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FIELD EFFICACY OF DOW AGROSCIENCES MXB-13 TRANSGENIC COTTON FOR CONTROL OF COTTON BOLLWORM AND TOBACCO BUDWORM

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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 cotton bollworm (*Helicoverpa zea [Boddie]*) and tobacco budworm (*Heliothis virescens[F.]*), as it was demonstrated by the percent damaged terminals, damaged squares and bolls, the presence of lepidopterous larvae in Bt and non-Bt cotton, and yields.

Introduction

Since 1996, transgenic Bt cotton 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 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 fruiting-structure lepidopteran activity on this new DAS transgenic event.

Materials and Methods

The Cry1Ac:Cry1F stack event, MXB-13, was tested for efficacy against cotton bollworm and tobacco budworm during 2001-2002 (Table 1). This event was compared to a non-Bt cotton. Thirteen studies were conducted across the major US cotton growing areas. The primary targets were cotton bollworm (*Helicoverpa zea*) and tobacco budworm (*Heliothis virescens*). Test sites were selected based on a likelihood of their developing a significant natural pest infestation. Artificial infestations were made at those locations where significant natural populations did not occur.

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. A modified design was used at some locations in which main-plot treatments were not randomized. Plot size was generally 2 or 4 rows wide X 30 to 40 ft long. Standard artificial infestation techniques were used in the unsprayed plots in those trials that were artificially inoculated.

The tests were categorized into five groups: artificially infested tobacco budworm tests, artificially infested cotton bollworm tests, naturally infested tobacco budworm tests, naturally infested cotton bollworm tests, and naturally infested heliothine (mix of cotton bollworm and tobacco budworm) tests. To facilitate presentation, the artificially infested tobacco budworm and cotton bollworm test means were averaged across observation dates to calculate a seasonal average for each test. An analysis of variance was then conducted on the seasonal averages within and across locations. The LSD at P=0.05 was then calculated and used to determine differences between treatments. For the naturally infested tests, percent damaged squares or bolls were chosen for the analysis across locations. Within each location, the observation period which produced the maximum level of damage in the non-Bt treatment was chosen to be used in the across location summary and analysis. Analysis of variance was used to determine significant differences for the seasonal averages within and across locations. Means separation between treatments within a location as well as averaged across locations was determined using the LSD at P=0.05.

Results and Discussion

Artificially Inoculated Tobacco Budworm Tests

Overall, the artificial infestations were successful in producing moderate damaging levels of tobacco budworm in the tests. Only data from the unsprayed plots are presented for the insect damage and infestation observations. Statistically significant reductions in infested terminals were noted for MXB-13 versus the non-Bt variety at three of the four locations (Table 2). The non-Bt variety sustained almost 9 times more damaged squares than MXB-13 on average across locations (Table 3). The average percent infested squares results show MXB-13 with statistically less than the non-Bt treatment in Table 4. Significant reductions were observed with MXB-13 versus non-Bt at all four test locations for damaged boll counts (Table 5). Across locations the non-Bt averaged 10.3% damaged bolls while MXB-13 had only 1.6%. Yields were available for the tests conducted in 2001 and only Wayside 2002 at the time of this writing. Because the main plots were not randomized at these locations, yield data are presented separately for the unsprayed and sprayed portions of the tests (Tables 6a and 6b). The unsprayed yields at Wayside were statistically superior for MXB-13 in 2001 and numerically greater in 2002 (Table 6a). The 2002 Wayside yields are low because of uncontrolled plant bug infestations plus the harvest was done very late due two hurricanes causing very wet soil conditions. Most of the lint had been dislodged and was lying on the ground before the harvest could be accomplished. The yields were relatively high for the unsprayed non-Bt in 2001, particularly at Starkville, indicating that the cotton plants were able to compensate for the fruit lost to the artificially infested tobacco budworms. The sprayed portion of the Starkville test had statistically similar yields for both test lines in 2001(Table 6b). Yields for the sprayed plots at the Wayside locations were numerically greater for MXB-13 in 2001 and significantly greater in 2002. In summary, MXB-13 sustained statistically less tobacco budworm damage than the non-Bt line and the reduced damage translated into higher yields at two of the three sites.

