2003 BELTWIDE COTTON CONFERENCES, NASHVILLE, TN – JANUARY 6-10

FIELD EFFICACY OF DOW AGROSCIENCES MXB-13 TRANGSGENIC COTTON FOR CONTROL OF FOLIAR FEEDING LEPIDOPTEROUS INSECTS

Vernon B. Langston, Xinpei Huang, Carlos A. Blanco, Ralph B. Lassiter, Randy M. Huckaba, L.B. Braxton, Fikru Haile, Jesse M. Richardson, and John Pellow
Dow AgroSciences, LLC
Indianapolis, IN

Abstract

Dow AgroSciences, LLC has genetically modified cotton (*Gossypium hirsutum* L.) to express two separate insecticidal crystal proteins from the bacterium *Bacillus thuringiensis* (Bt) for the control of key lepidopteran pests. Cotton genotype GC510 was transformed to contain the genes that express full-length synthetic protoxins (synpro) of Cry1F or Cry1Ac. Transgenic lines were backcrossed with a non-transgenic elite variety, PSC-355. Subsequently, Cry1F(synpro) and Cry1Ac(synpro) lines were crossed to produce the stacked product, MXB-13. Dow AgroSciences transgenic cotton event MXB-13 provided excellent control of the secondary foliar feeding lepidopterous insects such as beet armyworm (*Spodoptera exigua*), cabbage looper (*Trichoplusia ni*), soybean looper (*Pseudoplusia includens*), southern armyworm (*Spodoptera eridania*) and fall armyworm (*Spodoptera frugiperda).* Significantly lighter foliar damages or fewer foliage-feeding larvae were detected on unsprayed MXB-13 plots, as compared to those on the unsprayed non-Bt cotton variety.

Introduction

Since 1996, transgenic Bt cotton (*Gossypium hirsutum* L.) has been commercially available in the United States. Since that time, modifying the cotton to express a second gene encoding another Bt protein has created a new generation of Bt cotton. Dow AgroSciences, LLC has genetically modified cotton (*Gossypium hirsutum* L.) to express two separate insecticidal crystal proteins from the bacterium *Bacillus thuringiensis* (Bt) for the control of key lepidopteran pests. Cotton genotype GC510 was transformed to contain the genes that express full-length synthetic protoxins (synpro) of Cry1F or Cry1Ac. Transgenic lines were backcrossed with a non-transgenic elite variety, PSC-355. Subsequently, Cry1F(synpro) and Cry1Ac(synpro) lines were crossed to produce the stacked product, MXB-13. An application for registration of this stacked trait has been submitted to EPA. In this study, evaluations were made to determine the levels of foliage-feeding lepidopteran activity on these new DAS transgenic events.

Materials and Methods

The Cry1Ac:Cry1F stack event, MXB-13, was tested for efficacy against foliar feeding lepidopteran pests during 2001-2002 (Table 1). This event was compared to a near-isoline non-Bt cotton, PSC355. Thirteen studies were conducted across the major US cotton growing areas. The primary targets were beet armyworm (*Spodoptera exigua*), cabbage looper (*Trichoplusia ni*), soybean looper (*Pseudoplusia includens*), southern armyworm (*Spodoptera eridania*) and fall armyworm (*Spodoptera frugiperda*). Populations of these species were from either natural or artificial infestations.

A split plot design with 4 replications was employed in the field studies. Areas of "sprayed" and "unsprayed" were designated as the main plots, and events (entries) as the sub-plots. In sprayed main plots, conventional insecticides were used for optimum control of all insect pests. In unsprayed main plots; however, only non-lepidopteran pests were controlled. Plot size was generally 2 or 4 rows wide X 30 to 40 ft long. Standard artificial infestation techniques were used in those trials that were artificially inoculated.

To confirm field performance, lab bioassays were conducted with field-collected leaves or squares against beet armyworm and fall armyworm at Stoneville, MS and Starkville, MS.

