WIDESTIKETM INSECT PROTECTION AGAINST HELIOTHINE INSECTS R.M. Huckaba, L.B. Braxton, M.M Willrich, J.S. Richburg, R.B. Lassiter, V.B. Langston, R.A. Haygood, J.M. Richardson, F.J. Haile, J.W. Pellow, G.D. and Thompson J.P. Mueller Dow AgroSciences

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<u>Abstract</u>

Field studies were conducted at seventeen test sites across the cotton belt in 2004. Results showed that cotton expressing the WideStrikeTM trait (PHY 440 W and PHY 470 WR) provided very good control of both cotton bollworm (*Helicoverpa zea*) and tobacco budworm (*Heliothis virescens*). Management of high populations of cotton bollworm, particularly those sustained over an extended period of time, may require application of a foliar insecticide to prevent exceeding the treatment threshold and to achieve optimum yields in WideStrike cotton. No high populations of tobacco budworm occurred at any of the test sites in 2004, but previous studies indicate no additional insect control measures would be needed to manage this pest in WideStrike cotton.

Introduction

Insect-resistant transgenic cotton, *Gossypium hirsutum* L., varieties have been available to producers since 1996. These varieties were engineered to express the Cry1Ac protein from the bacterium, *Bacillus thuringiensis* Berliner (*Bt*). Single-gene *Bt* cottons have revolutionized cotton insect management by providing complete control of key lepidopteran pests, including tobacco budworm, *Heliothis virescens* (F.), and pink bollworm, *Pectinophora gosypiella* (Saunders). Control of bollworm, *Helicoverpa zea* (Boddie), has been less reliable and supplemental insecticides are commonly applied to prevent economic losses. Today, transgenic cotton varieties are available which express a second gene encoding another *Bt* protein. The rationales for deploying multiple insect resistant traits are to aid in resistance management and to broaden the spectrum of activity.

Dow AgroSciences LLC has genetically modified cotton to express two separate insecticidal *Bt* proteins: Cry1Ac and Cry1F. The simultaneous expression of these two proteins is characteristic of WideStrike. WideStrikeTM *Insect Protection* received deregulated status for cotton from the U.S. Department of Agriculture (USDA), completed Premarket Biotechnology Notice consultations with the U.S. Food and Drug Administration, and full registration from the U.S. Environmental Protection Agency (EPA) during 2004. The new cotton trait will be introduced into the market and available in PhytoGen varieties in 2005. WideStrike cotton varieties will provide improved control of bollworm and secondary Lepidopteran pests as compared to single-gene *Bt* varieties.

Cotton bollworm and tobacco budworm are primary pests of cotton in the United States (Williams 2002). Damaging population levels are frequently encountered by cotton producers across much of the cotton belt. Effective management of these pests is essential for the economically successful production of commercial cotton. This paper summarizes the performance of WideStrike cotton against heliothine pests in field trials conducted in 2004.

Materials and Methods

Field trials were conducted across the U.S. during 2004 to characterize the efficacy of WideStrike cotton against cotton bollworm and tobacco budworm pests (Table 1). All trials compared a WideStrike variety (PHY440W or PHY 470 WR) to a non-*Bt* variety (PHY 410 R). A modified split plot design with 4 replications was employed in the field studies. Areas of "sprayed" and "unsprayed" were designated as the main plots and generally were not randomized, and varieties as the sub-plots were randomized within the main plots. Test sites were selected based on a likelihood of their developing a significant natural pest infestation.

Data were collected on plant damage (terminals, flowers, squares, and bolls) and larval numbers on the same plant structures. Because of the volume of data only information on squares and bolls are presented in this paper. Mean separation between treatments within a location was determined using the LSD at P=0.05. Data are presented for locations that received moderate to high infestation levels.

Results and Discussion

WideStrike expressed in PHY 470 WR gave excellent control of a moderate population of primarily cotton bollworm at Corpus Christi, TX (Table 2). PHY 470 WR and PHY 410 R unsprayed and sprayed either on a Bt threshold or on a non-Bt threshold were compared. PHY 470 WR sustained no more square or boll damage in the unsprayed compared to either of the sprayed regimes. PHY 410 R (non-Bt variety) sprayed three times received numerically greater square and boll damage than unsprayed PHY 470 WR. Yields were numerically greater for PHY 470 WR unsprayed than PHY 410 R sprayed three times. PHY 470 WR receiving three insecticide applications had only a 52 lb/A yield increase over the same variety unsprayed and the difference was not statistically significant.

