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FIELD AND LABORATORY PERFORMANCE OF WIDESTRIKE™ INSECT PROTECTION AGAINST SECONDARY LEPIDOPTERAN PESTS

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Abstract

Field and laboratory studies conducted during 2004 have indicated WideStrike cotton to be effective against important secondary lepidopteran pests of cotton. WideStrike varieties, including PHY440W and PHY470WR, were highly effective against fall armyworm, *Spodoptera frugiperda;* soybean looper, *Pseudoplusia includens;* cabbage looper, *Trichoplusia ni*; European corn borer, *Ostrinia nubilalis;* citrus peelminer, *Marmara gulosa;* cotton leaf perforator, *Bucculatrix thurberiella*; and omnivorous leafroller, *Playnota stultana*. Efficacy of WideStrike was demonstrated against low-level field infestations of black cutworm, *Agrotis ipsilon.* Results from artificially infested efficacy trials against beet armyworm, *Spodoptera exigua,* suggest larval survival and foliage feeding may be greater than other species evaluated. The deregulation and registration of WideStrike will enable additional testing under more realistic field conditions.

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 referred to as WideStrike. WideStrike™ *Insect Protection* received deregulated status for cotton from the U.S. Department of Agriculture (USDA), completed Pre-market 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.

Non-heliothine lepidopteran larvae have the potential to cause significant damage in cotton by feeding on foliage and fruiting structures. Important secondary lepidopteran larvae include the following species: soybean looper, *Pseudoplusia includens* (Walker); cabbage looper, *Trichoplusia ni* (Hübner); fall armyworm, *Spodoptera frugiperda* (J. E. Smith); beet armyworm, *Spodoptera exigua* (Hübner); southern armyworm, *Spodoptera eridania* (Cramer); European corn borer, *Ostrinia nubilalis* Hübner; salt marsh caterpillar, *Estigmene acrea* (Drury); and black cutworm, *Agrotis ipsilon* (Hufnagel). In 2003, fall armyworm ranked eighth among all cotton pests and first among secondary lepidopteran in total losses (Williams 2004a). An average of less than one foliar insecticide was applied per acre against the secondary lepidopteran complex across the United States (Williams 2004b). The following paper summarizes results from field and laboratory trials conducted during 2004, or for trials previously not reported, on the performance of WideStrike cotton against secondary lepidopteran pests.

Materials and Methods

Field and laboratory trials have been conducted across the U.S. to characterize the efficacy of WideStrike cotton against lepidopteran pests. All trials compared a WideStrike variety (PHY440W or PHY470WR) to a non-*Bt* variety (PSC355 or PHY410R). Cotton varieties were arranged in either a randomized complete block design or as sub-plots within each of a “Lepidoptera Sprayed” and “Non-Sprayed” treatment regime (modified split plot design). Field trials were established in geographical regions that historically receive lepidopteran infestations. However, artificial infestations were employed if natural infestations were negligible. Number of larvae were obtained using shake sheet samples or by visual examination of artificial-infested plants. Visual damage ratings were also obtained for quantifying beet armyworm (Table 1) and cotton leafperforator foliage feeding. In laboratory trials, neonates or first instar larvae were infested on field grown cotton leaves. Individual leaflets were placed in Petri dishes containing an agar medium to prevent tissue desiccation. Variables measured included larval mortality, larval development, and tissue damage.

Results and Discussion

Survival of fall armyworm on WideStrike cotton in laboratory infestations was significantly lower compared to that on conventional cotton at 3, 4, 5 and 7 days after infestation in three trials (Table 2). Survival on these rating dates ranged from 0.0 to 12.5%. In replicated field trials, number of fall armyworm and damage was significantly lower in WideStrike plots compared to PHY410R plots (Table 3, 4). Fewer larvae were also observed in WideStrike-Sprayed plots compared to PHY410R-Sprayed plots. Langston et al. (2003) and Haile et al (2004) reported similar results for fall armyworm.

