RESISTANCE MONITORING IN BT COTTON: FIRST YEAR OBSERVATIONS

D. D. Hardee
Director
USDA, ARS, Southern Insect Management Laboratory
Stoneville, MS
D. A. Streett
Research Entomologist
USDA, ARS, Southern Insect Management Laboratory
Stoneville, MS
L. C. Adams
Entomologist
USDA, ARS, Southern Insect Management Laboratory
Stoneville, MS
G. W. Elzen
Research Entomologist
USDA, ARS, Subtropical Agricultural Research Laboratory
Weslaco, TX

Abstract

Resistance in insects to *Bacillus thuringiensis* Berliner (*B.t.*) delta endotoxin proteins has recently received considerable interest both nationally and internationally for three primary reasons: (1) unprecedented interest on the part of the environmental community and organic producers; (2) the recent registration and deployment of transgenic plants in many countries; and (3) laboratory and field resistance to *B.t.* in 10-12 insect species. Preliminary *B.t.* resistance monitoring in cotton in populations of cotton bollworm (CBW), *Helicoverpa zea* (Boddie), and tobacco budworm (TBW), *Heliothis virescens* (F.) was initiated in 1996 by (1) subjecting 23 different populations of these insects collected in Arkansas, Mississippi, Oklahoma, and Texas to field doses of MVP® II biological insecticide in spray chamber bioassays (the toxic protein in MVP® II is the closest in toxicological properties of all *B.t.* insecticides to the CryIA(c) protein expressed in transgenic cotton), and (2) subjecting larvae of CBW and TBW from 3 sites in Mississippi to *B.t.* delta endotoxin to select the optimum diagnostic dose in rearing diet. Preliminary monitoring results from both methods showed no shifts in baseline susceptibility levels of bollworm and budworm to *B.t.* insecticide (and by inference to *B.t.* cotton). These studies will continue and expand in 1997.

Introduction

More than 30 crop species have been genetically engineered to express delta endotoxin proteins of *Bacillus thuringiensis* Berliner *(B.t.)* which are highly toxic to several insect pests (Ives 1996). Most pertinent examples include corn, cotton, and potatoes. The Monsanto Company has developed cotton plants with the Bollgard® gene that is derived from *B.t.* and provides protection against attack from the cotton bollworm (CBW), *Helicoverpa zea* (Boddie), tobacco budworm (TBW), *Heliothis virescens* (F.), and the pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Barton 1995). Approximately 12% (1.7 million acres) of the cotton in the United States was planted in 1996 with *B.t.* cotton seed (Thomas A. Kirby, Delta and Pine Land Company, Scott, MS, Cotton, Inc. 1996 personal communication). Insects such as the Indianmeal moth, *Plodia interpunctella* (Hübner) (McGaughey 1985), the diamondback moth, *Plutella xylostella* (L.), (Tabashnik et al. 1990) and at least nine other species have developed resistance to *B.t.* insecticides in the laboratory and/or field. In resistance selection studies, Gould et al. (1995) developed a strain of TBW with a resistant gene frequency to *B.t.* of 0.001. Concerns have been raised by regulatory agencies (EPA), environmentalists, scientists, industry, and producers about the long-term effectiveness of transgenic plants through the use of genes for *B.t.* endotoxins. Thus, *B.t.* resistance management has become a highly important research activity in agricultural entomology today. We report herein our preliminary test results on monitoring *B.t.* cotton for signs of resistance in CBW and TBW. We have no data to report on PBW since it is found primarily in western cotton regions.

Methods and Materials

Spray chamber We encouraged several entomologists and consultants in several cotton states to collect, place on artificial diet (supplied by ARS), and ship to ARS at Stoneville, MS, any 3rd instar or larger larva of CBW and TBW found in *B.t.* cotton. In addition, we collected in several counties in Mississippi (1) eggs and larvae in large plantings of *B.t.* cotton (250 to 4,000 ha) grown for seed, and (2) moths in light traps and sweep nets in *B.t.* fields of varying sizes. Similar collections were made in non-*B.t.* cotton and handled similarly for comparison. All larvae collected or hatched from eggs were reared on artificial diet and held at 29 ± 3°C, 55-60% RH and a photoperiod of 14:10 (L:D) h until they emerged as moths, at which time they were mated, eggs collected, and placed on artificial diet and held under the conditions described. Methods and materials used in spray chamber bioassays were previously detailed by Elzen et al. (1990) and were originally adapted from Luttrell et al. (1987). 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 pics. Each insecticide treatment consisted of three replications of 15 terminals each.