Results and Discussion

Under natural infestations, a high level of resistance to beet armyworm was observed with the stacked line (MXB-13) in Fresno, CA (Figure 1). The total larvae observed on 10 plants per plot were counted. Unsprayed MXB-13 had a significant decrease in the number of larvae found as compared to the unsprayed non-Bt line. In the Stoneville, MS trial with artificial infestation of the unsprayed plots, a significant difference was found in the amount of foliar feeding (Table 2). With successful infestation, the rating for foliar feeding is always at least a rating of one because larvae must feed to ingest the Cry1F/Cry1Ac protein. The MXB-13 had significantly less foliar feeding than the non-Bt variety. Likewise, the MXB-13 had significantly fewer larvae than the unsprayed non-Bt variety. In subsequent counts, the number of small larvae (1^{st} - 2^{nd} instar) and large larvae (3^{rd} – 5^{th} instar) on the MXB-13 line was significantly less than on the non-Bt variety. Also, the number of large larvae was greater than the number of small larvae in the non-Bt variety indicating substantial growth was occurring while the MXB-13 line had no large larvae. Even though there were a few small larvae still present on the MXB-13 after 12-14 days, there was no indication of development to later instars. A field study using artificial inoculation at Fresno, CA conducted in 2002 indicated the high level of activity that this stacked line has on beet armyworm (Table 3). In the unsprayed plots, a significant reduction in larval counts and larval growth stage was found for unsprayed MXB-13 when compared to the non-Bt cotton variety. Similar results were obtained from a bioassay conducted in Stoneville, MS in 2002 (Table 4). The trial results from 2001 and 2002 indicate that MXB-13 is able to effectively control BAW infestations under field conditions. The data indicate that there were significantly fewer larvae on the MXB-13 line. Although early instars can be found on MXB-13 and limited feeding occurs, the larvae are not able to develop to larger instars.

Excellent control of loopers by the transgenic event was also demonstrated in trials at Fresno, CA; Wayside, MS; College Station, TX; Stoneville, MS and Winnsboro, LA. In the Fresno, CA study, one or fewer cabbage looper larvae were found at 49 days after planting on the unsprayed MXB-13 plots, as compared with 6 larvae/10 plants in the non-Bt plots (Figure 2). In a trial conducted in Wayside, MS, MXB-13 provided significantly lower numbers of cabbage loopers than the non-Bt variety in six out of nine evaluations (Table 5). In over nine evaluations in this trial, the total number of cabbage loopers counted on MXB-13 was two as compared to the total of 619 on the non-Bt variety. Similar results were obtained in another trial conducted in College Station, TX (Table 5). Total number of cabbage loopers counted was one in the MXB-13 plots versus 1102 counted in the non-Bt plots. Soybean loopers were evaluated in 2001-2002 under natural infestations in Texas, Louisiana and Mississippi. The number of soybean loopers found on unsprayed non-Bt cotton on Sept 5 in the LA trial was 24 times the number found on unsprayed MXB-13 (Figure 3). The number of loopers decreased in unsprayed non-Bt cotton on Sept 12, but the difference between this entry and MXB-13 was still significant. The efficacy of MXB-13 against soybean loopers was further confirmed at 83 days after planting at Stoneville, MS (Figure 4). Soybean loopers were also evaluated in a trial conducted at College Station, TX (Table 5). Over the nine week evaluation period, there were no soybean loopers counted in MXB-13 plots versus 157 found in the non-Bt plots.

A heavy natural infestation of southern armyworm occurred at a location in AL. Southern armyworm caused more severe damage on unsprayed non-Bt cotton at 101 and 116 days after planting (Figure 5). The damage on the MXB-13 event was minimal. While insecticide applications provided effective control of the pest in sprayed non-Bt cotton, significantly more southern armyworm larvae were found on the unsprayed non-Bt cotton at 101 days after planting (Figure 6). If the larval infestation data in Figure 6 are evaluated alone, the spray treatments appear to be more effective at controlling southern armyworm than the MXB-13. However, there was a complicating factor present in the trial. A high density of morningglory weeds developed throughout the plots. Since morningglory is a good food source for southern armyworm, the weed infestation allowed for the larvae to survive and grow in the unsprayed plots including the MXB-13. The larvae counts were found to be unusually high in the unsprayed plots, and the morningglory leaves were severely consumed. Even though a large population of larvae existed within the unsprayed MXB-13 plots and the larvae were able to develop into later-instar larvae on the morningglory, the percent defoliation of MXB-13 was only 0.8%. However, in the unsprayed non-Bt plots, the percent defoliation was significantly higher (16.3%). In essence, the MXB-13 was being constantly exposed to large numbers of later instar mature larvae and despite this extreme challenge, the MXB-13 performed exceptionally well as evidenced by the minimal defoliation rating.

