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EFFICACY OF WIDESTRIKE COTTON AGAINST HELIOTHINE INSECTS

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Abstract

Dow AgroSciences, LLC (DAS) 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, PHY 440W. Dow AgroSciences PHY 440W provided excellent control of bollworm (*Helicoverpa zea [Boddie]*) and tobacco budworm (*Heliothis virescens[F.]*), as it was demonstrated by the percent damaged terminals, flowers, squares and bolls, Heliothine larvae incidence and seed cotton yield.

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* 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, PHY 440W. 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 variety, PHY 440W, was tested for efficacy against bollworm and tobacco budworm during 2003 (Table 1). This event was compared to a non-Bt cotton (PSC 355). Fifteen studies were conducted across the major US cotton growing areas. The primary targets were bollworm (*Helicoverpa zea*) and tobacco budworm (*Heliothis virescens*). Test sites were selected based on a likelihood of their developing a significant natural pest infestation.

A modified split plot design was used at all locations in which main-plot treatments were not randomized across replications. Treatments were replicated four times. Areas of "sprayed" and "unsprayed" were designated as the main plots, and varieties (PHY 440W and PSC 355) 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 4 rows wide X 30 to 40 ft long.

Trial locations were classified into two categories based on natural insect pressure at the site: Low to moderate or high Heliothine pressure as determined by damage and larval incidence in the unsprayed PSC 355 treatment. Beginning with the first observation period when insect damage or larval incidence was recorded, all observations at all locations were averaged together to calculate a seasonal average for each treatment. An analysis of variance was then conducted on the seasonal averages across locations. Treatments were subjected to ANOVA means separation with a LSD at P=0.05.

Results and Discussion

Eight of the fifteen trials conducted in 2003 had low to moderate infestations of Heliothine pests (Table 1). All eight trials were infested with natural populations of bollworm and three of the trials also had a population of tobacco budworm develop. The three sites with tobacco budworm development occurred late season, after the initial population of bollworm. The ratios or infestation patterns at these sites were determined by monitoring adult moths captured in pheromone traps. The remaining seven trials in had high natural infestations of Heliothine pests, with three of the seven locations also having tobacco budworm-bollworm complex.

Terminal Damage

Only 1.5% and 2.8% terminal damage was observed in PHY 440W plots at mod - low and high insect pressure levels respectively (Table 2). Under high insect pressure, terminal damage to unsprayed PHY 440W was equal to sprayed non-Bt and sprayed PHY 440W. Under mod - low insect pressure, terminal damage to unsprayed PHY 440W was equal to sprayed PHY 440W, and significantly less than seen in sprayed non-Bt cotton.

Flower Damage

Flower damage to the non-Bt cotton was 14.5 % and 3.5% in the high and mod - low infestation level trials respectively (Table 3). Under high infestation levels, both PHY 440W treatments and the sprayed non-Bt treatment provided statistically significant reductions in the level of flower damage when compared to the non-sprayed non-Bt plot. There were no statistical differences between these three treatments on flower damage ratings however. In the mod - low infestation trials, a similar trend was observed. However, there was a statistically significant reduction in the percent of flower damage with PHY 440W being sprayed when compared to the sprayed non-Bt treatment.

Square Damage

Square damage to the non-Bt cotton was 25.3 % and 7.0% in the high and mod - low infestation level trials respectively (Table 4). In the high infestation level trials, both PHY 440W treatments and the sprayed non-Bt treatment provided statistically significant reductions in the level of square damage when compared to the non-sprayed non-Bt plot. However, the sprayed non-Bt plot had significantly higher square damage (7.4%) than the sprayed PHY 440W plot (2.3%). A similar trend was observed in the mod - low level insect pressure trials. Irregardless of insect pressure, square damage was significantly less in the unsprayed PHY 440W cotton than observed in the sprayed non-Bt cotton, but not significantly different than the sprayed PHY 440W cotton.

Larvae Infestation

Mean seasonal larval infestation levels observed in the unsprayed non-Bt cotton were 32.7 % and 10.7% in the high and mod - low infestation level trials respectively (Table 4). At high infestation levels, both PHY 440W treatments and the sprayed non-Bt treatment provided statistically significant reductions in the levels of larval infestations when compared to the non-sprayed non-Bt plot. There were no statistical differences between these three treatments on larval infestation levels, however. At mod - low infestation levels, both PHY 440W plots provided statistically significant reductions in levels of larval infestations when compared to the sprayed non-Bt plot.