Treatments in spray chamber bioassays included Cymbush (0.09 kg AI/ha), Larvin (1 kg AI/ha), Curacron (1.12 kg AI/ha), and MVP® II (2 l/ha), the biological insecticide

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:880-882 (1997)
National Cotton Council, Memphis TN

closest in toxicological properties to the CryIA(c) protein expressed in transgenic cotton (Gould et al. 1995). Controls were treated with water. The spray table was calibrated to deliver 56 liters per ha at 2,109 g/cm^2 pressure with one TX-6 hollow-cone nozzle travelling at a speed of 3.2 km/h. The spray nozzle was positioned 30.5 cm above the spray surface. One third instar (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 Tables 1 and 2. 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.

Study Sites Three study sites were selected in the Mississippi Delta that have a high proportion of transgenic cotton in the area. The sites were near Gunnison, MS (N34°00.352' W90°56.177'), Holly Bluff, MS (N32°50.935' W90°40.879') and Dunleith, MS (N33°27.634' W90°48.781'). A light cage was established at each of these sites and CBW and TBW moths collected daily for rearing in the laboratory. Colonies were established for each species from each field site. The colonies were maintained at 28°C and fed a 5% sucrose solution. Eggs were collected for colony maintenance and neonate larval bioassays. The USDA Stoneville Rearing facility supplied CBW and TBW neonate larvae for comparative bioassays.

Bioassay Dose response bioassays were conducted with artificial diet obtained from the USDA Stoneville Rearing facility. MVP® II (Mycogen Corp., San Diego, CA) was used as a source of CryIA(c) delta endotoxin. The diet was allowed to cool to ca. 50°C and then seven concentrations of MVP® II were incorporated into the diet by dispensing each delta endotoxin concentration in 30 mls of distilled water and blending with 270 mls of diet. The treated diet with each delta endotoxin concentration was added to 32 wells (1-1.5 mls per cell) in each set of bioassay trays (C-D International, Pitman, NJ) and allowed to harden. Each cell was infested with a single neonate larva, sealed with a cover, and incubated at 27°C for 7 days. Mortality data were recorded after 7 days and dose response bioassays were analyzed with SAS PROC Probit (SAS Institute, 1989).

Results

Spray Chamber Because of exceptionally low populations of TBW across the Cotton Belt, data from only 3 colonies of this insect were collected, and none of these was from *B.t.* cotton. Most of the results shown in Tables 1 and 2 are for CBW collected in Arkansas, Missisippi, Oklahoma, and Texas.

The data in Tables 1 and 2 are very preliminary in nature but show no detectable change in susceptibility of CBW and TBW to MVP® II in the spray chamber. Surprisingly, CBW colonies were equally or more susceptible to MVP® II than TBW, which is contrary to the accepted view and the results of Sims et al. (1996), and indicates the difficulty of comparing field and laboratory results. The results in Table 1 show the basic need for *B.t.* cotton in that the susceptibility of TBW populations, especially late in the season, was generally less to all classes of insecticides tested than that needed to effectively manage populations of this insect in cotton.

Diet The susceptibility of the laboratory and field colonies of CBW and TBW to *B.t.* delta endotoxin is summarized in Table 3. The susceptibility of TBW ranged from an LC_{50} value of 0.14 μg/ml (Stoneville) to 0.34 μg/ml (Holly Bluff) in diet. The LC_{50}'s for CBW ranged from 1.27 μg/ml (Stoneville) to 2.84 μ/g/ml (Dunleith) diet. No substantial differences in susceptibility were observed between the laboratory and field colonies for either species. The range in LC_{50} values for CBW were higher than for TBW which corroborates earlier reports (MacIntosh et al., 1990: Stone and Sims, 1993).

Discussion

Our data show no decrease in susceptibility in CBW and TBW to *B.t.* proteins in biological insecticides. However, because of the variability in response to these materials, especially in CBW (Stone and Sims 1993), our results are very preliminary. We do feel, however, that a monitoring system is now in place to continue and expand resistance monitoring in 1997 and monitor for shifts from the baseline susceptibility level in pest populations, especially in CBW. Still lacking, however, are threshold levels for remedial action, as well as the remedial actions themselves.

