

**PERFORMANCE OF PHYTOGEN™ COTTON VARIETIES EXPRESSING THE WIDESTRIKE™
INSECT PROTECTION TRAIT WHEN GROWN IN COMMERCIAL TYPE STRIP TRIALS**

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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 (Acala) was used in the transformations which allowed for additional selections for improved fiber characteristics. Larger strip plot trials were conducted from North Carolina to Louisiana in 2003 and 2004 to further characterize the efficacy and agronomic performance of the new WideStrike varieties PHY 440 W and PHY 470 WR. The trials demonstrated that varieties expressing the WideStrike trait provide high levels of control of tobacco budworm (*Heliothis virescens*[F.]), soybean looper (*Pseudoplusia includens*), cabbage looper (*Trichoplusia ni*), and fall armyworm (*Spodoptera frugiperda*) as well as good to excellent control of bollworm (*Helicoverpa zea* [Boddie]). The PhytoGen cottonseed varieties PHY 440 W and PHY 470 WR showed high yield potential and very good fiber quality in larger blocks in multiple environments.

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 lepidopteran 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). The use of an Acala variety in the transformation process allowed for the selection of improved fiber characteristics.

Small plot trials conducted in 2002 and 2003 demonstrated that WideStrike provides season long, broad spectrum control of the major Lepidoptera (lep) pests of cotton. Larger strip plot trials were conducted from North Carolina to Louisiana in 2003 and 2004 to further characterize the efficacy and agronomic performance of the WideStrike varieties PHY 440 W and PHY 470 WR. WideStrike received deregulated status for cotton from the U.S. Department of Agriculture (USDA), full registration from the U.S. Environmental Protection Agency (EPA) and completed Pre-market Biotechnology Notice consultations with the U.S. Food and Drug Administration during 2004. PhytoGen cottonseed varieties expressing the WideStrike trait will be introduced commercially in 2005.

Materials and Methods

Large, commercial-like strip trials (normally 2 to 5 acres) were conducted at 8 locations in 2003 to evaluate efficacy and agronomic performance of PHY 440 W and PSC 355 when lepidopteran pests were controlled versus when they were not controlled. The WideStrike trials were normally planted with PHY 440 W in a block 24 to 48 rows wide and 300 to 500 feet long. A similar number of rows of the conventional variety PSC 355 were planted on each side of PHY 440W. A 40 – 50 foot buffer of PSC 355 was planted on both ends of the plots. One exception was the WideStrike trial at Prattville, AL in which PHY 440 W was planted 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.

In all trials, one-half of the plot which contained both varieties was treated as needed for control of lepidopteran 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 lepidopteran pests. The entire test area was treated for non-lepidopteran pests as needed.

In 2004, the same plot design was used to initiate 19 trials, but the WideStrike variety tested was PHY 470 WR and the non-Bt was PHY 410 R. Both of these varieties are most similar to PSC 355. However, rather than one half of the plot being treated as needed for control of lepidopteran pests every time the spray threshold was achieved on the conventional non-Bt variety, the section was only treated when the spray threshold was achieved for the WideStrike variety PHY 470 WR, or when the cooperators saw that the local threshold for Bt cotton was not going to be reached and decided to make an application to evaluate the benefit of a lepidopteran insecticide application at sub-threshold levels.

Data collected in the trials included larval infestations, damage of terminals, squares, flowers and bolls, lint yields (hand or machine picked) and quality (HVI analysis). For consistency, hand picked yields were determined by picking all lint from four 13.8 to 14.5 foot row sections in each treatment. In most situations, lint analyses were conducted from samples taken from the picker or the bags of seed cotton picked by hand. In 2003, lint samples from sprayed and unsprayed plots were analyzed. In 2004, however, lint samples were only submitted for analyses from the treated PHY 410 R and PHY 470 WR plots in trials with moderate to high lepidopteran pressure or from untreated PHY 410 R and PHY 470 WR in trials where no lepidopteran sprays were made due to very low lepidopteran pressure.

