.2)	0.4
BLA	7.0 (3.1)	2.5 (1.7)	0.6 (3.0) (1.5)	0.6
ERA	0.8 (2.2)	1.6 (1.3)	0.7 (2.8) (2.6)	0.4
MIL	3.4 (2.6)	9.0 (3.0)	1.2 (2.6) (6.3)	0.6
TIF	0.8 (1.2)	ND	1.7 (6.9) (1.7)	0.8
TOB	6.0 (4.3)	5.1 (5.6)	1.0 (3.3) (1.8)	0.4
BLB	2.0 (1.8)	ND	ND	ND
ERB	1.0 (1.3)	ND	ND	ND
MIT	1.2 (3.1)	1.4 (5.2)	0.6 (5.7) (1.6)	0.3

ND = not determined