A bioassay on fall armyworm was conducted in Starkville, MS during 2001. Survival of fall armyworm was significantly lower with MXB-13 in a leaf tissue bioassay (Table 6). When larval weights were measured from the leaf tissue bioassay, a similar trend was observed (Table 7). Bioassay results for fall armyworm using square tissue was similar to that observed with the leaf tissue (Tables 8-9). MXB-13 had significantly lower fall armyworm survival and larval weights than the non-Bt cotton variety.

One trial was conducted in Puerto Rico in the winter of 2001-2002 to examine the efficacy of MXB-13 against lepidopteran pests that are common to that area. Natural infestations of both *Helicoverpa zea,* cotton bollworm (CBW) and *Spodoptera frugiperda,* fall armyworm (FAW) were encountered. Specific counts of larvae to determine lepidopteran populations were not taken. Observations of plots, other plantings in the immediate area and qualitative insect scouting information indicated the presence of and plant damage typical of cotton bollworm and fall armyworm. Plant mapping data were developed prior to harvest to demonstrate the boll distribution on the plants in the unsprayed treatments. Examining 10 plants per plot on each of four replications developed the plant map data. Each boll position was rated for the presence or absence of a boll. If a boll was present, the boll was rated as either open or not open. Data were then grouped by equivalent age. Equivalent age is defined as boll positions that had flowers bloom on the same date. Boll position bloom dates were calculated by assuming a six-day difference between subsequent positions on one fruiting branch and a three-day difference between the same boll position on subsequent fruiting branches.

The comparison of plant map data from the non-Bt variety and MXB-13 treatments that were unsprayed for lepidopteran infestation shows the effect of total lepidopteran activity (Figure 7). There were a low number of bolls in non-Bt variety corresponding to the equivalent age of less than 36 days. Therefore, the majority of the typical boll load was missing. Because of the low boll load in the non-Bt variety, the cotton plants continued to grow and set bolls at the top of the plant. The peak in boll set for this non-Bt variety corresponded to equivalent age between 36 and 45 days. Conversely, the MXB-13 plants had a typical boll load, resulting in limited late boll set at the top of the plant. The peak in boll set for MXB-13 corresponded to equivalent age between 18 and 24 days. Therefore, there are two main points that can be seen from these data: the total boll set on the non-Bt variety is much lower than MXB-13 and the bulk of the boll load for the non-Bt variety is shifted to later maturing bolls at the top of plant. The above description and interpretation of the plant map data resulting from infestations of cotton bollworm and fall armyworm can be visualized from the photograph in Figure 8. This photograph was taken just before harvest and at the same time the plant map data were gathered. The substantial loss of bolls for the non-Bt variety can be easily seen. Also, the remaining bolls are typically later maturing bolls located at the top of the plant or on the ends of the fruiting branches. The plant map data and photograph of the unsprayed plots demonstrate the large degree of protection that MXB-13 can provide under conditions that can cause very severe crop losses in non-transgenic cotton when insect control does not occur with the use of sprays.

Table 1. Trial location, year conducted, type of trial and insects for lepidopterous insects in Bt cotton trials in US, 2001-2002.

Location	Year Conducted	Trial Type	Insects Evaluated Common Name	Bayer Code
Fresno, CA	2001	Natural Inf.	Beet armyworm	LAPHEG
Stoneville, MS	2001	Artificial Inf.	Beet armyworm	LAPHEG
Fresno, CA	2002	Artificial Inf.	Beet armyworm	LAPHEG
Stoneville, MS	2002	Bioassay	Beet armyworm	LAPHEG
Fresno, CA	2001	Natural Inf.	Cabbage looper	TRIPNI
Wayside, MS	2002	Natural Inf.	Cabbage looper	TRIPNI
College Station, TX	2002	Natural Inf.	Cabbage looper	TRIPNI
Winnsboro, LA	2001	Natural Inf.	Soybean looper	PSEPIN
College Station, TX	2002	Natural Inf.	Soybean looper	PSEPIN
Stoneville, MS	2001	Natural Inf.	Soybean looper	PSEPIN
Loxley, AL	2001	Natural Inf.	Southern armyworm	PRODER
Starkville, MS	2001	Bioassay	Fall armyworm	LAPHFR
Puerto Rico	2001-02	Natural Inf.	Cotton bollworm	HELIAR
			Fall armyworm	LAPHFR

Table 2. Summary of beet armyworm, *Spodoptera exigua* evaluations under artificial infestation/unsprayed conditions. 2001. Stoneville, MS.

