Bt Cotton: Status of Heliothine Resistance in the United States

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ABSTRACT

Resistance to the delta endotoxin proteins of Bacillus thuringiensis (Bt) by insects is receiving considerable interest both nationally and internationally for three primary reasons: unprecedented interest on the part of the environmental community and organic producers; the registration and deployment of transgenic plants in many countries; and laboratory and field resistance to Bt in 10 to 12 insect species. Preliminary Bt resistance monitoring in 1996 showed that 23 cotton populations of cotton bollworm (CBW), Helicoverpa zea (Boddie) and tobacco budworm (TBW), Heliothis virescens (Fabricius) collected in Arkansas, Mississippi, Oklahoma, and Texas, showed no shifts in baseline levels of susceptibility to biological insecticides, compared to susceptible laboratory colonies. This program was expanded in 1997 to 67 colonies (24 TBW, 43 CBW) from 9 states and 27 co-operators. Although exposure to the MVP II toxin resulted in poor tolerance (i.e., continued susceptibility) for both species, there was a consistent trend of better survival in the field-collected colonies of TBW and CBW, suggesting a trend of tolerance to Bt in field populations of CBW and TBW compared to the laboratory colonies. Continued resistance monitoring in 1998 and beyond is vital to detect changes in this response.

Introduction

More than 30 crop species have been genetically engineered to express the delta endotoxin proteins of *Bacillus thuringiensis* (*Bt*) due to their insecticidal properties (Ives, 1996). Major crops that have been transformed include corn, cotton and potatoes. The Monsanto Company has developed a cotton plant with the Bollgard® gene, a *Bt* derivative that provides protection against attack from the cotton bollworm (CBW), *Helicoverpa zea* (Boddie), tobacco budworm (TBW), *Heliothis virescens* (Fabricius), and the pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Barton, 1995). Insects such as the Indian meal moth, *Plodia interpunctella* (Hübner) (McGaughey, 1985), the diamondback moth, *Plutella xylostella* (Linnaeus) (Tabashnik *et al.*, 1990), and at least nine others have developed resistance to *Bt* insecticides in the laboratory and/or field so concerns have been raised by environmentalists, scientists, industry and producers about the long-term effectiveness of genetically-engineered plants that express *Bt* endotoxins. *Bt* resistance management has become an important research activity in agricultural entomology today. This paper reports results of two years of monitoring to determine if significant levels of resistance to *Bt* cotton are present in wild populations of CBW and TBW. No data are reported on PBW since it is found primarily in western cotton regions.

Material and Methods

Several entomologists and consultants in all cotton-growing states collected any 3rd instar or larger larva of CBW and TBW found on *Bt* cotton, place them on artificial diet (supplied by ARS) and ship them to ARS at Stoneville, MS. Eggs and larvae were also collected in large plantings of *Bt* cotton (250 to 4,000 ha) grown for seed in several counties in Mississippi and moths were collected in light traps and sweep nets in *Bt* fields of varying sizes. Similar collections were made in non-*Bt* cotton for comparison. Several co-operators also sent eggs collected in cotton and moths captured in light traps around cotton, corn and soybean. Larvae received or hatched from eggs were reared on artificial diet and held at 29 ± 3°C, 55-60% RH with a photoperiod of 14:10 (L:D) h until they emerged as moths. These were mated, eggs were collected, placed on artificial diet and held under the same conditions. Moths received from co-operators were mass-caged and resulting eggs handled in the same way. Methods and material used in spray chamber bioassay, adapted from Luttrell *et al.* (1987). were detailed by Elzen *et al.* (1990). Cotton terminals (10 to 14 cm stem with three to four leaves and small buds) were clipped from plants grown in the greenhouse and placed in florist's water wicks. Each insecticide treatment consisted of three replications of 15 terminals each.

Treatments in spray chamber bioassays included cymbush (cypermethrin, Zeneca Ag Products, Wilmington, DE) (90 gm AI/ha), larvin (Thiodicarb, Rhone Poulenc Ag Company, Research Triangle Park, NC) (1 kg AI/ha), curacron (Profenofos, Novartis, Greensboro, NC) (1.12 kg AI/ha), and MVP II (*Bacillus thuringiensis* var. *kurstaki*, encapsulated delta endotoxin, Mycogen Crop Protection, San Diego, CA) (2 l/ha), the biological insecticide closest in toxicological properties to the Cry I A(c) protein expressed in transgenic cotton (Gould *et al.*, 1995). Controls were treated with water only. The spray table was calibrated to deliver 56 litres per ha at 2,109 g/cm^2 pressure with one TX-6 hollow-cone nozzle at 3.2 km/h, positioned 30.5 cm above the spray surface. One third-instar larva (20 ± 3 mg) from the selected strain was placed on each terminal 30 min after spraying and each plant was covered with a 590-ml ventilated paper cup. Sources of insect colonies are shown in Table 1. All treatments were held at 29 ± 3°C, 55-60% RH and a photoperiod of 14:10 (L:D) h. Treatment efficacy was determined 72 h after larvae were placed on the terminals. Numbers of moribund and dead larvae were used to calculate total mortality.

