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PERFORMANCE OF WIDESTRIKE™ INSECT PROTECTION FOR CONTROL OF LEPIDOPTERAN PESTS IN ALABAMA FROM 2001 THROUGH 2004

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Abstract

WideStrike Insect Protection, developed by Dow AgroSciences LLC, expresses the insecticidal crystal proteins Cry1F and Cry1Ac from the bacterium *Bacillus thuringiensis* (Bt). 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, or WideStrike Insect Protection. Small plot and larger strip plot trails were conducted in Alabama from 2001 thru 2004 to characterize the efficacy and agronomic performance of the 2 Bt proteins independently as well as when they are expressed together in the varieties PHY 440W and PHY 470 WR. PHY 440 W only expresses the WideStrike Insect Protection trait while PHY 470 WR is a stacked-trait variety which contains the WideStrike Insect Protection trait and RR trait. The trials demonstrated that cotton varieties expressing the WideStrike trait are effective tools in managing southern armyworm (*Spodoptera eridania),* tobacco budworm (*Heliothis virescens[F.]*) and bollworm (*Helicoverpa zea [Boddie]*). Supplemental insecticide applications may be needed to optimize yields when bollworm populations are high.

Introduction

Development of transgenic Bt cotton expressing two genes encoding different Bt proteins has resulted in varieties which offer season long, broad spectrum control of the major lep pests of cotton. Dow AgroSciences LLC genetically modified cotton to express two separate insecticidal crystal proteins from the bacterium *Bacillus thuringiensis* (Bt). 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 (or WideStrike Insect Protection). Field studies were conducted in Alabama from 2001 through 2004 to determine the efficacy of the trait in both small plot and larger strip type trials. Registration of this stacked trait was accepted by the EPA in 2004 and varieties expressing the WideStrike trait will be introduced commercially in 2005.

Materials and Methods

The Cry1Ac event (MXB–7), the Cry1F event (MXB-9), and the stacked event MXB-13, expressing both Cry1Ac and Cry1 F, were tested for efficacy compared to a non-Bt cotton variety at Loxley, AL in 2001 against natural field populations of lep pests. A split plot design with four replications was employed in the field study which was planted June 1. 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, only non-lepidopteran pests were controlled. Plot size was two rows wide X 30 ft long.

Larger strip type trials were conducted at the Auburn University Agricultural Experiment Station in Prattville, AL in 2003 and 2004. The WideStrike trial in 2003 was planted with PHY 440 W in a block 30 feet wide by 700 feet long. The block was surrounded on all four sides by a 40 foot buffer of PSC 355 which has a similar background to PHY 440 W. One-half of the plot which contained PHY 440 W was treated as needed for control of lep pests every time the spray threshold was achieved on the conventional non-Bt variety PSC 355. The other half of the plot was not treated for lep pests. The entire test area was treated for non-lep pests as needed.

In 2004, the same trial design was used, but the WideStrike variety tested was PHY 470 WR and the non-Bt was PHY 410 R which has a similar background to PHY 470 WR. However, rather than one half of the plot being treated as needed for control of lep pests every time the spray threshold was achieved on the conventional non-Bt variety, the “sprayed” section was to be treated when the spray threshold was achieved for the WideStrike variety PHY 470 WR.

Results and Discussion

Small plot replicated trial conducted in 2001

A natural infestation of southern armyworm developed at the Loxley, AL location in mid-August, 2001. This was the only lep pest population that developed in the plot. Tracer .067 + Decis .025 lb ai/a was applied August 21 and 29 to the “sprayed” main block. Number of larvae per 3 feet of row 100 days after planting ranged from 0.3 to 2.3 in the sprayed plot and from 20.5 to 60.3 in the unsprayed plots (Table 1). There were no significant differences in the number of larvae in the sprayed plots, but the number of larvae in the unsprayed WideStrike and MXB-9 (Cry1F) plots were significantly lower than in the MXB-7 (Cry1Ac) or unsprayed plots. There were no significant differences in the number of larvae in the unsprayed WideStrike and MXB-9 plots.

The larvae observed in the unsprayed WideStrike and MXB-9 plots did not cause major reductions in defoliation as the ratings were less than 1% 100 days after planting as compared to defoliation ratings of 11.3 and 16.3% for the unsprayed MXB 7 and PSC 355 plots, respectively (Table 2). The defoliation ratings of the unsprayed WideStrike and MXB-9 plots did not increase over the next 15 days as they did in the MXB 7 and unsprayed PSC 355 plots (up to 27.5 and 36.3, respectively).