Treatment		Foliar Damage * July 19	Total Larvae ** July 20	1st-2nd Instar** July 24	3rd-5th Instar** July 24
MXB-13	Unsprayed	1.45	6.3	0.5	0
Non-Bt Var.	Unsprayed	2.52	37.8	2.8	6.8
	LSD (0.05)	0.26	13.6	1.7	2.5

* 0-3 Scale:
0=no damage;
1=slight damage, little feeding, few worms;
2= moderate foliar damage, feeding and larvae easily noted;
3=severe foliar damage to the point of defoliation.
** Larvae obtained in 30 row-feet.

Table 3. Larval counts and growth stage six days after artificial innoculations of beet armyworm (LAPHEG) in Fresno, CA during 2002.

Test Lines		Larvae per 10 Leaves	Larval Instar
MXB-13	Unsprayed	10.1 b	1.00 b
Non-Bt	Unsprayed	22.7 a	2.00 a
	LSD (P=0.05)	9.7	0.15

Table 4. Summary of percent mortality and larval weight after nine days for beet armyworm (LAPHEG) terminal leaf bioassay. Adamczyk, Stoneville, MS, 2002. (1 neonate/plate, 10 plates /plot, 40 neonates/test line.)

		% Mortality				Larval Wt (mg)
Test Lines		**3 DAE**	**5 DAE**	**6 DAE**	**8 DAE**	**9 DAE**
MXB-13	Unsprayed	20 a	28 a	45 a	47 a	1.18 b
Non-Bt	Unsprayed	17 a	17 bc	18 b	22 b	7.39 a

Means followed by the same letter do not significantly differ as determined by paired t-tests.

Table 5. Number of cabbage looper (Trichoplusia ni) and soybean looper (Pseudoplusia includens) larvae on 40 meters of Bt-cotton (MXB-13) and non-Bt cotton plots in Wayside, MS and College Station, TX in 2002.

	MISSISSIPPI		TEXAS			
	Cabbage loopers		Cabbage loopers		Soybean loopers	
Evaluation	**MXB-13**	**Non-Bt**	**MXB-13**	**Non-Bt**	**MXB-13**	**Non-Bt**
1st week	0 b	112 a	0	0	0	0
2nd week	1 b	311 a	ND	ND	ND	ND
3rd week	0	8	ND	ND	ND	ND
4th week	1 b	40 a	0	0	0	0
5th week	0 b	29 a	0 b	453 a	0	10
6th week	0 b	102 a	1 b	317 a	0	22
7th week	0 b	8 a	0 b	236 a	0	34
8th week	0	5	0 b	61 a	0 b	84 a
9th week	0	4	0 b	35 a	0	7
Totals	2	619	1	1102	0	157

ND= No data taken.
Means in columns by trial location followed by different letters, differ statistically at P>0.05 level.

Table 6. Summary of percent survival after six days for fall armyworm (LAPHFR) leaf tissue bioassay at Starkville, MS in 2001.

	% Survival					
Test Line	**5-Jul-01**	**12-Jul-01**	**26-Jul-01**	**3-Aug-01**	**16-Aug-01**	**Mean across dates**
MXB-13	15.6	18.8	3.1	3.1	0.0	8.1
Non-Bt	59.4	90.6	90.6	84.4	93.8	83.8
LSD (P=0.05)	50.3	15.7	15.0	18.9	20.8	10.9

Table 7. Summary of larval weight after six days for fall armyworm (LAPHFR) leaf tissue bioassay at Starkville, MS in 2001.

	Mean Larval Weight (mg)					
Test Line	**5-Jul-01**	**12-Jul-01**	**26-Jul-01**	**3-Aug-01**	**16-Aug-01**	**Mean across dates**
MXB-13	0.08	0.33	0.03	0.03	0.00	0.09
Non-Bt	1.99	5.74	4.78	7.14	3.71	4.67
LSD (P=0.05)	1.31	1.68	1.99	1.17	0.52	1.41

Table 8. Summary of percent survival after six days for fall armyworm (LAPHFR) square tissue bioassay at Starkville, MS in 2001.

	% Survival		
Test Line	**26-Jul-01**	**3-Aug-01**	**Mean across dates**
MXB-13	37.5	0.0	18.8
Non-Bt	65.6	65.6	65.6
LSD (P=0.05)	35.3	23.1	25.8

Table 9. Summary of larval weight after six days for fall armyworm (LAPHFR) square tissue bioassay at Starkville, MS in 2001.

