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WIDESTIKE™ TRAIT EFFICACY UNDER VARIABLE INFESTATIONS OF HELICOVERPA ZEA IN GREENHOUSE AND FIELD TESTS

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Abstract

During 2003-2005 selected cotton varieties possessing the WideStrike™ trait (plant incorporated protectant expressing Cry 1F and Cry 1Ac insecticidal proteins) were evaluated for suppression of variable *Helicoverpa zea* (Boddie) infestations in greenhouse and field tests. Good suppression of *H. zea* damage to plant terminals and fruiting structures occurred under intense and moderate infestations with or without use of insecticide (Karate® @ 0.03 lbs ai/acre + Tracer® @ 0.062 lbs ai/acre) and was similar to cotton varieties expressing Bollgard I® and Bollgard II® traits. Insect control by WideStrike™ varieties tolerant to glyphosate were not affected by use of the herbicide on the cotton.

Introduction

WideStrike™ *Insect Protection* gene technology is a trait incorporated into certain cotton varieties produced by Phytogen Seed Company LLC where plants express insecticidal proteins, Cry 1F and Cry 1Ac, derived from *Bacillus thuringiensis* Berliner (*Bt*). WideStrike™ is registered as a plant incorporated protectant (PIP) and is known to express toxicity to various heliothine larvae. The greenhouse and field studies reported here were conducted during 2003-2005 as part of an insecticidal assessment of WideStrike™ varieties with and without RoundupReady® gene technology and in research comparing WideStrike™ to other *Bt* cotton varieties. Research emphasis was to evaluate WideStrike™ on the corn earworm, *H. zea*, in artificial greenhouse infestations and in field tests at a Georgia location with a high proportion of *H. zea* in heliothine infestations of cotton.

Methods

Two greenhouse tests were conducted in the Entomology greenhouses at the University of Georgia, Athens. Cotton was grown for eight weeks to R7-R10 stage of development in one-gallon pots filled with commercial potting media. When tests were initiated, the plants were arranged in stainless steel trays filled with 1 inch of water. A potted plant served as an entry (treatment) and each was replicated four times in a randomized complete block arrangement within the watering trays. The trays of water (moat system) handled irrigation and prevented insect migration between plants.

Certain treatments were sprayed weekly with insecticide for four weeks using a rotating, compressed air, boom sprayer with three TX-3 hollow cone nozzles (one central nozzle and two drop nozzles). The sprayer boom was housed in a Plexiglas-sided unit (4 ft^3) and rotated at 3 mph, applying a spray volume of 10 gal/acre to the potted cotton arranged on the floor of the unit. Certain plants were sprayed with Roundup WeatherMax® at a rate of 20 oz product/100 gal water 24 hours before the first insecticide applications were initiated.

Insect infestations were initiated 2 and 48 hours following insecticide applications on plants. Freshly hatched larvae (25 per plant) were placed on terminals, squares, flowers, and bolls using a fine brush. Terminals received 15 larvae and each fruiting structure 1 or 2 larvae at each infestation; at least 200 larvae were used per plant during a four-week period of testing. Plant terminals and fruiting structures were examined for damage and the presence and size of insects seven days following the last insect infestation.

Three field tests were conducted during 2003-2005 at the University of Georgia Southeastern Branch Research and Education Center (SEB) in Burke County, Georgia. Treatment plots were planted with a 4-row John Deere® vacuum planter in 40-foot long x 38-inch wide rows arranged in a randomized complete block experimental design

with 15-foot alleys separating blocks, replicated four times. Temik® 15G @ 3.5 lbs/acre was applied in the seed furrow of all cotton at planting for early season thrips control. Normal agronomic practices of fertilization, weed control, and irrigation used for cotton at the SEB were used in the tests. Each plot was separated from others by four buffer rows of a *Bt* cotton variety and the test fields were surrounded by at least 60 feet of *Bt* cotton. Treatments consisted of various varieties possessing either WideStrike™, Bollgard II®, or RoundupReady® traits or were non*Bt* cotton. The test fields were separated in half and all the treatments in one section were sprayed with insecticide when cotton sampling indicated insect infestations were reaching threshold levels either in the test field or in adjacent cotton fields at the SEB. The plots were sprayed with a high-cycle sprayer with a four-row boom utilizing three TX3 nozzles (one centered over the row and two drop nozzles angled to the side of rows) at a spray volume of 10 gal/acre. The two center rows of each plot were harvested with a mechanical spindle picker and weighed for assessment of yield.