Boll Damage

Boll damage ratings observed in the unsprayed non-Bt cotton were 25.5 % and 5.0% in the high and mod - low infestation level trials respectively (Table 5). At high infestation levels, both PHY 440W treatments and the sprayed non-Bt treatment provided statistically significant reductions in the level of boll damage when compared to the unsprayed non-Bt plot. There were no statistical differences between these three treatments on boll damage ratings however. At mod - low infestation, a similar trend was observed. However, there was a statistically significant reduction in the percent of boll damage observed with sprayed PHY 440W when compared to the sprayed non-Bt treatment.

Yield

There was no significant difference in seed cotton yields observed between PHY 440W, sprayed non-Bt and sprayed PHY 440W in 2003 (Table 6). The unsprayed non-Bt plot yielded an average of 1559 lbs per acre of seed cotton compared to 2298 to 2387 lbs per acre for the sprayed plots and non-sprayed PHY 440W. These data show PHY 440W under extremely heavy bollworm pressure sustaining only limited injury and little to no yield reduction, even when unsprayed in 2003 and when average across 2001 to 2003 (Table 6). Another way to look at the data is to compare all the yields of the plots back to the sprayed non-Bt plot (PSC 355) as a percent of the sprayed PSC 355 (Table 7). The non-sprayed PSC 355 in 2003 showed 72.3% of the sprayed PSC 355 and 67.8% for the three-year average or about a one third reduction in yield when these plots were not sprayed for Heliothine control. The PHY 440W provided 100% and 101.3% of the sprayed PSC 355 plots in 2003 and across the 3-yr period of 2001-2003, respectively. This indicates that under extremely heavy bollworm pressure unsprayed PHY 440W sustained only limited injury with no yield reduction. The addition of Heliothine sprays to PHY 440W provided 5.3% and 3.9% yield increases when compared to the sprayed PSC 355. When compared to the unsprayed PHY 440W, there was a 5.3% yield increase in 2003 from additional Heliothine sprays and a 2.6% yield increase for the 3-yr period of 2001-2003.

In summary, the efficacy of Widestrike™ cotton (PHY 440W) against *Heliothines* (*H. zea and H. virescens*) was similar to 2001-2002 results, but confirmed across a broader geography and under heavier bollworm pressure.

Table 1. Location, Heliothine species and pest pressure on Bt cotton trials conducted during 2003.

Location	Insects Evaluated Common Name	Bayer Code	Pest Pressure*
Tillar, AR	Bollworm	HELIZE	H
Chula, GA	Bollworm	HELIZE & HELIVI	H / M
Wallis, TX	Bollworm	HELIZE & HELIVI	H / M
Elko, SC	Bollworm	HELIZE	H
Edgecomb Co., NC	Bollworm	HELIZE	H
Jamesville, NC	Bollworm	HELIZE	H
Blackville, SC	Bollworm	HELIZE	H
Midville, GA	Bollworm	HELIZE	M
Santa Isabel, P. R.	Bollworm	HELIZE	M
Wayside, MS	Bollworm	HELIZE	M
Shoffner, AR	Bollworm	HELIZE	L
Portageville, MO	Bollworm	HELIZE	L
Rio Hondo, TX	Tobacco budworm & bollworm	HELIVI & HELIZE	L / L
St. Paul, TX	Tobacco budworm & bollworm	HELIVI & HELIZE	L / L
Winnsboro, LA	Tobacco budworm & cotton bollworm	HELIVI & HELIZE	L / L

*Pest Pressure: High (H), Moderate (M), Low (L)

Table 2. Mean seasonal observation of terminal damage occurring under different levels of natural Heliothine infestation in field studies during 2003.[1]

	Terminal Damage (%)	
	Mod – Low [2]	**High**
Treatment	***L* = 8, *n* = 40[3]**	**L = 7, n = 35**
PSC 355	9.4 a	29.4 a
PHY 440W	1.5 c	2.8 bc
PSC 355 – sprayed[4]	4.5 b	6.2 b
PHY 440W - sprayed	1.2 c	0.8 c

[1] Means within a column followed by the same letter do not differ according to LSD test at P ≤ 0.05.
[2] Grouping of locations based on level of insect pressure occurring at the site: Mod – Low = moderate to low insect pressure, High = high level of insect pressure.
[3] Number of locations (L) and total observations (n)
[4] Sprayed as needed for control of lepidopteran pests.

