EVALUATION OF TRANSGENIC BT COTTON LINES AGAINST HELIOTHINES IN NORTHEAST LOUISIANA

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Abstract

A series of field and laboratory tests evaluated the insecticidal and yield performance of selected transgenic cotton lines (BTK) from Delta Land and Pine Company, Cotton Seed International, and Stoneville Pedigreed Seed Company during 1997 in Louisiana containing the delta endotoxin of *Bacillus thuringiensis kurstaki*. All BTK cotton lines controlled the bollworm (BW), *Helicoverpa zea* (Boddie), and tobacco budworm (TBW), *Heliothis virescens* (F.), complex similar to that of the BTK standard cultivars, Coker 312-531 and NuCOTN 35B. All BTK lines also produced seedcotton or lint yields $\geq$ to that of the BTK standards in both non-sprayed and sprayed treatment regimes. In the laboratory studies, BW and TBW mortality on squares of BTK cotton lines ranged from 48.8 to 97.5% and 75.4 to 97.5%, respectively, and were not significantly different from that of Coker 312-531. Also in the laboratory, no significant difference was observed between a transgenic BTK cotton (NuCOTN 33B) and the non-BTK cotton cultivar (DP 5415) in the number of squares injured by L1 stage BW. Although the number of squares injured by L1 and L2 stage TBW was significantly lower for NuCOTN 33B than for DP 5415, both stages injured >11% of the squares in the study.

Introduction

The bollworm [BW], *Helicoverpa zea* (Boddie), and tobacco budworm [TBW], *Heliothis virescens* (F.), are the most common and widespread lepidopteran pests in Louisiana cotton and have caused devastating losses in many fields during recent years (Bagwell et al. 1996, 1997). Transgenic BTK cotton (Bollgard[R]) technology has been used to manage these Heliothine pests in Louisiana during 1996-97. Approximately 116,000 acres (13%) and 250,000 acres (40%) of Delta and Pine Land, Paymaster, and Stoneville cultivars containing Bollgard were planted in 1996 and 1997, respectively.

Previous research has shown that the TBW can be managed effectively with BTK cotton lines (Benedict et al. 1996, Leonard et al 1997). These cultivars were nearly immune to injury from this pest prior to 1997, and it has been difficult to achieve similar levels of TBW control with foliar insecticides. However, there are significant differences in the susceptibility of BTK cotton lines between TBW and BW (Leonard et al. 1997). BW larvae are capable of tolerating the delta endotoxin levels in the currently available BTK cotton lines and persistent field populations of BW can cause economic injury in some situations (Mahaffey et al. 1994, Hardee and Herzog 1997, Lambert et al. 1997, Leonard et al. 1997).

In 1996, BW populations were generally higher than that of TBW and constituted the majority of this pest complex in many areas of the Southern U.S. including Louisiana. Supplemental applications of pyrethroids were used to control persistent and high populations on both normal and BTK cotton cultivars during 1996. Over 60% of the BTK cotton fields in Louisiana were treated with at least one application of insecticide specifically to control BW (personal communication; R. Bagwell, LSU Agricultural Center). In 1997, BW population densities varied considerably, but were generally lower than that observed during 1996. Preliminary estimates of insecticide use patterns in Louisiana during 1997 indicate that over 75% of the acreage planted to BTK cultivars were treated with an insecticide specifically to control the BW/TBW complex.

During the 1997 season, agricultural consultants in Louisiana reported Heliothine feeding injury in plant terminals and on small squares. Additional observations by research and extension personnel supported these observations and suggested this damage was being produced by TBW as well as BW. The primary objective of this report is to discuss the performance of selected BTK cotton lines against the BW/TBW complex in Louisiana field trials. In addition, the efficacy of BTK cotton lines against BW and TBW in laboratory feeding studies is presented.

Materials and Methods

Field Trials

Three tests were conducted at the Macon Ridge location of the Northeast Research Station near Winnsboro, LA. Recommended cultural practices and integrated pest management strategies were used to maintain all plots in a similar manner within each test. Insecticides were applied across the entire test area to control non-target pests, primarily boll weevil, *Anthonomus grandis,grandis Boheman*, and tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois).