Acknowledgements

We gratefully acknowledge the assistance of Angela Wooley in conducting the diet bioassays.

Disclaimer

Mention of a proprietary product does not constitute an endorsement by the USDA.

References

Barton, G. F. 1995. Monsanto receives EPA approval for seed propagation plantings of insect-protected cotton. Press Release, 28 March 1995. Monsanto, St. Louis, MO.

Elzen, G. W., P. J. O'Brien, and G. L. Snodgrass. 1990. Toxicity of various classes of insecticides to pyrethroid-resistant *Heliothis virescens* larvae. Southwest. Entomol. 15: 33-38.

Gould, F., A. Anderson, A. Reynolds, L. Bumgardner, and W. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88: 1545-1559.

Ives, A. R. 1996. Evolution of insect resistance to *Bacillus thuringiensis*-transformed plants. Science 273: 1412-1413.

Luttrell, R. G., R. T. Roush, A. Ali, J. S. Mink, M. R. Reid, and G. L. Snodgrass. 1987. Pyrethroid resistance in field populations of *Heliothis virescens* (Lepidoptera: Noctuidae) in Mississippi in 1986. J. Econ. Entomol. 80: 985-989.

MacIntosh, S. C., T. B. Stone, S. R. Sims, P. L. Hunst, J. T. Greenplate, P. G. Marrone, F. J. Perlak, D. A. Fischoff, and R. L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebr. Pathol. 56: 258-266.

McGaughey, W. H. 1985. Insect resistance to the biological insecticide, *Bacillus thuringiensis*. Science 119: 193-194.

SAS Institute. 1989. Sas/STAT User's Guide, Version 6, 4th ed., Vols. 1 and 2, SAS Institute, Cary, NC.

Sims, S. R., J. T. Greenplate, T. B. Stone, M. A. Caprio, and F. L. Gould. 1996. Monitoring strategies for early detection of Lepidoptera resistance to *Bacillus thuringiensis* insecticidal proteins. Annu. Rev. Entomol. (In Press).

Stone, T. B., and S. R. Sims. 1993. Geographic susceptibility of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis*. J. Econ. Entomol. 86: 989-994.

Tabashnik, B. E., N. L. Cushing, N. Finson, and M. W. Johnson 1990. Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83: 1671-1676.

Table 1. Results of spray chamber bioassays in 1996.

Colony	Date Received	Stage Received	Source	Date Tested
St. *H. zea*[1]	05/21	larvae	rearing	05/21
Gen 196 *zea*[2]	05/7-31	larvae	geranium	06/12
Gen 296 *zea*[2]	06/6-28	eggs/larvae	non-bt cotton/velvetleaf	07/23
Gen 396 *zea*[2]	07/11-8/1	eggs/larvae	non-bt cotton	08/30
St. *H. vir*[1]	05/24	larvae	rearing	05/24
Gen 196 *vir*[2]	05/7-31	larvae	geranium	06/25
Gen 296 *vir*[2]	06/6-28	eggs/larvae	non-bt cotton/velvetleaf	07/31
Gen 396 *vir*[2]	07/11-8/1	eggs/larvae	non-bt cotton	09/13
TAMU *vir*[3]	08/6	pupae	non-bt cotton	09/13

[1] Susceptible colony reared at Stoneville, MS, since 1969.
[2] Collected in Washington County, MS.
[3] Collected near Corpus Christi, TX by Dr. John Benedict.

Table 1. Continued

Colony	% mortality to: Cymbush (90 gm/ha)	Larvin (1 kg/ha)	Curacron (1.12 kg/ha)	MVP II (2 l/ha)	Check
St. *H. zea*[1]	100.0	100.0	100.0	44.4	6.7
Gen 196 *zea*[2]	100.0	100.0	100.0	48.9	11.1
Gen 296 *zea*[2]	100.0	100.0	100.0	66.7	4.5
Gen 396 *zea*[2]	100.0	95.6	100.0	48.9	4.5
St. *H. vir*[1]	93.3	100.0	100.0	64.5	2.2
Gen 196 *vir*[2]	62.2	71.1	93.3	40.0	0.0
Gen 296 *vir*[2]	51.1	71.1	84.4	44.5	2.2
Gen 396 *vir*[2]	37.8	75.5	100.0	44.4	0.0
TAMU *vir*[3]	48.9	62.2	75.6	28.9	2.2

[1] Susceptible colony reared at Stoneville, MS, since 1969.
[2] Collected in Washington County, MS.
[3] Collected near Corpus Christi, TX by Dr. John Benedict.