	Mean Larval Weight (mg)		
Test Line	**26-Jul-01**	**3-Aug-01**	**Mean across dates**
MXB-13	0.54	0.00	0.27
Non-Bt	1.96	1.42	1.69
LSD (P=0.05)	1.27	0.53	0.71

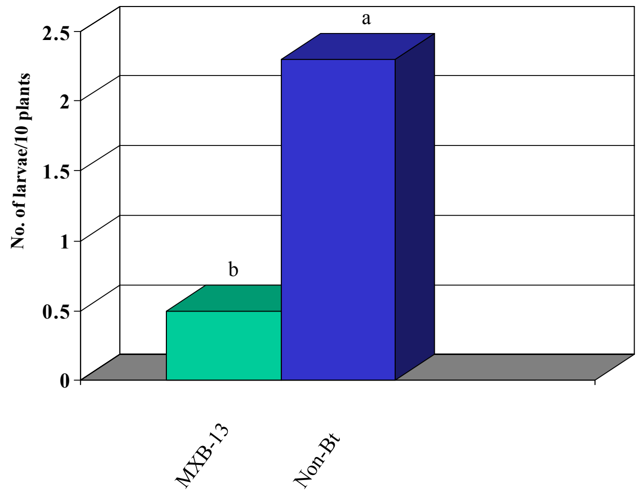

Figure 1. Control of beet armyworm, *Spodoptera exigua* 84 days after planting under natural infestation/unsprayed conditions. 2001 Fresno, CA. Tukey's HSD, P=0.05

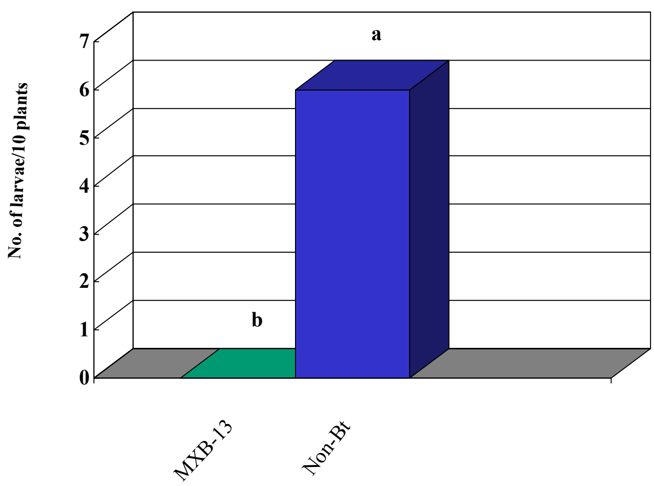

Figure 2. Control of cabbage looper, *Trichoplusia ni* 49 days after planting under natural infestation/unsprayed conditions. Fresno, CA. Tukey's HSD, P=0.05

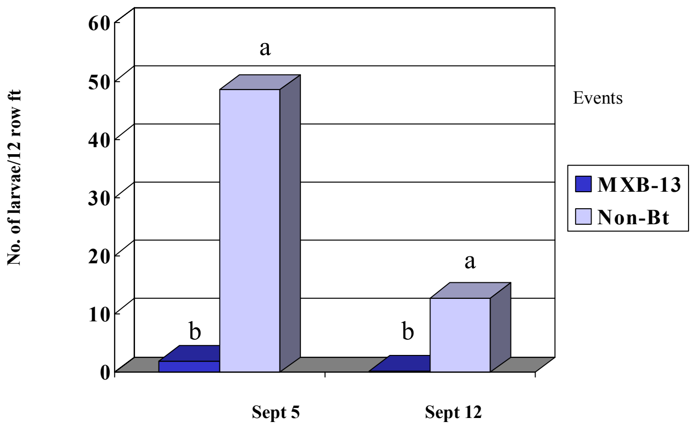

Figure 3. Control of soybean looper, *Pseudoplusia includens* at 86 and 93 days after planting under natural infestation/unsprayed conditions. 2001 Winnsboro, LA. Tukey's HSD, P=0.05