Each test was designed as a multi-factor experiment to evaluate the interaction of insecticide oversprays (main plots) and selected BTK lines (subplots) on Heliothine control and seedcotton yields. Treatment combinations were grouped in a split-plot arrangement within a RBCD and replicated 4 times. Subplots within each trial consisted of 2-4 rows (40 inch centers) x 45 ft. The main plots in each test included an untreated control and insecticide treatment for BW/TBW. Subplots in Test MRDP97were

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DP 20, DP 20B (BTK), DP 50B (BTK), NuCOTN 35B (BTK), Stoneville 474, Stoneville STX001 (BTK), and PM 1330BG (BTK). Subplots in Test MRCSI97 included Gumbo 500, Coker 312-531(BTK), , DP 32B (BTK), CSI 1003, CSI 1011 (BTK), CSI 1012 (BTK), CSI 1014 (BTK), and CSI 1015 (BTK). Subplots in Test MRCG97 included Stoneville 474, Stoneville STX001 (BTK), Coker 312-531 (BTK), and DP 32B (BTK). Seed of Coker 312-531, DP and NuCOTN cultivars, Stoneville and STX001 lines, and CSI lines were obtained from Monsanto Company, Delta Land and Pine Company, Stoneville Pedigreed Seeds, and Cotton Seed International, respectively. Seed of Gumbo 500 was received from the Louisiana State University Agricultural Center's cotton breeding laboratories.

Insecticide treatments were applied to eggs and <4 day old BW and TBW larvae in the appropriate plots. These treatments included a pyrethroid, pyrethroid + Larvin 3.2F (thiodicarb), pyrethroid + Tracer 4SC (spinosad), or pyrethroid + Pirate 3F (chlorfenapyr). Formulated samples of pyrethroids, Larvin 3.2F (Rhone Poulenc Ag, Research Triangle Park, NC), Tracer 4F (DowElanco, Indianapolis, IN), and Pirate 3F (American Cyanamid, Princeton, NJ), were obtained from the manufacturers. Four-five applications of each treatment were made within each test. Applications in all tests were made with a tractor mounted boom and compressed air delivery system calibrated to deliver 6 gallons total spray/acre through Teejet TX-8 hollow cone nozzles (2/row) at 42 psi.

Treatment efficacy against BW and TBW was determined by examining 25-50 fruiting forms (squares or bolls)/plot. Plots were sampled at 3-7 days after treatment for evidence of damage and larvae infesting squares. The center two rows of each plot in selected tests were mechanically harvested twice to estimate seed cotton yields. Results were subjected to two-way ANOVA to determine significant treatment effects. Duncan's Multiple Range test ($\underline{P}$=0.05) was used, wherever appropriate, to compare treatment means within each respective test (SAS Institute 1988).

Laboratory Feeding Studies

Two cotton square feeding bioassays were used to evaluate the efficacy of the delta endotoxin in BTK cotton lines against L1 stage BW and TBW larvae. The non-BTK cotton lines in the tests included DP 20, Stoneville 474, Gumbo 500 and CSI 1003. The transgenic BTK lines included Coker 312-531, DP 20B, DP 50B, DP 32B, PM 1330 BG, NuCOTN 33B, STX001, CSI 1010, CSI 1011, CSI 1012, CSI 1014, and CSI 1015.

BW and TBW colonies were established by collecting eggs and/or larvae from field corn and cotton, respectively, at the Macon Ridge location of the Northeast Research Station near Winnsboro, LA in 1997. These colonies were reared in the laboratory for one generation to obtain enough insects at the proper stage for the bioassays. The two colonies were reared in a similar manner. Adults were confined in one gallon cardboard cartons covered with cotton gauze as an oviposition substrate and were fed a 10% sugar water solution. Eggs were removed at least every other day and allowed to hatch at room temperature. Larvae were reared on a pinto bean and wheat germ diet according to procedures described by Leonard et al. (1987).

Cotton squares were collected from the upper one-third of field-grown cotton plants, washed, and allowed to air dry in the laboratory. Squares were removed only from non-sprayed plots. BW and TBW larvae were individually placed in one oz. plastic cups and offered two squares. Each larvae was checked daily for mortality and surviving larvae were offered additional squares. The mortality values for each comparison were determined from five replicates of 50 insects per cotton line. After infestation, the larvae were held at 27±3°C and 55-65% RH under a 14:10 light:dark (L:D) photoperiod. Mortality was determined at 72 hours after infestation (HAI). The criterion for mortality was inability of a larva to move within 15 seconds after being prodded with a blunt probe. Results were analyzed using a microcomputer based ANOVA (SAS Institute 1988).