Table 2. Results of spray chamber bioassays in 1996.

Colony	Date Received	Stage Received	Source	Date Test
Gunnison I *zea*[1]	07/23	eggs/larvae	bt cotton	07/31
Gunnison II *zea*[1]	08/12	eggs/larvae	bt cotton	09/20
Summer I *zea*[2]	07/16	eggs	bt cotton	07/23
McGehee, AR I *zea*[3]	07/16	eggs	bt cotton	07/23
McGehee, AR II *zea*[3]	07/31	eggs	bt cotton	08/06
McGehee, AR III *zea*[3]	08/22	eggs	bt cotton	08/30
McGehee, AR VI *zea*[3]	09/19	eggs	bt cotton	09/27
Monticello, AR *zea*[3]	07/24	eggs/larvae	bt cotton	07/31
Pieralisi *zea*[4]	07/15	moths	bt cotton	07/23
TAMU I *zea*[5]	08/20	eggs	bt cotton	08/27
TAMU II *zea*[5]	08/20	eggs	bt cotton	08/27
TAMU *vir*[5]	08/23	eggs	bt cotton	08/27
OKI *zea*[6]	08/16	eggs	non-bt ctn	08/22
Starkville *zea* [7]	08/16	larvae	bt cotton	09/20

[1] Cooperator -- Justin Shuford, Clarksdale, MS.
[2] Cooperator -- Wade Worley, Sumner, MS.
[3] Cooperator -- Charles Allen, McGehee, AR.
[4] Collected in Washington County, MS.
[5] Cooperator -- Stanley Nemec, Snook, TX.
[6] Cooperator -- Miles Karner, Altus, OK.
[7] Cooperator -- Blake Layton, Mississippi State, MS.

Table 2. Continued

Colony	% mortality to MVP II (2 l/ha)	Check
Gunnison I *zea*[1]	55.5	2.2
Gunnison II *zea*	53.3	2.2
Summer I *zea*2	57.8	2.2
McGehee, AR I *zea*[3]	75.5	2.2
McGehee, AR II *zea*[3]	55.7	2.2
McGehee, AR III *zea*[3]	55.6	0.0
McGehee, AR VI *zea*[3]	51.1	4.5
Monticello, AR *zea*[3]	57.8	2.2
Pieralisi *zea*[4]	75.5	0.0
TAMU I *zea*[5]	60.6	2.2
TAMU II *zea*[5]	55.5	6.7
TAMU *vir*[5]	48.9	0.0
OKI *zea*[6]	42.2	0.0
Starkville *zea*[7]	55.5	4.5

[1] Cooperator -- Justin Shuford, Clarksdale, MS.
[2] Cooperator -- Wade Worley, Sumner, MS.
[3] Cooperator -- Charles Allen, McGehee, AR.
[4] Collected in Washington County, MS.
[5] Cooperator -- Stanley Nemec, Snook, TX.
[6] Cooperator -- Miles Karner, Altus, OK.
[7] Cooperator -- Blake Layton, Mississippi State, MS.

Table 3. Susceptibility of *H. zea* and *H. virescens* to the *B.t.* delta endotoxin in artificial diet.

Species	Location	Slope±(SE)	LC_{50} [1,2]	95% CI
H. zea	Stoneville	1.42(0.48)	1.27	(0.70-1.70)
	Gunnison	1.20(0.36)	2.43	(0.67-4.68)
	Dunleith	2.03(0.66)	2.84	(0.58-5.86)
	Holy Bluff	1.72(0.45)	1.86	(1.26-2.94)
H. virescens	Stoneville	2.64(0.54)	0.14	(0.10-0.22)
	Gunnison	2.13(0.86)	0.23	(0.17-0.36)
	Dunleith	1.86(0.37)	0.16	(0.08-0.29)
	Holy Bluff	2.84(0.76)	0.34	(0.14-0.66)

[1] (μg/ml diet)
[2] N=3 replications for each population