WideStrike expressed in both PHY 440 W and PHY 470 WR gave good performance against a mixed population of cotton bollworm and tobacco budworm (60:40) at Chula, GA (Fig. 1 and 2). PHY 410 R unsprayed had peak damage levels for squares and bolls of 50% and 30%, respectively. Square damage levels in the unsprayed PHY 440 W and PHY 470 WR peaked on Aug. 16 at 1.3% and 6.5%. The sprayed PHY 410 R sustained about 16% square damage on Aug. 9. Boll damage levels were minimal never exceeding 1.5% in the unsprayed PHY 440 W and PHY 470 WR indicating very few larvae survived to infest the bolls. The sprayed PHY 410 R plots had 13.3% damaged bolls on Aug. 9.

A heavy population of 89% cotton bollworm and 19% tobacco budworm developed at the Blackville and Elko, SC test sites. Data for the date when the greatest insect damage was recorded is presented in Table 4. Percent damaged squares and bolls remained at or below 3% for both PHY 440 W and PHY 470 WR unspraved treatments at Blackville. The unsprayed PHY 410 R treatment sustained damage of 42% for squares and 34% for bolls. Boll damage levels reached 19% even in the sprayed PHY 410 R treatment that received four insecticide applications at Blackville. Achieving timely insecticide applications with the tractor equipment was not possible due to inclement weather preventing entry into the test area at Blackville. For the Elko site, peak damage levels reached 59% for squares and 40.6% for bolls in the unsprayed PHY 410 R. The unsprayed PHY 440 W and PHY 470 WR treatments under this intense pressure had peak square damage levels of 8.1% and 7.5%, respectively. Boll damage levels were 10.6% for PHY 440 W and 8.1% for PHY 470 WR. The sprayed regime at Elko received weekly applications by backpack sprayer for seven weeks. The lint yields for Blackville and Elko are presented in Table 5. The yield results at Blackville are statistically similar for both the sprayed and unsprayed PHY 440 W and PHY 470 WR treatments. The unsprayed PHY 410 R yields were reduced by about two-thirds compared to the WideStrike cottons by the uncontrolled insects. Overall at Blackville, four insecticide applications added one bale of lint/A to the non-Bt cotton while four insecticide applications increased WideStrike expressing cotton lint yields by only 100 lbs/A. Additionally, the WideStrike expressing cottons without insecticide applications increased lint yields by about two bales/A over the unsprayed non-Bt. At Elko, the unsprayed PHY 410 R yield was only 49 lb/A while the unsprayed PHY 440 W and PHY 470 W had 1122 lb/A and 1307 lb/A, respectively. The sprayed treatments had statistically greater yields versus the unsprayed at Elko demonstrating the need for a timely insecticide application to WideStrike cotton under such heavy and extended cotton bollworm pressure.

Heavy cotton bollworm pressure developed at the test in Jamesville, NC (Table 5). The unsprayed PHY 410 R sustained high fruit damage levels with percent damaged squares ranging from 33.8% to 65.8% and boll damage levels ranging from 27% to 56.3% across the sample period. Both PHY 440 W and PHY 470 WR had statistically less fruit damage than the non-Bt variety. The peak damage levels for squares (10% and 11.3%) and bolls (5.8% and 7.5%) for the two WideStrike expressing cottons suggest that a timely insecticide application may be needed under such heavy cotton bollworm pressure.

The Rocky Mount, NC site developed high levels of primarily cotton bollworm (Table 6). Percent damaged bolls peaked at 54% in the PHY 410 R. At this location, the sprayed PHY 470 WR was to receive applications only when the spray threshold was triggered. The threshold was never reached, so neither PHY 470 WR treatment was sprayed for heliothines. Boll damage ranged from 2% to 6% for PHY 470 WR which was statistically less than unsprayed PHY 410 R and statistically similar to the sprayed PHY 410 R. Lint yields were numerically superior by 621 lb/A in the unsprayed PHY 470 WR to the sprayed PHY 410 R, although the difference was not statistically significant.

The test conducted at Suffolk. VA had three spray regimes: 1) unsprayed 2) sprayed once on initial egg threshold 3) sprayed on egg threshold and then again 5 to 7 days later. Percent damaged bolls peaked at 54% on Aug. 20 in

the PHY 410 R unsprayed (Table 7). PHY 410 R sprayed on either of the spray regimes resulted in boll damage being limited to 10% or less. PHY 470 WR sustained no more than 1% boll damage under any of the spray regimes including unsprayed. Lint yield was greatly reduced in the unsprayed PHY 410 R versus the other treatments. Although only very low levels of insect damage, there was about a 100 lb/A yield increase for PHY 470 WR when sprayed twice versus unsprayed. The reason for this was not noted, but sucking insects including stinkbugs and lygus bugs may have been controlled by the two insecticide applications.

In summary, the results generated from the tests in 2004 demonstrate that cottons expressing the WideStrike trait are effective tools in managing cotton bollworm and tobacco budworm. The findings reported in this paper are supportive of previous work with WideStrike cotton (Huckaba et. al 2003, Langston et al. 2004).