Fewer beet armyworm larvae were observed on WideStrike cotton compared to conventional cotton at 11 to 15 days after artificial field infestation (Table 5). Significant reductions were observed in WideStrike plots at the New Mexico location for late-instar (third-fifth) and total number of beet armyworm larvae as compared to PSC355. However, number of larvae across all stages was not significantly different between WideStrike and PSC355 treatments in Mississippi and California. WideStrike cotton exhibited foliar damage at levels significantly less than damage observed on PSC355 in New Mexico and Mississippi. In the California trial, however, foliar damage by beet armyworm was not significantly different between WideStrike and PSC355 cotton.

Results from previous artificial field infestations have also indicated reductions in number of beet armyworm larvae in WideStrike cotton compared to a non-*Bt* control (Adamczyk et al. 2003). However, data from the present field trials suggest beet armyworm may be capable of surviving on WideStrike cotton. Development of larvae could be expected in the field under specific circumstances. This may include periods of extended egg lay or during an outbreak, which are typically the consequence of intense broad-spectrum insecticide use and subsequent elimination of natural enemies.

WideStrike cotton demonstrated excellent activity against soybean looper and cabbage looper. Number of soybean looper in WideStrike plots was 9.8 to 20.6-fold less compared to PSC355 in two small-plot studies (Table 6). Additionally, WideStrike was efficacious against a mixed infestation of cabbage looper and soybean looper (Table 7). Previous trials have also indicated satisfactory control of Plusiinae larvae with WideStrike cotton (Langston et al. 2003, Haile et al. 2004).

Survival of European corn borer on WideStrike cotton was significantly less than on PSC355 in a laboratory infestation (Table 8). European corn borer survival ranged from 6.0-8.0% on WideStrike cotton. Other lepidopteran pests infesting cotton in the southwestern U.S. include the citrus peelminer, *Marmara gulosa* Guillen and Davis, and cotton leafperforator, *Bucculatrix thurberiella* Busck. Field data suggest WideStrike cotton to be effective against both species (Figure 1, Table 9). Efficacy of WideStrike was also demonstrated against omnivorous leaf roller, *Playnota stultana* (Table 10). WideStrike (no lepidopteran insecticides applied) provided control of omnivorous leaf roller equivalent to PHY410 that was over-sprayed with three applications of Tracer.

Cotton plant stands were significantly improved for WideStrike varieties, planted alone or in combination with a conventional insecticide treatment program, compared to non-*Bt* varieties treated for control of black cutworm in artificial infestations (Table 11). In contrast, stand reduction in a natural black cutworm infestation was not significantly different between WideStrike (non-treated) or conventional cotton (non-treated) varieties (Table 12). These data suggest control of low-level black cutworm infestations may be expected with WideStrike cotton varieties. Furthermore, this control is likely to be as good or better if compared to a conventional insecticide spray regime. However, planting of WideStrike cotton into existing infestations of late-instar black cutworm may result in unacceptable stand protection if supplemental chemical treatments are not used.

References

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Table 1. Beet armyworm damage rating scale.

Rating	Description
0	Feeding damage absent.
1	Window paned area absent. Pinholes evident, but not more than pinhead size; scattered near the location of egg mass.
2	2% to <5% of the interveinal leaf consumed or damaged. Window paned areas present, but not covering more than one contiguous area approximately the size of a dime or less.
3	5 to <10% of the interveinal leaf consumed or damaged; one or more contiguous window paned areas.
4	10 to < 20% of the interveinal leaf area consumed or damaged; two or more contiguous window paned areas.
5	20 to <50% of the interveinal leaf area consumed or damaged.
6	50 to <80% of the interveinal leaf area consumed or damaged.
7	80 to 100% of the interveinal leaf area consumed or damaged.

Table 2. Survival of fall armyworm (first instar) on fully expanded leaves, 2004.