This trial demonstrated that WideStrike and MXB-9 under moderate southern armyworm pressure sustained only limited injury as determined by defoliation ratings of less than 1%. However, the MXB-7 trait did not provide significant reductions in defoliation as compared to the non-Bt variety PSC 355. Visual observations of the plots indicated that the relatively high number of larvae found in the MXB-13 and MXB-9 plots were due to feeding on morningglory weeds within the plots.

2003 WideStrike Strip Trial

On July 17 eggs and neonate bollworm larvae were discovered in the terminals of nearly 100% of the plants in the test area. Additional egg deposition continued for approximately 14 days. The lep treated area of the trial was sprayed a total of three times with a combination of Karate plus Tracer (July 21 and 30, and Aug. 12). Infestation and damage data was collected on 6 dates, at approximately 6-8 day intervals from July 28 until September 2. All insecticide treatments made to the plots are provided in Table 3.

Terminal damage was heavy, over 50%, in the unsprayed PSC 355 for approximately 3 weeks (July 17-August 11). During this same period, terminal damage in the unsprayed PHY 440 W ranged between 10 and 20%. Larvae infested terminals were about 25% in the PSC 355 on the July 28 and August 4 observation dates while the number recorded for the PHY 440 was just under 15% on July 28 and zero on August 4 (Table 4). Square damage in the unsprayed PSC 355 was over 75% for three consecutive observation dates (July 28, August 4 and August 11, Table 5). Square damage in the unsprayed PHY 440 ranged from 20 to 35% on July 28 and August 4 but had fallen to 10% by August 11. Small larvae (less than one-fourth inch in size) were recorded in 25% of the squares in the unsprayed PSC 355 on July 28 but dropped to 4% on August 4. The unsprayed PHY 440 W had between 10 and 15% of the squares infested with small larvae on both the July 28 and August 4 observation dates. Large larvae (> ¼”) were found in about 28% of the unsprayed PSC 355 squares on July 28 while none were found in the unsprayed PHY 440 W or any of the following observation dates.

It was apparent that both PSC 355 and PHY 440 W were infested in a similar manner. On both varieties, egg deposition and larval survival in the terminals was followed by small larvae damage and survival in squares. However, there was a noticeable absence of large larvae in the squares of the PHY 440 W. There was also a noticeable difference in the number of blooms with larval feeding, with the unsprayed PSC 355 ranging from 30 to 80% on three observation dates. The unsprayed PHY 440 W had less than 10% flower damage on one date and none on the other dates. On the July 28 observation date, the unsprayed PSC 355 had 25% of the blooms with small larvae while the unsprayed PHY 440 W had none. On August 11 about 32 % of the blooms were damaged in the unsprayed PSC 355 as compared to 8% in the unsprayed PHY 440 W. Boll damage ranged from 20-95% in the unsprayed PSC 355 (Table 6). The unsprayed PHY 440 W had 10% boll damage on the August 21 and September 9 dates. Up to 35% of the unsprayed PSC 355 bolls had large larvae on August 4 while no larvae were found in the unsprayed PHY 440 W bolls on any observation date.

When all observation dates were averaged together (Table 7), terminal damage in the unsprayed PSC 355 was over 45% while the unsprayed PHY 440 W was about 12%. Terminal larval numbers were less than 15% in the unsprayed PHY 440 W compared to about 65% in the unsprayed PSC 355. Square damage across all observation dates was near 50% in the unsprayed PSC 355, compared to about 13% in the unsprayed PHY 440 W. However, the difference in the number of small larvae in squares was not that great between the unsprayed PSC 355 and unsprayed PHY 440 W, 33% compared to 26% respectively. Large larvae were another story—with a seasonal average of over 35% of the squares having large larvae in the unsprayed PSC 355 compared to none in the unsprayed PHY 440 W. Wide differences were also recorded in the number of large larvae and damage in blooms. The unsprayed PSC 355 had about 30% of the blooms damaged by worms while the unsprayed PHY 440 W had about 2%. No small larvae were found in the blooms of unsprayed PHY 440 W compared to over 30% found in unsprayed PSC 355 blooms. Large larvae varied from about 37% in the unsprayed PSC 355 blooms to 8% in unsprayed PHY 440 W blooms.