One additional experiment compared the feeding characteristics of BW and TBW on squares produced by NuCOTN 33B (BTK) and DP 5415. All methods were similar to those previously described for the larval mortality comparisons. However, L1 and L2 stage larvae were individually placed in one oz. plastic cups and offered two squares. Each comparison consisted of three-four replicates of 25-50 insects per cultivar. At 48 HAI, square injury was recorded. Injury was defined as complete penetration through the petals or calyx to the interior floral structures. Data analyses were identical to those previously reported.

Results and Discussion

Field Tests

The results of these tests characterize the susceptibility of selected transgenic (BTK) cotton lines to the BW/TBW complex in Louisiana during 1997. Each test included at least one non-BTK normal variety (DP 20, Stoneville 474, Gumbo 500, or CSI 1003) and a transgenic BTK standard variety (NuCOTN 35B or Coker 312-531). Population densities of BW and TBW were relatively low during most of 1997 and damage to squares never exceeded 18% on any evaluation date. In addition, the species composition of this insect complex varied considerably during 1997 with the BW being most common during Jul through early Aug, and the TBW being the dominant species after mid-Aug.

In Test MRDP97, all non-sprayed BTK lines had significantly fewer damaged squares and larvae in squares compared to that for the non-sprayed standard DP 20 and Stoneville 474 cotton varieties (Table 1). Lint yields for all non-sprayed BTK cotton lines except NuCOTN 35B were significantly higher than the yield of the non-sprayed

Stoneville 474 line. In the sprayed plots, all BTK lines had significantly fewer damaged squares than Stoneville 474, but not DP 20. Lint yields in the sprayed plots were similar except for the DP 20 plots which produced significantly less lint than all other plots. Insecticide treatment significantly increased lint yields above the untreated plots by ca. 54%. All BTK lines controlled the BW/TBW complex similar to that of NuCOTN 35B and produced lint yields $\geq$ to that of NuCOTN 35B in the non-sprayed and sprayed treatment regimes.

All non-sprayed BTK lines had significantly fewer damaged squares compared to that for the non-sprayed Gumbo 500 in Test MRCSI97 (Table 2). The non-sprayed BTK cotton lines, except CSI 1012, produced seedcotton yields similar to the non-sprayed Gumbo 500 and CSI 1003 cotton lines. With the exception of CSI 1014 in the sprayed plots, all BTK lines had significantly fewer damaged squares and larvae in squares than in the Gumbo 500 and CSI 1003 cotton lines. DP 32B was the only BTK cotton line to yield significantly more seedcotton than Gumbo 500 and CSI 1003 in the sprayed plots. Insecticide treatment significantly increased lint yields above the untreated plots in this test by ca. 34%. All BTK lines controlled the BW/TBW complex similar to that of the standard Coker 312-531 in the non-sprayed and sprayed treatment regimes. Except for the non-sprayed plots of CSI 1012, all BTK lines produced seedcotton yields $\geq$ to that of Coker 312-531 in the non-sprayed and sprayed treatment regimes.

In Test MRCG97, all non-sprayed BTK lines had significantly fewer damaged squares and larvae in squares compared to that for the non-sprayed Stoneville 474 (Table 3). The lint yield of unsprayed DP 32B was significantly higher than that in the non-sprayed Stoneville 474. In the sprayed plots, there were no significant differences in BW and TBW damaged squares and larvae in squares among cotton lines. Lint yields for all sprayed BTK cotton lines, except for Coker 312-531, were not different from that of sprayed Stoneville 474. Insecticide treatment significantly increased lint yields above the untreated plots by ca. 40%. All BTK lines controlled the BW and TBW complex similar to that of Coker 312-531 and produced lint yields $\geq$ to that of Coker 312-531 in the non-sprayed and sprayed treatment regimes.

The yield increases from the insecticide sprays in these tests are likely the result of controlling other cotton pests in addition to BW and TBW. Benedict et al. (1996) reported yield increases of 6.2-23.9% in Texas by overspraying BTK cottons with a pyrethroid. In North Carolina, yields of BTK lines were increased by 11 and 23% with pyrethroid applications (Lambert et al. 1997). BTK cottons are target specific for lepidopteran insects and will need to be supplemented with foliar insecticides to provide optimum control of the complete pest spectrum that exists in a cotton field.