	Location / % Survival			
Variety	Dow AgroSciences, Wayside, MS[1]		USDA-ARS, J. Jenkins, Starkville, MS[2]	USDA-ARS, J. Adamczyk, Stoneville, MS[1]
	3 DAI[3]	7 DAI	5 DAI	4 DAI
PHY440W	12.5b	3.0b	0.0b	4.0b
PSC355	85.8a	81.7a	-----	96.0a
PHY410R	-----	-----	81.3a	-----

Columns means followed by the same letter are not significantly different (*P*<0.05; DMRT).
[1]Laboratory infestations using mainstem leaves located five nodes below the terminal.
[2]Laboratory infestations using mainstem leaves located four nodes below the terminal.
[3]Days after infestation.

Table 3. Comparison of WideStrike cotton varieties for fall armyworm control under two lepidopteran treatment regimes, Clemson University, M. Sullivan, 2004.

Treatment Regime	Variety	Number of larvae/row-m
Non-Sprayed	PHY470WR	0.0b
	PHY440W	0.0b
	PHY410R	1.0a
Sprayed	PHY470WR	0.0b
	PHY440W	0.3b
	PHY410R	0.5ab

Column means followed by the same letter are not significantly different (*P*<0.05).

Table 4. Efficacy of WideStrike cotton against fall armyworm in a replicated, small-plot trial, University of Arkansas, Glenn Studebaker, 2004.

Variety	Mean Number Per 25 Bolls			
	27 Aug		3 Sept	
	Larvae	Damage	Larvae	Damage
PHY470WR	0.0b	0.0b	0.0b	0.0b
PHY410R	1.0a	1.0a	1.0a	1.0a

Column means followed by the same letter are not significantly different (*P*<0.05; DMRT).

Table 5. Number and larval size of beet armyworm, and damage rating of artificially infested leaves[1], 2004.

Location	Variety	DAI[2]	Mean Number Per Plant			Damage Rating
			L1-2	L3-5	Total Larvae	
New Mexico State University, Scott Bundy	PHY440W	15	0.8a	0.8b	1.6b	2.0b
	PSC355		1.1a	13.3a	14.4a	5.5a
Dow AgroSciences, Wayside, MS	PHY440W	14	0.2a	2.3a	2.5a	1.9b
	PSC355		0.0a	6.9a	6.9a	3.8a
Dow AgroSciences, Fresno, CA	PHY440W	11	35.4a	1.6a	37.0a	2.9a
	PSC355		41.9a	20.1a	62.0a	5.5a

Column means within each location followed by the same letter are not significantly different (*P*<0.05).
[1]3 leaves infested per plant; 1 egg mass per leaf (mainstem leaf located 2, 3, and 4 nodes below the terminal).
[2]Days after infestation.

Table 6. Number of soybean looper larvae in WideStrike and conventional cotton, 2004.

Variety	Location / Number of Larvae	
	Dow AgroSciences, Wayside, MS[1]	USDA-ARS, John Adamczyk, Stoneville, MS[2]
PHY440W	0.5a	1.6b
PSC355	-----	15.6a
PHY410R	10.3a	-----

Means with the same letter are not significantly different (P<0.05).
[1]NAWF 3; Number of larvae per 7.3 row-m; DMRT.
[2]NAWF 5; Number of larvae per 5.5 row-m; Fisher's Protected LSD.

Table 7. Number of soybean looper larvae in WideStrike and conventional cotton, Donalsonvile, GA, 2004.

		Mean Number of Loopers[1]/3.3 row-m			
Treatment Regime	Variety	4 Aug	11 Aug	19 Aug	27 Aug
Non-Sprayed	PHY470WR	0.0	0.0	0.0	0.0
	PHY410R	3.0	5.0	30.0	9.0
Sprayed	PHY470WR	0.0	0.0	0.0	0.0
	PHY410R	3.0	6.0	25.0	8.0

[1]Mixed infestation of cabbage looper and soybean looper.

Table 8. Survival of European corn borer (first instar) on fully expanded leaves[1] in laboratory infestations (n = 600 larvae), Dow AgroSciences, Wayside, MS, 2004.