References

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| Trial Location | Researcher | Pest | Pest Levels | |
|--------------------|----------------------------|------------------------------------|-------------|--|
| Holland, VA | Herbert, VPI | Heliothine (1° cotton bollworm) | High | |
| Rocky Mount, NC | Bradley, NCSU | Heliothine (1° cotton bollworm) | High | |
| Jamesville, NC | Bradley/Van Duyn, NCSU | Heliothine (1° cotton bollworm) | High | |
| Blackville, SC | Sullivan, Clemson | Heliothine (81% cotton bollworm) | High | |
| Elko, SC | McCarty, Carolina Ag. Res. | Heliothine (81% cotton bollworm) | High | |
| Chula, GA | Moore, SE AG Res. | Heliothine (60% cotton bollworm) | High | |
| Midville, GA | All, UGA | Heliothine (1° cotton bollworm) | Moderate | |
| Headland, AL | Smith, Auburn | Heliothine | Low | |
| Greenville, MS | Horn, Stoneville R&D | Heliothine | Low | |
| Greenville, MS | Willrich, Dow AgroSciences | Heliothine | Low | |
| Starkville, MS | Jenkins, USDA ARS | Heliothine | Low | |
| Winnsboro, LA | Leonard, LSU | Heliothine | Low | |
| Newport, AR | Heap, Schoffner Res. Farm | Heliothine (1° cotton bollworm | Low | |
| Portageville, MO | Boyd, U of MO | Heliothine (1° cotton bollworm | Low | |
| St. Paul, TX | Hopkins, Hopkins Ag. Ser. | Heliothine (1° cotton bollworm | Low | |
| Richmond, TX | Visoski, Visoski Res. Ser. | Heliothine (1° cotton bollworm | Moderate | |
| Corpus Christi, TX | Parker, TA&M | Heliothine (1° cotton bollworm | Moderate | |

Table 1. Trial location, researcher, pest, and pest levels in trials for WideStrike in US, 2004.

Table 2. Comparison of transgenic cottons for heliothine control under different treatment thresholds, Corpus Christi, TX, 2004.¹

| Variety ² | Percent damaged ³ | Percent damaged bolls ³ | Lint yield |
|----------------------|------------------------------|------------------------------------|------------|
| | squares | | (lb/A) |
| PHY 410 R (0) | 16.0a | 5.9a | 553bc |
| PHY 470 WR (0) | 1.3bc | 0.0b | 756a |
| PHY 410 R (1) | 20.0a | 6.7a | 417c |
| PHY 470 WR (1) | 1.3bc | 0.4b | 768a |
| PHY 410 R (3) | 6.3b | 1.3b | 672ab |
| PHY 470 WR (3) | 0.3b | 0.0b | 820a |

⁷Means within a column followed by the same letter do not significantly differ (P=0.05, LSD). ² (0) no insecticide; (1) 7/26 application based on Bt threshold; (3) 7/2, 7/15, 7/26 applications based on non-Bt threshold.

³Seasonal average.

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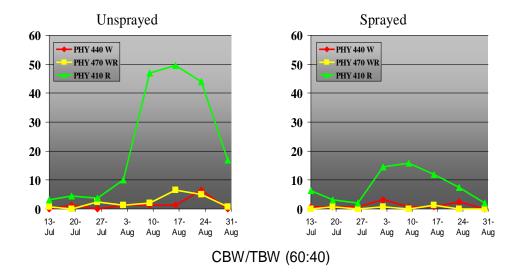
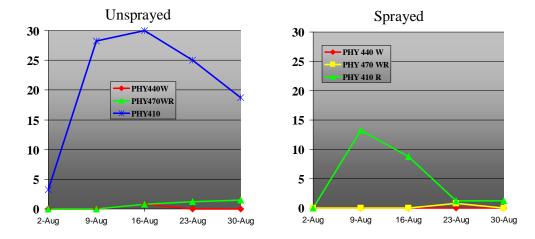


Fig. 1. Comparison of transgenic cottons for heliothine control, percent damaged squares, Chula, GA, 2004.



Fiq. 2. Comparison of transgenic cottons for heliothine control, percent damaged bolls, Chula, GA, 2004.

Table 3. Comparisons of transgenic cottons for heliothine control, Blackville, SC and Elko, SC, 2004.^{1,2}

| Treatment Regime | Variety | Percent damag | ed squares | Percent damaged bolls | | |
|------------------|------------|----------------|------------|-----------------------|----------|--|
| | | Blackville, SC | Elko, SC | Blackville, SC | Elko, SC | |
| Unsprayed | PHY 440 W | 3.0c | 8.1b | 2.0c | 10.6b | |
| | PHY 470 WR | 2.0c | 7.5bc | 0.0c | 8.1bc | |
| | PHY 410 R | 42.0a | 59.0a | 34.0a | 40.6a | |
| Sprayed | PHY 440 W | 1.0c | 0.0c | 3.0c | 0.0c | |
| | PHY 470 WR | 1.0c | 0.0c | 0.0c | 0.6bc | |
| | PHY 410 R | 25.0b | 0.0c | 19.0b | 0.6bc | |

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

²Heliothine population composed of 81% cotton bollworm and 19% tobacco budworm during study period at both locations.