Results and Discussion

2003 WideStrike Strip Trials

Bollworms were the primary lepidopteran pest in most plots in 2003. Results of the Prattville, AL and Wayside, MS trials represented the good efficacy of WideStrike against bollworms (Tables 1 and 2). A heavy, sustained bollworm flight occurred in the Prattville trial starting in mid-July. Eggs and small newly hatched larvae were discovered in the terminals of nearly 100% of the plants on July 17. The lepidopteran 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). When all observation dates were averaged together, terminal damage in the unsprayed PSC 355 was over 45% while the unsprayed PHY 440 W was about 12%. Square damage across all observation dates was near 50% in the unsprayed PSC 355, compared to about 13% in the unsprayed PHY 440 W. The unsprayed PSC 355 had about 30% of the blooms damaged by worms while the unsprayed PHY 440 W had about 2%. 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.

The unsprayed PHY 440 W yielded 1,480 lbs. of lint (machine harvested) compared to 483 lbs. in the unsprayed PSC 355. When lepidopteran controls were made, the PSC 355 yielded 1,318 lbs. of lint and the PHY 440 W yielded 1,530 lbs.

In the Wayside, MS trial, the bollworm pressure was moderate. The lepidopteran treated area of the trial was sprayed a total of three times with either Karate or a combination of Karate plus Tracer. On July 30, terminal damage on the unsprayed PSC 355 plot was 22% while it was less than 4% in both the unsprayed PHY 440 W plot as well as in the sprayed PSC 355 and PHY 440 W plots. Similar results were observed for the damage ratings of squares, flowers and bolls. Machine harvesting resulted in the following yields presented as pounds of lint: unsprayed PHY 440 W = 1,080; unsprayed PSC 355 = 898, sprayed PHY 440 W = 1,296 and sprayed PSC 355 = 1,156. As expected, the hand picked yields were higher, but the same trends were observed.

When 8 trials were subjected to analysis of variance with each trial representing a replicate, there were no significant differences in yields of the PHY 440 W plots sprayed or not sprayed for lepidopteran pests and the PSC 355 sprayed for lepidopteran pests (Table 3). However, yields of the unsprayed PSC 355 plots were significantly lower. The average micronaire of PHY 440 W was 0.5 units lower than PSC 355. The lint turnout was significantly higher for PHY 440 W than PSC 355.

2004 WideStrike Strip Trials

Low lepidopteran pressure occurred in 13 of 19 locations. In those trials where pressure was moderate to high, the WideStrike Insect Protection trait continued to perform very well. For example, high levels of control of cotton bollworms were achieved in the PHY 470 WR plot in the Bossier City, LA trial as compared to the unsprayed PHY 410 R plot (Table 4). Hand picked yields of these 2 plots were 229 and 983 pounds of lint, respectively. In the lepidopteran control plots, applications of Karate at .03 lbs ai/acre were made to the PHY 410 R plot on 7/6, 7/14, 7/26 and 8/04.

The PHY 470 WR plot was sprayed once with Karate at the same rate on 7/6. Lint yield of the sprayed PHY 410 R plot was 987 pounds while the yield of the non-sprayed PHY 470 WR plot was 983 pounds.

A high sustained tobacco budworm population began in early July at the Donalsonville, GA trial and continued into August. Moderate bollworm, fall armyworm populations developed in July and August and continued into early September. Soybean and cabbage looper populations developed in August. The spray threshold in the WideStrike plot was not reached, but one half of the plot was sprayed on August 13 to determine if a pyrethroid application would increase yields of the PHY 470 WR plot under these conditions. Seasonal averages of percent damage caused by these leps to the fruiting forms are shown in Tables 5 and 6. Ratings for defoliation caused by a mixed population of soybean and cabbage loopers were 45% in the unsprayed PHY 410 R plot and 0 % in the unsprayed PHY 470 WR plot. The WideStrike trait provided high levels of control of Heliothines, fall armyworms and loopers season long. The high yield potential of PHY 470 WR was clearly demonstrated as the lint yield of the lep unsprayed plot was estimated by hand picking to be 1,895 pounds. (Table 6).

Lint quality of PHY 410 R and PHY 470 WR was very good in the 2004 trials (Table 7). Data available from 11 trials at the time of this report showed that the average micronaire levels of PHY 470 WR and PHY 410 R were 4.4 and 4.6 units, respectively.