Artificially Inoculated Cotton Bollworm Tests

The artificial cotton bollworm inoculations were conducted at four locations in 2001-2002. Percent infested terminals were determined at three locations (Table 7). The MXB-13 cotton event had an average of 1.1% terminals infested while the non-Bt variety had 9.3%. Percent damaged squares were observed at each of the four locations and MXB-13 had statistically less at 3.5% than the non-Bt at 18.8% (Table 8). A statistically greater number of percent infested squares was observed for the non-Bt than MXB-13 for all but one of the locations (Table 9). At the fourth location MXB-13 was numerically superior to the non-Bt variety. Percent damaged bolls in the unsprayed non-Bt were about 10% at three of the locations and 4.2% in the Starkville 2002 test site (Table 10). MXB-13 had statistically fewer damaged bolls at three sites and was numerically less at the Starkville 2002 site. Means for percent infested bolls followed the same trend as percent damaged bolls with MXB-13 having statistically fewer than non-Bt at three locations and numerically fewer at the fourth (Table 11). Yields were not available at this writing for the 2002 Starkville test, however the results for the other three tests are presented in Tables 12a and 12b. Yields in the unsprayed MXB-13 plots were statistically greater than the non-Bt at both Wayside tests and numerically greater at the Starkville 2001 site (Table 12a). The MXB-13 gave an impressive 4.9 fold yield increase over the non-Bt at Wayside in 2002. Yields were statistically similar for MXB-13 and non-Bt in the sprayed portion of the tests (Table 12b). In summary, MXB-13 was effective in greatly reducing feeding damage by artificially inoculated cotton bollworm, which translated into a yield increase, particularly in the unsprayed comparisons.

Natural Tobacco Budworm Infestation Test

A tobacco budworm natural infestation occurred at the Winnsboro, LA location in 2001. Percent damaged squares were 28% and 21% in the unsprayed and sprayed non-Bt cotton, respectively (Table 13). This infestation occurred toward the end of August and wet weather prevented the planned spray applications. The inability to make a timely insecticide application resulted in high damage levels in the sprayed plots and highlights the advantage of the transgenic cotton event, MXB-13, which expresses insecticidal crystal proteins. MXB-13 gave excellent control of this infestation with only 1 % damaged squares occurring in the unsprayed portion of the test. Percent infested squares results showed a statistically significant reduction in percent infested squares for MXB-13 in both sprayed and unsprayed plots. Yield results reveal high damage levels from the tobacco budworm infestation (Table 14). MXB-13 gave statistically significant yields that were greater by 812 lb/A (36%) in the sprayed comparison and 1079 lb/A (49%) in the unsprayed comparison than the non-BT. The MXB-13 cotton event gave similar levels of yield whether sprayed or unsprayed, demonstrating the high level of efficacy of this transgenic cotton event against tobacco budworm.

Natural Cotton Bollworm Infestation Tests

A natural infestation of predominantly cotton bollworm occurred at two test locations in 2001-2002. The tests were located in NC. High damage levels (> 55%) boll damage were observed in the unsprayed non-Bt plots both years (Table 15). MXB-13 gave a statistically significant reduction in boll damage for the unsprayed portion of the test in 2001 and for both sprayed and unsprayed plots in 2002. The yields for the unsprayed plots showed an average of 2608 lb/A for the MXB-13 and only 1410 lb /A in the non-Bt. These data show MXB-13 under extremely heavy cotton bollworm pressure sustaining only limited injury and little to no yield reduction, even when unsprayed.

Natural Heliothine Infestation Tests

Both cotton bollworm and tobacco budworm infested the tests at Winnsboro, LA and Malbis, AL in 2002. The Winnsboro test had cotton bollworm infestations from early season until late August and then a tobacco budworm infestation occurred in September. The exact ratios or infestation patterns at Malbis are not known. Percent damage square counts reflect high infestation levels at Winnsboro (50%) and moderate levels of 23.7% at Malbis in the unsprayed non-Bt (Table 17). MXB-13 sustained very low levels of damaged squares that were statistically less than the unsprayed non-BT. The application of insecticides significantly reduced the damage in the non-BT at both locations. Yields were only available for the Winnsboro test at this writing. MXB-13 unsprayed was statistically superior, yielding 2177 lb/A, while the non-Bt unsprayed gave only 990 lbs/A (Table 18). MXB-13 sprayed was numerically superior to the non-Bt sprayed, 1992 lb/A versus 1954 lb/A, respectively. These data indicate a high level of efficacy for MXB-13 against both cotton bollworm and tobacco budworm. Overall, MXB-13 gave excellent control of both artificial and natural infestations of tobacco budworm and cotton bollworm. Plant damage evaluations and ultimately yields showed that MXB-13 when subjected to high levels of these pests sustained only low levels of damage to the plants fruiting structures.