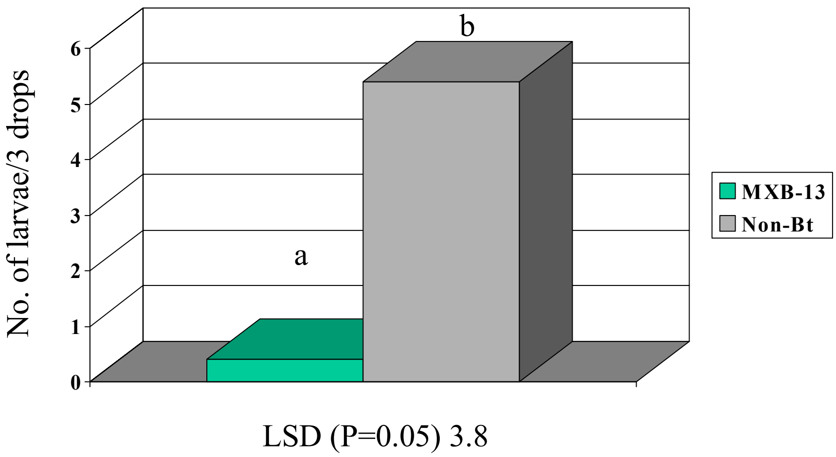

Figure 4. Control of soybean looper, *Pseudoplusia includens* at 83 days after planting under natural infestation/unsprayed conditions. 2001 Stoneville, MS.

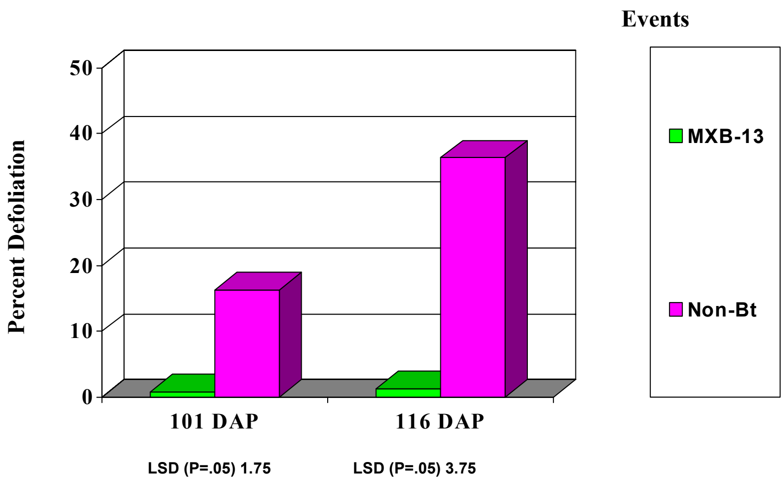

Figure 5. Defoliation of cotton by southern armyworm, *Spodoptera eridania* in under natural infestation/unsprayed conditions. 2001 Baldwin, AL.

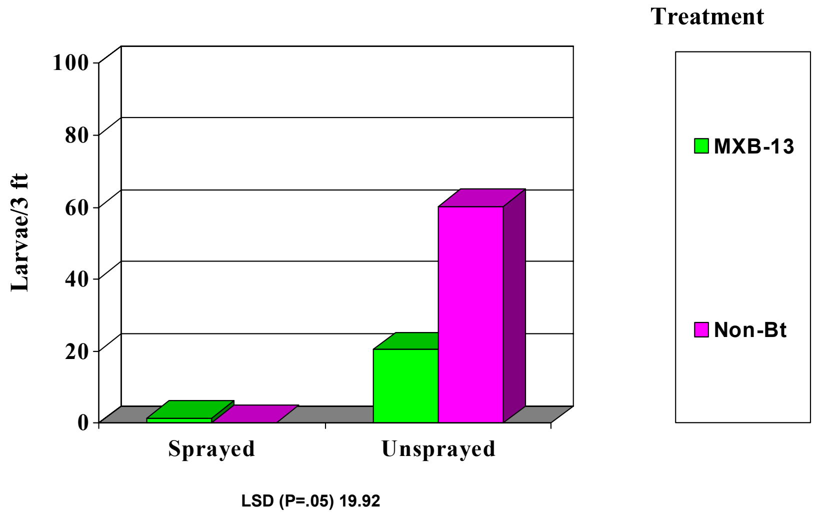

Figure 6. Control of southern armyworm, *Spodoptera eridania* at 101 days after planting under natural infestation conditions. 2001 Baldwin, Al.