In addition, in 1997 preparations of MVP II overlays were made (J.T. Greenplate (Monsanto) unpublished) and one neonate larva (CBW and TBW were kept separate) was added to each covered well and incubated for 5 days under these conditions (or when 85% or more of larvae in control trays without MVP II powder reached 3rd instar). For each test, 30-128 larvae/colony were assayed. Concentrations of MVP II in agar were 0.05 and 5.0 µg/ml (EC98) for TBW and CBW, respectively. Dead or stunted larvae (<3rd instar) in treated and control overlays were recorded. A paired *t*-test compared mortality levels for each colony with the laboratory colony (SAS, 1987).

Results and Discussion

The spray chamber application of MVP II in 1996 effectively killed larvae of both TBW and CBW (Table 1). Lab and field-collected TBW produced larvae that suffered significantly greater mortality in the MVP II treatment than in the check treatment ($\bar{x}_{Difference}$ ± SEM = 44.10 ± 4.75%, t = 9.28, df = 5, P = 0.0002). Furthermore, the average percentage mortality of field-collected TBW (41.34 ± 3.41%) was significantly lower than the percentage mortality of the Stoneville colony (64.5%; t = -6.78, df = 4, P = 0.0025). Larvae of lab and field-collected CBW also suffered significantly more mortality in the MVP II treatment than in the check treatment ($\bar{x}_{Difference}$ ± SEM = 53.09 ± 2.55%, t = 20.79, df = 16, P = 0.0001). However, the average percentage mortality of field-collected CBW (57.26 ± 2.24%) was significantly greater than the percentage mortality of the Stoneville colony (44.4%; t = 5.75, df = 15, $P < 0.0001$).

In paired comparisons of 1997 using the MVP II overlay assay, field-collected TBW suffered less mortality/stunting than the Stoneville control strain (Table 2; 97.87 ± 0.62 vs. 99.86 ± 0.13, respectively). A paired t-test found the average difference between field colonies and the control strain to be significantly different from zero ($\bar{x}_{Difference}$ ± SEM = -2.00 ± 0.57%, t = -3.51, df = 14, P = 0.0034). The difference in mortality/stunting rates of field colonies and the control strain on the non-toxic diet was not significant (t = 1.30, df = 14, P = 0.2159).

Field populations of CBW also suffered less mortality/stunting than the Stoneville control strain (Table 3; 98.38 ± 0.28 vs. 99.97 ±

0.03, respectively). The average difference between field colonies and the control strain were significant from zero ($\bar{x}_{Difference} \pm SEM = -1.59 \pm 0.29\%$, $t = -5.51$, $df = 38$, $P = <0.0001$). The difference in mortality/stunting rates of field colonies and the control strain on non-toxic diet was not significant ($t = -1.10$, $df = 36$, $P = 0.2793$). Although exposure to the MVP II toxin in the overlay assays resulted in poor survival/growth for both species, there was a consistent trend of better survival/ growth in the field-collected colonies of TBW and CBW relative to the laboratory control strains. These results suggest a trend of tolerance to *Bt* in field populations of CBW and TBW compared to the Stoneville lab colony. In both years, CBW colonies were as susceptible to MVP II as TBW in the spray chamber. This contradicts the accepted view and the results of Sims *et al.* (1996). Unpublished results (Hardee and Adams) show the need for *Bt* cotton in that TBW populations, especially late in the season, were generally less susceptible to all insecticides than needed to manage populations of this insect in cotton effectively. A resistance monitoring system now in place should continue and expand in 1998 and beyond to look for shifts from the baseline susceptibility level in pest populations, especially in CBW. However, threshold levels and remedial action are still lacking.

Disclaimer

This paper reports results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

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Table 1. Percentage mortality of larvae to MVP II (2 l/ha) from spray chamber bioassays during 1996.