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

Location	Year Conducted	Trial Type	Insects Evaluated Common Name	Bayer Code
Wayside, MS	2001	Artificial Inf.	Tobacco budworm	HELIVI
Wayside, MS	2002	Artificial Inf.	Tobacco budworm	HELIVI
Starkville, MS	2001	Artificial Inf.	Tobacco budworm	HELIVI
Starkville, MS	2002	Artificial Inf.	Tobacco budworm	HELIVI
Winnsboro, LA	2001	Natural Inf.	Tobacco budworm	HELIVI
Winnsboro, LA	2002	Natural Inf.	Tobacco budworm & Cotton bollworm	HELIVI & HELIZE
Wayside, MS	2001	Artificial Inf.	Cotton bollworm	HELIZE
Wayside, MS	2002	Artificial Inf.	Cotton bollworm	HELIZE
Starkville, MS	2001	Artificial Inf.	Cotton bollworm	HELIZE
Starkville, MS	2002	Artificial Inf.	Cotton bollworm	HELIZE
Jamesville, NC	2001	Natural Inf.	Cotton bollworm	HELIZE
Jamesville, NC	2002	Natural Inf.	Cotton bollworm	HELIZE
Malbis, AL	2002	Natural Inf.	Tobacco budworm & cotton bollworm	HELIVI & HELIZE

Trials with Artificial Tobacco Budworm Infestations:

Table 2. Seasonal average percent infested terminals in field studies artificially inoculated with HELIVI at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Starkville 2002	Wayside 2002	Avg.
MXB-13	Unsprayed	0.8 b	6.9 b	0.8 a	0.9 b	2.4 b
Non-Bt	Unsprayed	2.7 a	19.4 a	4.4 a	11.1 a	9.4 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 3. Seasonal average percent damaged squares in field studies artificially inoculated with HELIVI at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Starkville 2002	Wayside 2002	Avg.
MXB-13	Unsprayed	1.5 b	2.3 b	1.9 b	5.2 b	2.7 b
Non-Bt	Unsprayed	24.4 a	19.4 a	13.3 a	37.1 a	23.5 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 4. Seasonal average percent infested squares in field studies artificially inoculated with HELIVI at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Starkville 2002	Wayside 2002	Avg.
MXB-13	Unsprayed	0.2 b	0.2 b	0.6 b	0.6 b	0.4 b
Non-Bt	Unsprayed	5.9 a	8.3 a	2.5 a	10.4 a	6.8 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 5. Seasonal average percent damaged bolls in field studies artificially inoculated with HELIVI at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Starkville 2002	Wayside 2002	Avg.
MXB-13	Unsprayed	1.6 b	1.7 b	0.6 b	2.9 b	1.7 b
Non-Bt	Unsprayed	13.7 a	7.1 a	5.0 a	15.5 a	10.3 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 6a. Yield (pounds of seed cotton/acre) in field studies artificially inoculated with HELIVI at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001[3]	Starkville 2001	Starkville 2002[2]	Wayside 2002
MXB-13	Unsprayed	2978 a	3100 a	na	798 a
Non-Bt	Unsprayed	2190 b	3146 a	na	178 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).
[2]Data not available at time of this writing.
[3]Due to missing plots LS MEANS were used in analysis for means comparison for the Wayside location, but actual means are reported in table.

Table 6b. Yield (pounds of seed cotton/acre) in field studies artificially inoculated with HELIVI at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Starkville 2002[2]	Wayside 2002
MXB-13	Sprayed	2883 a	3911 a	na	1407 a
Non-Bt	Sprayed	2868 a	4030 a	na	1037 b

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).
[2]Data not available at time of this writing.

Trials with Artificial Cotton Bollworm Infestations:

Table 7. Seasonal average percent infested terminals in field studies artificially inoculated with HELIZE at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Wayside 2002	Starkville 2002	Avg.
MXB-13	Unsprayed	0.0 b	2.3 b	-	1.0 b	1.1 a
Non-Bt	Unsprayed	4.5 a	16.0 a	-	7.5 a	9.3 b