Surveys of insect infestation and plant injury were done weekly or at other specified intervals during the season after cotton fruiting had begun. Plant terminals and two each of squares, flowers, and bolls on 20 plants selected at random in the center two rows of each plot were examined for injury and the presence of larvae. Hartstack-style traps were located near the test fields, one each baited with sex pheromone of *H. zea* and *Heliothis virescens* (Fabricius). Moth captures were monitored weekly during the season. Data analysis utilized SAS (Statistical Analysis System) for ANOVA at $P<0.05$ with mean separation using Tukey's Studentized Range Test for percent damage.

Results and Discussion

Tables 1, 2, and 3 present data from two sample dates for each year of the three years of tests. Weekly pheromone trapping data showed *H. zea:H. virescens* seasonal moth capture ratios of 95:5, 80:20, and 85:15 for 2003, 2004, and 2005, respectively, indicating that the cotton infestations were mostly *H. zea*. In all three years, the WideStrike™ cotton had significantly better control of insect infestations and a trend for higher yield than the non*Bt* cotton. Examination of trends in the data during the three years indicates that use of Karate® @ 0.03 lbs ai/acre + Tracer® @ 0.062 lbs ai/acre increased insect control and improved yield in both non*Bt* and *Bt* cotton, but separating the test fields into sprayed and unsprayed halves did not allow for statistical comparison of the different cotton varieties with and without insecticide treatment.

Table 1. *H. zea*[1] infestations of sprayed and unsprayed WideStrike™ cotton in Burke County GA at two selected sampling dates during 2003.

	% Damage[3]				
	August 7				
Treatment[2]	Terminals	Squares	Flowers	Bolls	
PS355	48.8 a	19.4 a	20.8 a	6.3 a	
P440W	0.0 b	0.0 b	0.8 b	0.0 a	
PS355S	6.3 a	2.5 a	3.5 a	1.9 a	
P440WS	0.0 b	0.0 a	0.0 a	0.0 a	
		August 22			Yield[4]
PS355	22.5 a	18.8 a	6.3 a	5.6 a	2328 a
P440W	0.0 b	3.8 b	1.3 a	1.3 b	2696 a
PS355S	0.0 a	0.0 a	0.6 a	0.0 a	2817 a
P440WS	0.0 a	0.0 a	0.0 a	0.6 a	2656 a

[1] Seasonal sex pheromone trapping had a ratio of 95:5 *H. zea*:*H. virescens*.
[2] PS355 = non-WideStrike™ variety; P440W = WideStrike™ variety; S = cotton was sprayed weekly with Karate® 2.09CS @ 0.03 lbs ai/acre + Tracer® 4SC @ 0.062 lbs ai/acre.
[3] Data analysis of sprayed and unsprayed blocks were done separately using SAS, ANOVA ($p = 0.05$, mean separation using Tukey's Studentized Range).
[4] Yield = lb seed cotton/acre.

Table 2. *H. zea*[1] infestations of sprayed and unsprayed WideStrike™ (RoundupReady® and non-RoundupReady®) cotton in Burke County GA at two selected sampling dates during 2004.