Large differences were observed in the level of boll damage between the unsprayed PSC 355 compared to the unsprayed PHY 440 W. Slightly more than 50% of the unsprayed PSC 355 had bolls damaged by bollworm larvae while the unsprayed PHY 440 W had only about 4%, when all observation dates were averaged. On the July 28 and August 4 observation dates, the unsprayed PSC 355 had 28 and 37% of the bolls with large larvae compared to none in the unsprayed PHY 440 W.

Lint yields support the extent and differences in the damage to the various treatments in this test (Table 8). The unsprayed PHY 440 W yielded 1,480 lbs. of lint (machine harvested) compared to 483 lbs. in the unsprayed PSC 355. When lep controls were made, the PSC 355 yielded 1,318 lbs. of lint and the PHY 440 W yielded 1,530 lbs.

2004 WideStrike Strip Trial

On July 13 a moderate bollworm flight was detected in the test area. Egg deposition continued for approximately 14 days. Infestation and damage data was collected on 5 dates, at approximately 6-8 day intervals from July 13 until August 9. The percent damaged terminals of PHY 410 R increased from 25 % on July 13 to 50 % on July 19 while the square damage increased form 0 to 30 % (Table 10). The % of live larvae also increased during this time frame (Table 11). However, this was not the case with PHY 470 WR as no live larvae or damaged terminals or squares were detected on these two dates. On July 26, significant larval infestations as well as damage levels were reached on fruiting structures in the unsprayed PHY 410 R plot, but not in the PHY 470 WR plot (Table 12). A 2% infestation of live larvae > ¼” long was detected in the PHY 470 WR plot.

The trial design called for a pyrethroid application to be applied when the spray threshold for PHY 470 WR was reached. However, it did not appear that the spray threshold of 3 – 5 % live larvae > ¼” long in bolls would be met, so the decision was made to make a pyrethroid application on July 27 at the 2 % infestation level to determine the benefit of an application at that time. Data from the sprayed PHY 410 R plot is not shown as spray was not applied based on the non-Bt cotton threshold, thus significant injury had already occurred to the fruiting structures and the data did not contribute to the objectives of the trial.

Based on the data collected on August 2 and 3, square damage on the unsprayed PHY 410 R plot had increased to 28 % while the boll damage had increased to 15%. This compared to only 7% of the squares and 2 – 2.5 % of the bolls damaged in the sprayed and unsprayed PHY 470 WR plots. On August 9, there were 20.8% damaged bolls in the PHY 410 R plot and only 3.3 – 3.8% damaged bolls in the PHY 470 WR sprayed and unsprayed plots.

Overall, WideStrike provided good control of natural infestations of cotton bollworm in the Prattville, AL plots in both 2003 and 2004. Plant damage evaluations and ultimately yields showed that WideStrike when subjected to high levels of cotton bollworms sustained only low levels of damage to the plants fruiting structures as compared to the unsprayed non-Bt varieties. The findings reported in this paper are supportive of previous work with WideStrike cotton (Huckaba et. al 2003, Langston et al. 2004).

The data suggests that although the varieties expressing the WideStrike trait have very good efficacy against cotton bollworm, they may benefit from supplemental insecticide sprays when heavy, sustained bollworm flights occur.

Therefore, cotton varieties expressing WideStrike Insect Protection, as well as other Bt proteins, should be scouted regularly and supplemental insecticide sprays should be made when local spray thresholds are met.

References

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Table 1. Larval infestations of southern armyworm in various candidate cotton lines 100 DAP, 2001[1]

	Sprayed	Unsprayed
WideStrike	1.5	20.5
MXB 9 (CRY1F)	1.8	24.8
MXB 7 (CRY1AC)	2.3	52.8
PSC 355 (Non-Bt)	0.3	60.3

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

Table 2. Percent defoliation caused by southern armyworm in various candidate cotton lines 100 and 115 DAP, 2001[1]

	100 DAP	115 DAP
WideStrike	0.8	1.3
MXB 9 (CRY1F)	1.0	1.3
MXB 7 (CRY1AC)	11.3	27.5
PSC 355 (Non-Bt)	16.3	36.3

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

Table 3. Insecticide applications applied to the plot sprayed for all insect pests of cotton and to the plot sprayed for non-lep pests only in 2003 strip trial..