Table 3. Mean seasonal observation of flower damage occurring under different levels of natural Heliothine infestation in field studies during 2003.[1]

	Flower Damage (%)	
	Mod – Low [2]	**High**
Treatment	***L* = 8, *n* = 42[3]**	***L* = 7, *n* = 28**
PSC 355	3.5 a	14.5 a
PHY 440W	0.6 bc	3.8 b
PSC 355 – sprayed[4]	1.4 b	2.9 b
PHY 440W - sprayed	0.2 c	1.9 b

[1] Means within a column followed by the same letter do not differ according to LSD test at P ≤ 0.05.
[2] Grouping of locations based on level of insect pressure occurring at the site: Mod – Low = moderate to low insect pressure, High = high level of insect pressure.
[3] Number of locations (L) and total observations (n)
[4] Sprayed as needed for control of lepidopteran pests.

Table 4. Mean seasonal observation of square damage occurring under different levels of natural Heliothine infestation in field studies during 2003.[1]

Treatment	Square Damage (%) Mod – Low[2] *L = 8, n = 46*[3]	Square Damage (%) High *L = 7, n = 33*
PSC 355	7.0 a	25.3 a
PHY 440W	1.1 bc	4.0 bc
PSC 355 – sprayed[3]	2.3 b	7.4 b
PHY 440W - sprayed	0.3 c	2.3 c

[1] Means within a column followed by the same letter do not differ according to LSD test at $P \leq 0.05$.
[2] Grouping of locations based on level of insect pressure occurring at the site: Mod – Low = moderate to low insect pressure, High = high level of insect pressure.
[3] Number of locations (L) and total observations (n)
[4] Sprayed as needed for control of lepidopteran pests.

Table 5. Mean seasonal observation of larval infestation occurring under different levels of natural Heliothine infestation in field studies during 2003.[1]

Treatment	Larvae Infestation (%) Mod – Low[2] *L = 8, n = 46*[3]	Larvae Infestation (%) High *L = 7, n = 38*
PSC 355	10.7 a	32.7 a
PHY 440W	0.9 c	4.2 b
PSC 355 – sprayed[3]	3.3 b	5.5 b
PHY 440W - sprayed	0.4 c	1.1 b

[1] Means within a column followed by the same letter do not differ according to LSD test at $P \leq 0.05$.
[2] Grouping of locations based on level of insect pressure occurring at the site: Mod – Low = moderate to low insect pressure, High = high level of insect pressure.
[3] Number of locations (L) and total observations (n)
[4] Sprayed as needed for control of lepidopteran pests.

Table 6. Mean seasonal observation of boll damage occurring under different levels of natural Heliothine infestation in field studies during 2003.[1]

Treatment	Boll Damage (%) Mod – Low[2] *L = 8, n = 41*[3]	Boll Damage (%) High *L = 7, n = 30*
PSC 355	5.0 a	25.5 a
PHY 440W	0.7 bc	4.0 b
PSC 355 – sprayed[3]	1.8 b	4.4 b
PHY 440W - sprayed	0.4 c	1.3 b

[1] Means within a column followed by the same letter do not differ according to LSD test at $P \leq 0.05$.
[2] Grouping of locations based on level of insect pressure occurring at the site: Mod – Low = moderate to low insect pressure, High = high level of insect pressure.
[3] Number of locations (L) and total observations (n)
[4] Sprayed as needed for control of lepidopteran pests.

Table 7. Seed cotton yields averaged across field efficacy trials: 1-yr. vs. 3-yrs. results.[1]

	Mean seed cotton yield (lbs./A)	
Treatment	2003 *(9 locations)*	2001-2003 *(13 locations)*
PSC 355	1559 b	1744 b
PHY 440W	2329 a	2397 a
PSC 355 – sprayed[2]	2298 a	2412 a
PHY 440W - sprayed	2387 a	2493 a

[1] Means within a column followed by the same letter do not differ according to LSD test at $P \leq 0.05$.
[2] Sprayed as needed for control of lepidopteran pests.

Table 8. Seed cotton yields as a percent of sprayed PSC 355 averaged across field efficacy trials: 1-year vs. 3-year results.

	Yield as a percent of sprayed PSC 355 (%)	
Treatment	2003 *(9 locations)*	2001-2003 *(13 locations)*
PSC 355	72.3	67.8
PHY 440W	100.0	101.3
PSC 355 – sprayed[1]	--	--
PHY 440W - sprayed	105.3	103.9

[1] Sprayed as needed for control of lepidopteran pests.