Variety	% Survival	
	3 DAI[2]	5 DAI
PHY440W	8.0b	6.0b
PSC355	72.2a	70.5a

Column means follwed by the same letter are not significantly different (P<0.05).
[1]Mainstem leaves located six nodes below the terminal.
[2]Days after infestation.

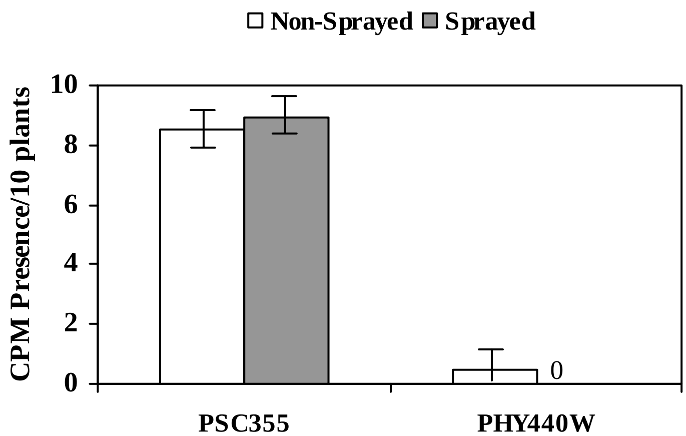

Figure 1. Average number of cotton mainstems infested with mature mines of the citrus peelminer (CPM) per 10 consecutive plants, University of Arizona, Peter Ellsworth, 2000.

Table 9. Efficacy of WideStrike cotton against cotton leafperforator, New Mexico State University, Scott Bundy, 2003.

Variety	Leaf Damage[1,2]	Total Larvae	Total Pupae
PHY440W	0.88	1	11
PSC355	1.71	18	71

[1]Average rating across four dates (1Oct-23Oct); 10 leaves (mainstem leaf located five nodes below the terminal) rated/plot/date.
[2]Rating scale: 0 (no damage); 1 (light damage, <20%); 2 (moderate damage, 20-50%); 3 (heavy damage, >50%).

Table 10. Efficacy of WideStrike cotton against omnivorous leaf roller, Texas Agricultural Experiment Station, Nueces, County, TX, Roy Parker, 2004.

Variety	Lepidopteran Treatments	% Damage[1]
PHY410R	0	20.0a
	1[2]	17.5a
	3[3]	3.8bc
PHY470WR	0	5.0bc
	1	10.0b
	3	0.0c

Means follwed by the same letter are not significantly different (*P*<0.05, LSD).
[1]25 terminals inspected.
[2]Tracer applied 26 Jul.
[3]Tracer applied 2, 15 and 26 Jul.

Table 11. Stand reduction by black cutworm in artificially infested field trials[1], Dow AgroSciences, 2004.

Variety	Percent Stand Reduction: 14DAI[2]			
	Brinson, GA		Fresno, CA	
	Non-Sprayed	Sprayed[3]	Non-Sprayed	Sprayed
PHY470WR	26.6a	10.9a	-----	-----
PHY410R	45.3b	45.3b	-----	-----
PHY440W	-----	-----	13.0a	5.0a
PSC355	-----	-----	48.0b	34.0b

Column means followed by the same letter are not significantly different (*P*<0.05; LSD).
[1]Barrier infestation at cotyledon stage: 24 plants/barrier; 24 larvae (3rd instar)/barrier.
[2]Days after infestation.
[3]Lorsban 4E (1 lb ai/A) at planting followed by Karate Z (1.28 oz/A) at 2 DAI.

Table 12. Stand reduction by black cutworm in a naturally infested field trial[1], Dow AgroSciences, Wayside, MS, 2004.

Variety	Percent Stand Reduction: 14DAI[2]
PHY440W	45.6a
PSC355	48.9a

Means followed by the same letter are not different (*P*<0.05; LSD).
[1]Burndown herbicide treatment applied two days before planting.
[2]Days after infestation.