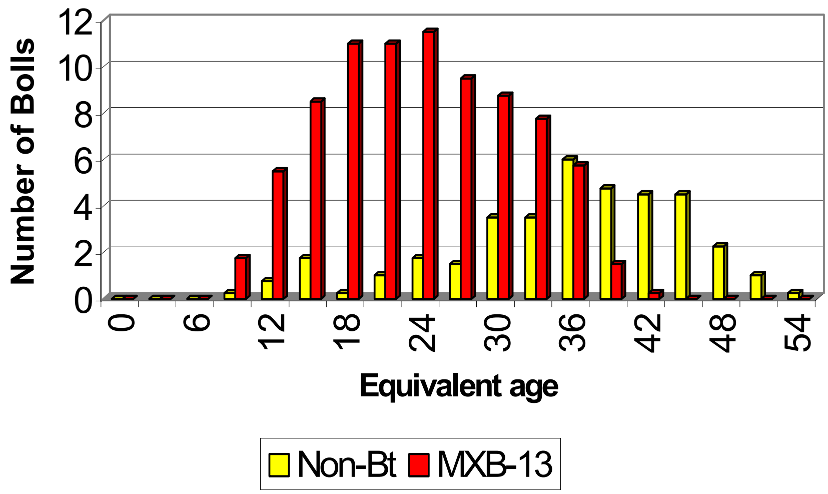

Figure 7. Plant Map Data for Unsprayed MXB-13 vs the non-Bt variety in a trial conducted in Puerto Rico 2001-02.

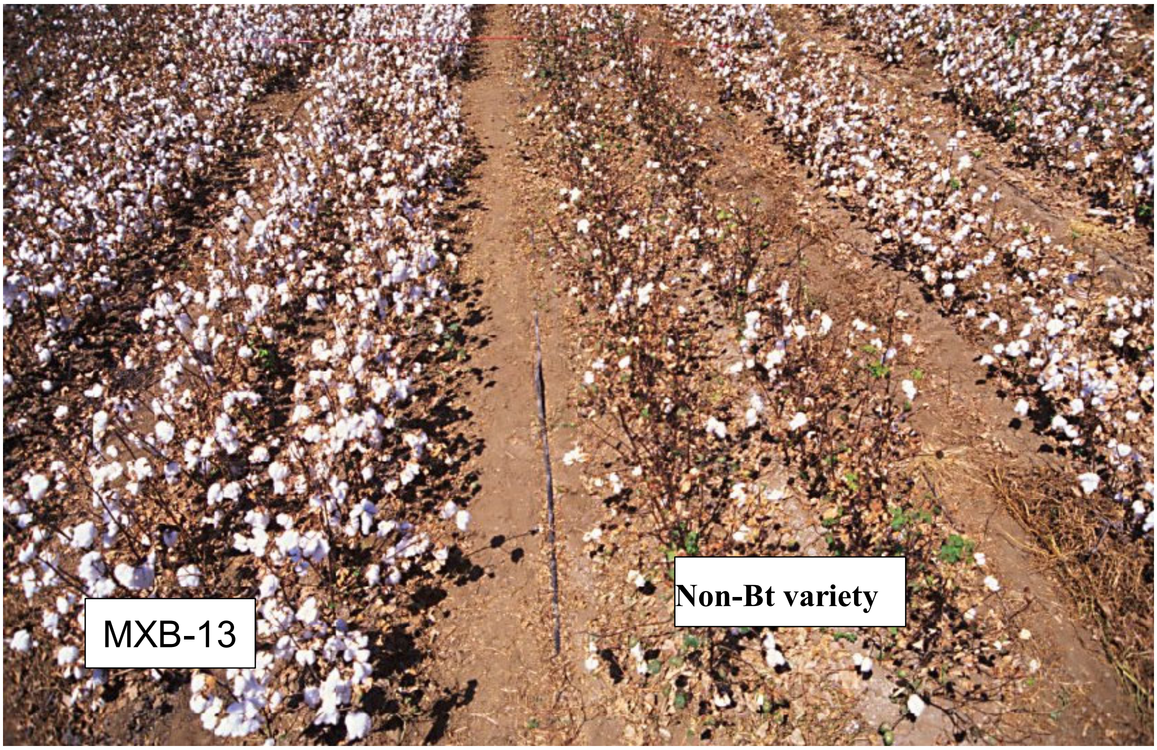

Figure 8. Productivity of MXB-13 vs. non Bt variety under unsprayed conditions. Puerto Winter 2001-2002.