State	Location	Host	% Mortality[4]			
			H. virescens		*H. zea*	
			MVP II	Check	MVP II	Check
MS	Stoneville	Lab Colony	64.5	2.2	44.4	6.7
	Washington Co.	Geranium[1]	40.0	0.0	48.9	11.1
	Washington Co.	Non-Bt Cotton[2]	44.5	2.2	66.7	4.5
	Washington Co.	Non-Bt Cotton[3]	44.4	0.0	48.9[5]	4.5[5]
	Four Counties	Bt Cotton	---	---	60.0	2.2
TX	Snook	Bt Cotton	48.9	0.0	58.0[6]	4.4[6]
	Corpus Christi	Non-Bt Cotton	28.9	2.2	---	---
AR	McGehee	Bt Cotton	---	---	59.5[7]	2.2[7]
	Monticello	Bt Cotton	---	---	57.8	2.2

OK	Altus	Bt Cotton	---	---	57.8	2.2
_	All Locations	All Hosts	45.2	1.1	55.8	4.4

[1]Collected 15 May from weed hosts; [2]Collected 15 June from non-Bt cotton; [3]Collected 15 July from non-Bt cotton; [4]Data shown are from one test unless otherwise noted.
[5]Five tests; [6]Two tests; [7]Four tests.

Table 2. Results of Bt resistance monitoring with MVP II overlays for *Heliothis virescens* (1997).

State	Location	Host	% Dead/Stunted	
			Wild[1]	Control[2]
AL	Enterprise	non-Bt cotton	100	100
	Prattville	non-Bt cotton	98	100
GA	Decatur	non-Bt cotton	95	100
LA	Franklin Parish	---	95	100
	Franklin Parish	---	97	100
	Franklin Parish	---	98	100
MS	Washington Co.	non-Bt cotton	100	100
	Washington Co.	non-Bt cotton	100	100
	Washington Co.	Geranium	100	100
	Washington Co.	non-Bt cotton	100	100
	Washington Co.	non-Bt cotton	100	100
	Holly Bluff	non-Bt cotton	98	100
	Belzoni	Bt cotton	94	98
NC	Rocky Mount	non-Bt cotton	94	100
TX	College Station	Bt cotton	97	100
	Snook	Bt cotton	100	100
_	------	---	97.9	97.9

[1] Field-collected colony submitted by cooperator. [2] Rearing colony from USDA-ARS, Stoneville, MS.

Table 3. Results of Bt resistance monitoring studies with MVP II overlays and *Helicoverpa zea* (1997).

State	Location	Host	% Dead/Stunted	
			Wild	Control
AL	Prattville	non-Bt corn	100	99
	Enterprise	non-Bt cotton	98	100
	Houston Co.	non-Bt cotton	97	100
	Bullock Co.	non-Bt cotton	97	100
	Headland	Bt cotton	100	100
	Mobile	Bt cotton	97	100
	Prattville	Bt cotton	100	100
	Prattville	Bt cotton	100	100
	Semi-Springs	Bt cotton	100	100
AR	Monticello	non-Bt cotton	98	100
	Monticello	non-Bt cotton	100	100
	Monticello	non-Bt cotton	97	100
FL	Jackson Co.	Bt cotton	100	100
	Escambia	Bt cotton	100	100

GA	Decatur	non-Bt cotton	98	100
	Brinson	Bt cotton	100	100
	Jakin 1	Bt cotton	98	100
	Jakin 2	Bt cotton	97	100
	Omega	Bt cotton	100	100
MS	Washington Co.	non-Bt cotton	100	100
	Washington Co.	Geranium	100	100
	Washington Co.	non-Bt cotton	100	100
	Washington Co.	non-Bt cotton	98	100
	Washington Co.	Bt cotton	97	100
	Winterville	Bt cotton	97	100
	Tribbett	Bt cotton	100	100
	Stoneville	Bt cotton	100	100
	Tunica	Bt cotton	97	100
	N. Bolivar Co.	Bt cotton	95	100
	Yazoo City	Bt cotton	95	100
NC	Rocky Mount	non-Bt cotton	97	100
SC	Hampton Co.	non-Bt cotton	94	100
	Blackville	non-Bt cotton	100	100
TX	College Station	non-Bt corn	95	100
	Corpus Christi	non-Bt cotton	98	100
	Snook	Bt cotton	99	100
	Snook	Bt cotton	100	100
	Snook	Bt cotton	98	100
	Snook	Bt cotton	100	99
	---	---	98.0	99.9