Overall, WideStrike provided good to high levels of control of natural infestations of cotton bollworms in trials conducted from Louisiana to North Carolina. 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 fruiting structures as compared to the unsprayed non-Bt varieties. In addition, very high control levels of fall armyworm and looper populations were achieved. The high yield potentials of PSC 355, PHY 440 W, PHY 410 R and PHY 440 W were demonstrated across a large geography. Lint of these varieties was shown to have very good quality under the growing conditions of these trials. It was noticeable both years that the WideStrike varieties offered lower micronaire levels as compared to the non-Bt varieties to which they were compared.

The data suggests that varieties expressing the WideStrike trait may sometimes benefit from supplemental insecticide sprays, especially when heavy, sustained bollworm flights occur. Therefore, cotton varieties expressing WideStrike, 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. Seasonal average of percent damaged fruiting structures and yields (machine picked) in 2003 strip trial conducted in Prattville, AL. S= sprayed for leps and NS = not sprayed for leps.

	Terminals	Squares	Flowers	Bolls	Lint yields
PSC 355 - S	8	11	1.2	3	1,318
PSC 355 - NS	47	48	29.5	51	483
PHY 440 W - S	3.3	4	1.7	0	1,530
PHY 440 W - NS	12.5	12.6	1.2	3.3	1,480

Table 2. Percent damaged fruiting structures on July 30, 2003 and yields (machine picked) in strip trial conducted in Wayside, MS. S= sprayed for leps and NS = not sprayed for leps.

	Terminals	Squares	Flowers	Bolls	Lint yields
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PSC 355 - S	4	16	4	1	1,156
PSC 355 - NS	22	27	14	19	898
PHY 440 W - S	1	3	3	2	1,296
PHY 440 W - NS	2	2	3	1	1,080

Table 3. Yields and lint quality of 8 strip trials conducted in 2003. S= sprayed for leps and NS = not sprayed for leps.

Variety	Yield	Lint %	Mic	Length	Strength
PSC 355 - S	1241 a	39.2 b	4.86 a	1.12 a	30.84 a
PSC 355 - NS	989 b	38.8 b	4.77 a	1.12 a	31.28 a
PHY 440 W - S	1358 a	40.6 a	4.28 b	1.13 a	30.08 b
PHY 440 W - NS	1270 a	40.5 a	4.27 b	1.13 a	30.14 b

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

Table 4. Seasonal averages of percent damage of fruiting forms and yields (hand picked) in the Bossier City, LA. trial conducted in 2004. S= sprayed for leps and NS = not sprayed for leps.

	Terminals	Flowers	Squares	Bolls	Lint Yield
PHY 410 R - S	6.3	1.0	2.6	3.3	987
PHY 410 R - NS	32.0	11.0	20.0	23.3	229
PHY 470 WR - S	1.8	1.0	0.7	2.3	889
PHY 470 WR - NS	8.0	2.3	3.6	3.8	983

Table 5. Seasonal averages of percent damaged fruiting forms caused by Heliothines and yields (hand picked) in the Donalsonville, GA trial conducted in 2004. S= sprayed for leps and NS = not sprayed for leps.

	Terminal	Square	Flower	Boll	Lint Yield
PHY 410 R - NS	38.7	40.1	40.1	32.9	487
470 WR - S	2.1	0.0	1.4	0.0	1,896
470 WR - NS	2.7	0.0	1.1	0.0	1,847

Table 6. Seasonal averages of percent damaged fruiting forms caused by fall armyworm in the Donalsonville, GA trial conducted in 2004. S= sprayed for leps and NS = not sprayed for leps.

	Terminal	Square	Flower	Boll
PHY 410 R - NS	0.9	6.1	1.4	6.3
PHY 470 WR S	0	0.6	0	0
PHY 470 WR - NS	0	0	0	0

Table 7. Lint quality of 11 strip trials conducted in 2004.

	% Lint	Mic	Length	Strength	Uniformity
PHY 470 WR	0.42	4.4	1.14	29.2	85.7
PHY 410 R	0.41	4.6	1.14	29.5	85.8