	% Damage[3]			
	July 20			
Treatment[2]	Terminals	Squares	Bolls	
P410R	15.0 a	11.9 a	4.4 a	
P470WR	5.0 b	1.9 b	1.3 b	
P440W	1.3 b	1.9 b	0.6 b	
P410RS	3.8 a	2.5 a	1.3 a	
P470WRS	0.0 a	0.0 a	0.0 a	
P440WS	0.0 a	0.0 a	0.0 a	
		July 27		Yield[4]
P410R	20.0 a	12.5 a	8.1 a	1959 a
P470WR	5.0 b	2.5 b	0.6 b	2441 a
P440W	5.0 b	5.6 b	0.6 b	2269 a
P410RS	1.3 a	2.5 a	0.6 a	2234 a
P470WRS	2.5 a	0.0 a	0.0 a	2407 a
P440WS	1.3 a	0.0 a	0.0 a	1815 a

[1] Seasonal sex pheromone trapping had a ratio of 80:20 *H. zea*:*H. virescens*.

[2] P410R = non-WideStrike™, RoundupReady® variety; P470WR = WideStrike™, RoundupReady® variety; P440W = WideStrike™, non-RoundupReady® variety; S = cotton was sprayed weekly with Karate® 2.09CS @ 0.03 lbs ai/acre + Tracer® 4SC @ 0.062 lbs ai/acre.

[3] Data analysis of sprayed and unsprayed blocks were done separately using SAS, ANOVA (p = 0.05, mean separation using Tukey's Studentized Range).

[4] Yield = lb seed cotton/acre.

Field tests in 2004 and 2005 showed that WideStrike™ cotton either with or without a RoundupReady® trait had no significant differences in insect control rate (Tables 2 and 3). In 2005, three WideStrike™ varieties were tested with a Bollgard II®/RoundupReady® variety (DP543B2/RR) and results showed that insect control and yield were statistically similar among the *Bt* varieties and all had significantly better insect control than the non*Bt* cotton (Table 3).

Table 3. *H. zea*[1] infestations of sprayed and unsprayed WideStrike™ and Bollgard II® cotton in Burke County GA at two selected sampling dates during 2005.

	% Damage[3]				
	August 2				
Treatment[2]	Terminals	Squares	Flowers	Bolls	
P410R	27.5 a	10.0 a	1.9 a	0.6 a	
P470WR	7.5 b	1.9 b	0.0 a	0.0 a	
P475WRF	2.5 b	1.3 b	0.0 a	0.0 a	
P440W	6.3 b	3.1 ab	0.0 a	0.0 a	
DP543B2/RR	2.5 b	0.6 b	0.0 a	0.0 a	
P410RS	16.3 a	7.5 a	1.9 a	0.0 a	
P470WRS	5.0 b	1.3 b	0.6 a	0.0 a	
P475WRFS	1.3 b	3.1 ab	0.0 a	0.0 a	
P440WS	5.0 b	2.5 ab	0.0 a	0.0 a	
DP543B2/RRS	1.3 b	1.9 b	0.0 a	0.0 a	
		August 9			Yield[4]
P410R	31.3 a	11.3 a	5.0 a	1.3 a	1998 a
P470WR	3.8 b	4.4 ab	0.6 a	0.0 a	1448 a
P475WRF	7.5 b	1.3 b	0.6 a	0.0 a	1931 a
P440W	5.0 b	3.8 b	0.6 a	0.0 a	1727 a
DP543B2/RR	2.5 b	0.0 b	0.6 a	0.0 a	2210 a
P410RS	17.5 a	5.0 a	2.5 a	0.0 a	2896 a
P470WRS	3.8 b	0.0 b	0.0 a	0.0 a	2708 a
P475WRFS	6.3 b	2.5 b	0.0 a	0.0 a	2528 a
P440WS	2.5 b	1.3 b	0.6 a	0.0 a	2699 a
DP543B2/RRS	0.0 b	0.0 b	0.0 a	0.0 a	3006 a

[1] Seasonal sex pheromone trapping had a ratio of 85:15 *H. zea*:*H. virescens*.

[2] P410R = non-WideStrike™, RoundupReady® variety; P470WRS = WideStrike™, RoundupReady® variety; P475WRF = WideStrike™, RoundupReady® variety; P440WS = WideStrike™, non-RoundupReady® variety; DP543B2/RR = Bollgard II®, RoundupReady® variety; S = cotton was sprayed weekly with Karate® 2.09CS @ 0.03 lbs ai/acre + Tracer® 4SC @ 0.09 lbs ai/acre.