Area Treated for Leps	Area Untreated for Leps
6/2 Bidrin 0.25	6/2 Bidrin 0.25
7/10 Centric 2 oz/A	7/10 Centric 2 oz/A
7/21 Bidrin 0.33, Tracer .063, Karate .028	7/21 Bidrin 0.33
7/30 Bidrin 0.33,Tracer .063, Karate .028	7/30 Bidrin 0.33
8/12 Trimax 1 oz, Bidrin 0.4, Karate 0.028, Tracer 0.063	8/12 Trimax 1 oz, Bidrin 0.4
8/29 Bidrin 0.4	8/29 Bidrin 0.4

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Table 4. Percent total live bollworm larvae & damage on terminals not sprayed for leps in 2003 strip trial.

Variety and Observation Date		% Live larvae	% Damage
PSC 355	7-28	25	85
PHY 440 W	7-28	13	20
PSC 355	8-4	23	70
PHY 440 W	8-4	0	20
PSC 355	8-11	7	53
PHY 440W	8-11	0	15

Table 5. Percent total live bollworm larvae & damage on squares not sprayed for leps in 2003 strip trial.

Variety and Observation Date		Live larvae	Damage
PSC 355	7-28	53	85
PHY440W	7-28	13	20
PSC 355	8-4	4	80
PHY440W	8-4	12	35
PSC 355	8-11	4	80
PHY440W	8-11	0	10

Table 6. Percent total live bollworm larvae & damage on bolls not sprayed for leps in 2003 strip trial.

Variety	Live larvae	Damage
PSC 355	28	80
PHY440W	0	0
PSC 355	37	95
PHY440W	0	0
PSC 355	3	52
PHY440W	0	0

Table 7. Seasonal average percent damaged fruiting structures in lep sprayed and lep unsprayed plots in 2003 strip trial.

Variety and treatment	Terminals	Squares	Flowers	Bolls
PSC 355 - Sprayed	8	11	1.2	3
PSC 355 - Unsprayed	47	48	29.5	51
PHY440 W - Sprayed	3.3	4	1.7	0
PHY 440 W - Unsprayed	12.5	12.6	1.2	3.3

Table 8. Pounds of lint yield per acre in 2003 strip trial in Prattville, AL.

PHY 440 W Sprayed	PHY 440 W Unsprayed	PSC 355 Sprayed	PSC 355 Unsprayed
1530	1480	1318	483

Table 9. Insecticide applications applied to the plot sprayed for all insect pests of cotton and to the plot sprayed for non-lep pests only in 2004 strip trial.

Spray Date	Chemical (lbs ai/ac)	Overspray	Lep-Spray Area
05/26/2004	Bidrin 0.20	X	
06/04/2004	Orthene 0.25	X	
06/30/2004	Bidrin 0.30	X	
07/06/2004	Bidrin 0.03	X	
07/14/04	Bidrin 0.03	X	
07/26/04	Karate 0.033		X

Table 10. Percent damage on terminals not sprayed for leps in 2004 strip trial.

Variety	Observation Date	Terminals	Squares
PHY 410 R	7-13-04	25	0
PHY 470 WR	7-13-04	0	0
PHY 410 R	7-19-04	50	30
PHY 470 WR	7-19-04	0	0

Table 11. Percent live larvae on terminals not sprayed for leps in 2004 strip trial.

Variety	Observation Date	Terminals	Squares
PHY 410 R	7-13-04	5	15
PHY 470 WR	7-13-04	0	0
PHY 410 R	7-19-04	10	35
PHY 470 WR	7-19-04	0	0

Table 12. Percent live larvae on plants not sprayed for leps on July 26 in 2004 strip trial.

Variety	Terminals		Squares		Flowers		Bolls	
	< ¼”	> ¼”	< ¼”	> ¼”	< ¼”	> ¼”	< ¼”	> ¼”
PHY 410 R	36	39	15	6	0	30	0	7
PHY 470 WR	10	2	3	0	0	0	0	0

Table 13. Percent damage on terminals not sprayed for leps in 2004 strip trial.

Variety	Treatment	Squares	Bolls
PHY 410 R	Unsprayed for leps	28	15
PHY 470 WR	Unsprayed for leps	7	2
PHY 470 WR	Sprayed once for leps	7	2.5