Cotton Square Feeding Studies

The results of two laboratory tests evaluating the toxicity of selected transgenic BTK cotton lines against BW/TBW are summarized in Tables 4 and 5. With the exception of DP 50B and STX001, all BW mortality levels were significantly higher on BTK cotton lines compared to that on the non-BTK cotton lines in both tests. All BTK cotton lines produced BW mortality levels $\geq$ to that observed for Coker 312-531. TBW mortality levels were significantly higher on squares of all BTK cotton lines compared to that on squares of non-BTK cotton lines in both tests. All BTK cotton lines produced TBW mortality levels $\geq$ to that observed for Coker 312-531. With the exception of DP 32B (one test), CSI 1011, and CSI 1014, BW mortality was lower than that for TBW on each respective BTK cotton line. Leonard et al. (1997) previously reported BW mortality levels that were lower than that for TBW on Coker 312-531 and NuCOTN 33B. In that study, BW mortality levels were <10% on BTK lines, whereas in the present study BW mortality levels on BTK lines ranged from 48.8 to 97.5%. TBW mortality values on BTK lines were >80% in Leonard et al. (1997) tests, but ranged from 75.4 to 97.5% in the present study. The use of insecticide oversprays on BTK lines for BW is justified in some situations (Lambert 1997, Lambert et al. 1997).

BW and TBW injury to squares of NuCOTN 33B and DP 5415 are reported in Table 6. There were no significant differences in the number of BW damaged squares between cotton lines. The number of squares injured by L1 and L2 stage TBW was significantly lower for NuCOTN 33B compared to that for DP 5415. However, both stages of TBW did injure over 11% of NuCOTN 33B squares within 48 HAI. BW and TBW mortality levels were high, 54% and >74% respectively, on NuCOTN 33B. Even though BW and TBW larvae were capable of injuring squares, many of those larvae were morbid or dead. Relatively low levels of BW and TBW square (<5%) to BTK cotton lines was reported in feeding studies by Benedict et al. (1993). However, earlier research by Jenkins et al (1990) found no differences in TBW square injury between normal and BTK lines which was considered to be the result of low delta endotoxin expression in the BTK lines. The data in the present study suggests that the larvae actually injured squares before ingesting a lethal dose. Previous research supports these observations with BW, but with the high susceptibility of TBW to the delta endotoxin in commercial BTK cultivars, these results are surprising. The reasons for TBW square injury on NuCOTN 33B are unknown, but may relate to interactions between the environment, insect, and delta endotoxin expression.

These data demonstrate the effectiveness of several new transgenic BTK cotton lines against the BW and TBW in field and laboratory tests in Louisiana. The TBW still is being controlled with BTK cultivars, but the levels of square injury by TBW reported in the present study should warrants further evaluation. Additional tests are needed to re-define economic injury levels and action thresholds for BW on commercial BTK cultivars.

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Table 1. Susceptibility of selected BTK cotton lines to bollworm/tobacco budworm (BW/TBW) in field test MRDP97.

Treatment[1]	Variety	Damaged[2] squares (%)	Larvae in[2] squares (%)	Lint yield (lb/acre)
Non-sprayed	DP 20	7.3b	3.0b	435c
	Stoneville 474	10.1a	5.0a	303d
	DP 20B	1.7c	0.7c	578a
	DP 50B	2.0c	0.3c	604b
	NuCOTN 35B	2.0c	1.0c	413cd
	PM 1330BG	0.3c	0.3c	748a
	STX001	0.3c	0.0c	532bc
Sprayed	DP 20	4.7ab	1.3ab	950b
	Stoneville 474	6.0a	3.0a	1161a
	DP 20B	1.4b	0.5b	1112a
	DP 50B	0.3b	0.0b	1203a
	NuCOTN 35B	1.3bc	0.0b	1148a
	PM 1330BG	0.3b	0.0b	1129a
	STX001	0.7c	0.0b	1185a

Means in columns within each treatment followed by the same letter are not significantly different (P=0.05;DMRT).
[1] Treatments included insecticide combinations for BW/TBW.
[2] Mean of last three samples.

Table 2. Susceptibility of selected BTK cotton lines to bollworm/tobacco budworm (BW/TBW) in field test MRCSI97.