³ For the sprayed regime, the Blackville, SC location received 4 spray applications and Elko, SC was sprayed weekly for a total of 7 applications.

| Treatment Regime | Variety | Lint Yield (lbs/A) | |
|------------------|------------|--------------------|----------|
| | | Blackville, SC | Elko, SC |
| Unsprayed | PHY 470 WR | 1566a | 1122c |
| | PHY 440 W | 1451a | 1307b |
| | PHY 410 R | 570c | 49d |
| Sprayed | PHY 470 WR | 1695a | 1523a |
| | PHY 440 W | 1541a | 1587a |
| | PHY 410 R | 1059b | 1571a |

Table 4. Comparisons of transgenic cottons for heliothine control, Blackville, SC and Elko, SC, 2004. ^{1,2,3}

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

²Heliothine population composed of 81% cotton bollworm and 19% tobacco budworm during study period at both locations.

³ For the sprayed regime, the Blackville, SC location received 4 spray applications and Elko, SC was sprayed weekly for a total of 7 applications.

Table 5. Comparisons of transgenic cottons for heliothine control, Jamesville, NC, 2004.^{1,2}

| Treatment Regime | Variety | Percent damaged squares | | | Percent damaged bolls | | |
|---------------------|------------|-------------------------|---------|---------|-----------------------|---------|---------|
| | | 04Aug04 | 10Aug04 | 17Aug04 | 04Aug04 | 10Aug04 | 17Aug04 |
| Unsprayed | PHY 440 W | 3.3b | 10.0b | 11.3b | 3.8b | 2.5b | 5.8b |
| | PHY 470 WR | 3.8b | 7.0b | 7.0b | 3.8b | 5.0b | 7.5b |
| | PHY 410 R | 33.8a | 47.5a | 65.8a | 27.0a | 35.0a | 56.3a |
| Sprayed | PHY 440 W | 0.0b | 0.0c | 0.0c | 0.0b | 0.0b | 0.0b |
| | PHY 470 WR | 0.0b | 0.0c | 0.0c | 0.0b | 0.0b | 0.0b |
| | PHY 410 R | 0.0b | 0.0c | 0.0c | 0.0b | 0.0b | 0.0b |

¹Means within a column followed by the same letter do not significantly differ (P=0.05, LSD). ²Heliothine population composed primarily of cotton bollworm.

Table 6. Comparisons of transgenic cottons for heliothine control, Rocky Mount, NC, 2004.^{1,2}

| Treatment Regime | Variety | Percent damaged bolls | | | Lint yield (lb/A) |
|---------------------|-------------------------|-----------------------|---------|---------|-------------------|
| | | 04Aug04 | 19Aug04 | 26Aug04 | |
| Unsprayed | PHY 470 WR | 2.0b | 6.0b | 5.0b | 2671ab |
| | PHY 410 R | 42.0a | 54.0a | 35.0a | 1405d |
| Sprayed | PHY 470 WR ³ | 0.0b | 6.0b | 5.0b | 2448abc |
| | PHY 410 R | 2.0b | 6.0b | 7.0b | 2020a |

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

²Heliothine population composed primarily of cotton bollworm.

³PHY 470 WR did not reach treatment threshold and was not sprayed.

| Treatment Regime ³ | Variety | Percent damaged bolls | | Lint yield (lb/A) |
|----------------------------------|------------|-----------------------|---------|-------------------|
| | | 20Aug04 | 26Aug04 | |
| Unsprayed | PHY 410 R | 54.0a | 30.0a | 486c |
| | PHY 470 WR | 1.0b | 1.0c | 1360b |
| Sprayed once | PHY 410 R | 2.0b | 8.0b | 1210b |
| | PHY 470 WR | 1.0b | 0.0b | 1401ab |
| Sprayed twice | PHY 410 R | 3.0b | 10.0c | 1243ab |
| | PHY 470 WR | 0.0b | 1.0c | 1457a |

Table 7. Comparisons of transgenic cottons for heliothine control, Suffolk, VA, 2004. ^{1,2}

¹Means within a column followed by the same letter do not significantly differ (P=0.05, LSD). ²Heliothine population composed primarily of cotton bollworm.

³The sprayed once treatment received an application at the egg treatment threshold and the sprayed twice treatments received applications at the egg treatment threshold and again 5-7 days later.