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 8. Seasonal average percent damaged squares in field studies artificially inoculated with HELIZE at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Wayside 2002	Starkville 2002	Avg.
MXB-13	Unsprayed	3.1 b	5.2 b	3.5 b	2.3 b	3.5 b
Non-Bt	Unsprayed	22.9 a	17.1 a	22.1 a	12.9 a	18.8 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 9. Seasonal average percent infested squares in field studies artificially inoculated with HELIZE at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Wayside 2002	Starkville 2002	Avg.
MXB-13	Unsprayed	0.3 b	1.3 b	0.8 b	1.3 a	0.9 b
Non-Bt	Unsprayed	4.8 a	6.5 a	6.9 a	2.3 a	5.1 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 10. Seasonal average percent damaged bolls in field studies artificially inoculated with HELIZE at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Wayside 2002	Starkville 2002	Avg.
MXB-13	Unsprayed	0.8 b	1.3 b	2.5 b	0.4 a	1.2 b
Non-Bt	Unsprayed	9.4 a	10.0 a	10.9 a	4.2 a	8.6 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 11. Seasonal average percent infested bolls in field studies artificially inoculated with HELIZE at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Wayside 2002	Starkville 2002	Avg.
MXB-13	Unsprayed	0.1 b	0.4 b	0.4 b	0.0 a	0.3 b
Non-Bt	Unsprayed	1.5 a	6.7 a	3.8 a	2.9 a	3.7 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 12a. Yield (pounds of seed cotton/acre) in field studies artificially inoculated with HELIZE at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Wayside 2002	Starkville 2002
MXB-13	Unsprayed	2937 a	4015 a	1740 a	na[2]
Non-Bt	Unsprayed	1947 b	3826 a	357 b	na

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).
[2]Data not available at time of this writing.

Table 12b. Yield (pounds of seed cotton/acre) in field studies artificially inoculated with HELIZE at Wayside and Starkville, MS during 2001-02.[1]

Test Lines		Wayside 2001	Starkville 2001	Wayside 2002	Starkville 2002
MXB-13	Sprayed	2883 a	3910 a	1797 a	na[2]
Non-Bt	Sprayed	2868 a	4030 a	1527 a	na

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).
[2]Data not available at time of this writing.

Trial with Natural Tobacco Budworm Infestations:

Table 13. Percent damaged squares and percent infested squares from a natural infestation of HELIVI at Winnsboro, LA, August 30, 2001.[1]

Test Lines		% Damaged Squares	% Infested Squares
MXB-13	Unsprayed	1 b	1 b
Non-Bt	Unsprayed	28 a	9 a
MXB-13	Sprayed	0 b	0 b
Non-Bt	Sprayed	21 a	7 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 14. Yield (pounds of seed cotton/A) under natural infestation of HELIVI at Winnsboro, LA during 2001.[1]

Test Lines		Winnsboro 2001
MXB-13	Unsprayed	2218 a
Non-Bt	Unsprayed	1139 c
MXB-13	Sprayed	2256 a
Non-Bt	Sprayed	1444 b

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Trials with Natural Cotton Bollworm Infestations:

Table 15. Percent damaged bolls at peak natural infestation of HELIZE at Jamesville, NC, 2001-2002.[1]

		Jamesville		
Test Lines		2001	2002	AVG
MXB-13	Unsprayed	7.5 b	2.5 c	5.0 b
Non-Bt	Unsprayed	56 a	66.9 a	61.4 a
MXB-13	Sprayed	0.5 c	0.6 c	0.6 b
Non-Bt	Sprayed	3.5 bc	11.9 b	7.7 b

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 16. Yield (pounds of seed cotton/A) under natural infestation of HELIZE at Jamesville, NC during 2001 and 2002.[1]

		Jamesville	
Test Lines		2001	2002
MXB-13	Unsprayed	2608 ab	1701 a
Non-Bt	Unsprayed	1410 c	1031 b
MXB-13	Sprayed	2399 b	1955 a
Non-Bt	Sprayed	2798 a	1972 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Trials with Natural Heliothine Infestations:

Table 17. Percent damaged squares from a natural heliothine infestation at Winnsboro, LA and Malbis, AL, 2002.[1]

Test Lines		Winnsboro 2002	Malbis 2002	AVG
MXB-13	Unsprayed	2.5 b	0.6 b	1.6 b
Non-Bt	Unsprayed	50.0 a	23.7 a	36.9 a
MXB-13	Sprayed	2.5 b	3.1 b	2.8 b
Non-Bt	Sprayed	10.0 b	2.5 b	6.3 b

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).

Table 18. Yield (pounds of seed cotton/A) under natural infestation of HELISP (HELIVI and HELIZE) at Winnsboro, LA, 2002.[1]

Test Lines		Winnsboro 2002
MXB-13	Unsprayed	2177 a
Non-Bt	Unsprayed	990 b
MXB-13	Sprayed	1992 a
Non-Bt	Sprayed	1954 a

[1]Means within a column followed by the same letter do not significantly differ (P=0.05, LSD).