[3] Data analysis of sprayed and unsprayed blocks were done separately using SAS, ANOVA (p = 0.05, mean separation using Tukey's Studentized Range).

[4] Yield = lb seed cotton/acre.

The greenhouse test was conducted to simulate severe *H. zea* pressure on the *Bt* cotton varieties using at least 200 freshly hatched larvae on infestations of each plant during four weeks. Table 4 shows that in the first test infestations developed on all unsprayed cotton, but damage was significantly reduced on the WideStrike™, Bollgard I®, and Bollgard II® varieties. Treatments sprayed with Karate® @ 0.03 lbs ai/acre + Tracer® @ 0.062 lbs ai/acre produced good control on all of the cotton. Use of a single Roundup® application on certain treatments did not significantly influence *H. zea* infestations on either insecticide sprayed or unsprayed cotton.

Table 4. Greenhouse evaluations of the influence of Roundup® treatment on *H. zea* infestations of sprayed and unsprayed WideStrike™, Bollgard®, and Bollgard II® cotton.

	% Damage[2]			
	Test 1		Test 2	
Treatment[1]	Terminals	Fruiting Structures	Terminals	Fruiting Structures
Roundup®				
No Insecticide				
P410R NBt	100.0 a	53.2 a	75.0 a	35.3 ab
SG521 NBt	100.0 a	36.7 bc	50.0 ab	46.9 a
P470WR	25.0 b	14.4 def	25.0 bc	6.3 d
P480WR	25.0 b	6.1 efg	0.0 c	0.0 d
SG215BGIR	100.0 a	27.1 cd	75.0 a	6.3 d
FM960B2/RR	25.0 b	0.0 g	0.0 c	0.0 d
+ Insecticide				
P410R NBt+I	0.0 b	0.0 g	0.0 c	0.0 d
SG521 NBt+I	0.0 b	0.0 g	0.0 c	0.0 d
P470WR+I	0.0 b	0.0 g	0.0 c	0.0 d
P480WR+I	25.0 b	0.0 g	0.0 c	0.0 d
SG215BGIR+I	25.0 b	3.1 g	25.0 bc	3.1 d
FM960B2/RR +I	0.0 b	0.0 g	0.0 c	0.0 d
No Roundup®				
No Insecticide				
P410R NBt	100.0 a	42.0 ab	75.3 a	22.5 bc
SG521 NBt	100.0 a	52.5 a	50.0 ab	42.6 a
P470WR	100.0 a	40.2 ab	0.0 c	12.5 cd
P480WR	75.0 a	18.8 de	0.0 c	0.0 d
SG215BGIR	75.0 a	0.0 g	50.0 ab	8.2 d
FM960B2/RR	25.0 b	8.1 efg	0.0 c	0.0 d
+ Insecticide				
P410R NBt+I	0.0 b	0.0 g	0.0 c	0.0 d
SG521 NBt+I	0.0 b	0.0 g	0.0 c	0.0 d
P470WR+I	0.0 b	0.0 g	0.0 c	0.0 d
P480WR+I	0.0 b	0.0 g	0.0 c	0.0 d
SG215BGIR+I	25.0 b	0.0 g	25.0 bc	0.0 d
FM960B2/RR +I	0.0 b	1.6 fg	0.0 c	0.0 d

[1] P410R = non-WideStrike™, RoundupReady® variety; SG521 = non-WideStrike™, RoundupReady® variety; P470WR = WideStrike™, RoundupReady® variety; P480WR = WideStrike™, RoundupReady® variety; SG215BGIR = Bollgard®, RoundupReady® variety; FM960B2/RR = Bollgard II®, RoundupReady® variety; +I = cotton was sprayed weekly for 4 weeks with Karate® 2.09CS @ 0.03 lbs ai/acre + Tracer® 4SC @ 0.062 lbs ai/acre.

[2] Data analysis using SAS, ANOVA (p = 0.05, mean separation using Tukey's Studentized Range).