Treatment[1]	Variety	Damaged[2] squares (%)	Larvae in[2] squares (%)	Seedcotton yield (lb/acre)
Non-sprayed	Gumbo 500	7.3a	2.0a	1879ab
	CSI 1003	4.7ab	1.0a	1628bc
	Coker 312-531	1.7bc	1.0a	1868ab
	DP 32B	2.3bc	0.3a	2256a
	CSI 1010	2.0bc	0.7a	1559bc
	CSI 1011	4.0bc	1.3a	1966ab
	CSI 1012	2.0bc	1.7a	1366c
	CSI 1014	3.3bc	1.0a	1610bc
	CSI 1015	1.0c	0.3a	1609bc
Sprayed	Gumbo 500	5.0a	2.7a	2746bc
	CSI 1003	5.0a	3.7a	2335cd
	Coker 312-531	0.7b	0.0b	2137d
	DP 32B	0.0b	0.0b	3240a
	CSI 1010	0.7b	0.3b	2816abc
	CSI 1011	1.3b	0.3b	2471cd
	CSI 1012	1.0b	0.3b	2543cd
	CSI 1014	2.3ab	0.3b	2586bcd
	CSI 1015	1.0b	0.0b	3071ab

Means in columns within each treatment followed by the same letter are not significantly different (P=0.05;DMRT).
[1] Treatments included insecticide combinations for BW/TBW.
[2] Mean of last three samples.

Table 3. Susceptibility of selected BTK cotton lines to bollworm/tobacco budworm (BW/TBW) in field test MRCG97.

Treatment[1]	Variety	Damaged[2] squares (%)	Larvae in[2] squares (%)	Lint yield (lb/acre)
Non-sprayed	Stoneville 474	5.7a	2.3a	675b
	Coker 312-531	1.6b	0.3b	467c
	DP 32B	1.3b	0.0b	808a
	STX001	1.0b	0.0b	615b
Sprayed	Stoneville 474	1.3a	0.0a	1124a
	Coker 312-531	0.7a	0.3a	815b
	DP 32B	0.3a	0.3a	1186a
	STX001	0.0a	0.0a	1157a

Means in columns within each treatment followed by the same letter are not significantly different (P=0.05;DMRT).
[1] Treatments included insecticide combinations for BW/TBW.
[2] Mean of last three samples.

Table 4. Evaluation of selected BTK cotton lines against bollworm/tobacco budworm (BW/TBW) in a laboratory square feeding test, I.

	% mortality (L1 stage larvae) 72 HAI	
Cotton line	Bollworm	Tobacco Budworm
DP 20	24.4c	30.8b
Stoneville 474	30.0c	27.2b
Coker 312-531	72.4ab	84.4a
DP 20B	78.7ab	91.3a
DP 50B	48.8bc	94.4a
DP 32B	85.4a	97.5a
PM 1330 BG	89.4a	97.5a
NuCOTN 35B	84.4ab	97.2a
STX001	60.0abc	89.4a

Means in columns for each variety followed by the same letter are not significantly different (P=0.05;DMRT).

Table 5. Evaluation of selected BTK cotton lines against bollworm/tobacco budworm (BW/TBW) in a laboratory square feeding test, II.

	% mortality (L1 stage larvae) 72 HAI	
Cotton line	Bollworm	Tobacco Budworm
Gumbo 500	13.8d	22.8c
CSI 1003	25.8cd	13.9c
Coker 312-531	51.9bc	75.4ab
DP 32B	97.5a	90.0a
CSI 1010	71.9ab	91.9a
CSI 1011	92.5a	77.7ab
CSI 1012	77.5ab	78.1ab
CSI 1014	86.9a	61.3b
CSI 1015	78.7ab	88.2ab

Means in columns for each variety followed by the same letter are not significantly different (P=0.05;DMRT).

Table 6. Bollworm/tobacco budworm (BW/TBW) injury to squares of DP 5415 and NuCOTN 33B in a laboratory feeding study.

	% penetrated squares 48 HAI		
	Bollworm	Tobacco Budworm Stage	
Variety	L1, 1 day	L1, 1day	L2, 2 day
DP 5415	43.7a	28.7a	45.7a
NuCOTN 33B	35.1a	11.5b	24.2b

Means in columns for each variety followed by the same letter are not significantly different (P=0.05;DMRT).