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EFFICACY OF SINGLE AND DUAL GENE COTTON *GOSSYPIUM HIRSUTUM* (L.) EVENTS ON YELLOWSTRIPED ARMYWORM (LEPIDOPTERA: NOCTUIDAE) IN SOUTH TEXAS AND THE MISSISSIPPI DELTA[1]

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[1]Presented verbally at The Armyworm Symposium held in conjunction with the Entomological Society of America Southeastern Branch Meeting, March 6-10, 2010 in Atlanta.

Abstract

The yellowstriped armyworm (YSAW), *Spodoptera ornithogalli* (Guenée), has a broad host range and can be an economic threat to cotton in southern growing regions of the United States by consuming leaves and damaging fruiting forms. Field grown cotton varieties containing the endotoxins from *Bacillus thuringiensis,* (Cry1Ac = Bollgard®; Cry1Ac + Cry2Ab = Bollgard II®; Cry1F + Cry1Ac = Widestrike™) and a Non-Bt cotton were evaluated for YSAW susceptibility to cotton leaf tissue. Bioassays conducted at Stoneville, MS, from leaves collected from the lower, middle, and top canopy showed that YSAW were highly susceptible to Bollgard II® and WideStrike™ traits. No live larvae were collected from the WideStrike™ replicated plots and only 2 larvae that weighed 171.5 ± 53.5 mg were collected from Bollgard II® cotton. Larvae collected from Bollgard® and Non-Bt cotton were plentiful and averaged 886 ± 63.5 and 824.25 ± 51.53 mg, respectively. Additional bioassays from late-season cotton plots at both Stoneville and Weslaco, TX, indicated that WideStrike™ and Bollgard II® are very active against YSAW larvae. Results from these assays indicate that cotton producers have very effective options for controlling YSAW even late in the growing season.

Key Words: *Bacillus thuringiensis,* Cry1Ac, Cry2Ab, Cry1F + Cry1Ac, GMO, PIP, transgenic

Resumen

El gusano de rayas amarillas (GRA), *Spodoptera ornithogalli* (Guenée), tiene un amplio rango de hospedantes y puede ser una amenaza económica para las regiones productoras del algodón en el sur de los Estados Unidos por las hojas que consumen y el daño que hace a los capullos. Se evaluaron las variedades de algodón cultivadas en el campo que contienen las endotoxinas de *Bacillus thuringiensis* [=Bt], (Cry1Ac = Bollgard®; Cry1Ac + Cry2Ab = Bollgard II®; Cry1F + Cry1Ac = Widestrike™) y un algodón sin Bt para la susceptibilidad de GRA a los tejidos de las hojas de algodón. Los bioensayos realizados en Stoneville, MS, de las hojas recogidas de la parte baja, media y superior del dosel mostró que GRA era muy susceptible a Bollgard II® y caracteristicas de WideStrike™. No larvas vivas se obtuvieron en las parcelas replicadas de WideStrike™ y sólo dos larvas que pesaron 171,5 ± 53,5 mg se obtuvieron de algodón Bollgard II®. Las larvas recogidas del algodón Bollgard® y el algodón sin Bt eran abundantes con un promedio de 886 ± 63,5 y 824,25 ± 51,53 mg, respectivamente. Bioensayos adicionales realizados de las parcelas de algodón al final de la temporada, tanto en Stoneville y Weslaco, Texas, indicó que WideStrike™ y Bollgard II® son más activos contra las larvas GRA. Los resultados de estos ensayos indican que los productores de algodón tienen opciones muy efectivas para el control de GRA, incluso al final de la temporada de crecimiento.

The yellowstriped armyworm (YSAW), *Spodoptera ornithogalli* (Guenée), is one of the most innocuous lepidoterous pests of the 30 *Spodoptera* species that are known to exist. The distribution of YSAW ranges from the Canadian prairies to southern Texas and Florida, but is not found west of the Rocky Mountains (Pogue 2002). The host range for YSAW also is considered broad and versatile for an armyworm species, ranging from a number of vegetables crops, grasses, soybeans, alfalfa, and cotton; and consuming foliage as well as fruiting forms (King 1981; Capinera 2001). Larvae are considered one of the largest in *Spodoptera.* They develop at a high rate and are capable of consuming >80 cm^2 of leaf area of snapbean (*Phaseolus* sp.) within a 10 d feeding period (Parkman & Shepard 1981). First instars are generally considered tissue or leaf skeletonizers,

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while larger larvae consume all tissues. The high growth and consumption rates can make YSAW a serious economic pest when present on agricultural crops. Control options for YSAW are provided in most university extension publications as a pest of cotton where management options are generally given in the form of insecticide recommendations. The YSAW does not receive the attention that beet armyworm, *S. exigua* (Hübner), or fall armyworm, *S. frugiperda* (J. E. Smith), receive with regard to cotton because of sporadic, unpredictable, and often late season infestations. To our knowledge, transgenic cotton varieties containing different isolates of *Bacillus thuringiensis* traits have not been evaluated for control of YSAW in comparison with non-Bt varieties. We evaluated the efficacy of Bollgard® (Cry 1Ac); Bollgard II® (Cry1Ac + Cry2Ab); and Widestrike™ (Cry1Ac + Cry1F) cotton varieties in Stoneville, MS, and Weslaco, TX. Cotton varieties were grown in research plots in the field, and assays with fresh leaf tissue were used to determine the effects of the traits on neonate YSAW larvae.

Materials and Methods

Source of the yellowstriped armyworm (YSAW)

All YSAW used in the Stoneville, Mississippi bioassays were collected from cotton on the USDA-ARS research station in Stoneville, Mississippi, and those used in Weslaco, Texas bioassays were collected as larvae from cotton at the USDA-ARS south farm, Weslaco, Texas. Collections were reared to adults on meridic diet used for rearing lepidopteran species (Vanderzant 1962). The adults, larvae, and pupae were maintained in an environmental chamber (Percival Scientific, Perry, IA) at 29.4°C (85.0°F) and 13:11 (L:D) photoperiod. When larvae pupated, they were placed in petri dishes with a 0.5-cm layer of vermiculite. The adults were fed 10% sucrose solution (w:v) via a cotton wick and maintained in plexi-glass cages (10 × 10 cm) lined with paper toweling as an oviposition substrate. Adults were allowed to mate freely within the cages. Neonates were transferred to 20-mL diet cups containing 15 mL of meridic diet. The rearing process was repeated for 3 generations to generate enough larvae to conduct the bioassays.

Stoneville, Mississippi Experiments

Cotton varieties 'Deltapine® 444BR' (Bollgard®), 'Stoneville 4357 B2RF' (Bollgard II®), 'PhytoGen 485 WRF' (WideStrike™), 'PhytoGen 425 RF' (Non-Bt), and 'Coker 312' (Non-Bt) were planted in paired rows (1.0 m centers × 10.67 m) arranged in a randomized complete block with each variety replicated 4 times. The plots were maintained with standard agronomic practices, and no applications of insecticides active against Lepidoptera were used during the growing season. When the cotton was near physiological cutout defined as 5 nodes above the top white bloom (Bourland et al. 1992), leaves from the lower, middle, and upper parts of the canopy were collected from each plot, and transported to the laboratory in an ice-chest. In the laboratory, replicate samples of 10 single leaves from each cotton variety were placed individually in Petri dishes (150 × 10 mm) lined with Whatman® (Buffalo, New York) filter paper. Ten neonates were placed on each leaf for a total of 100 larvae for each variety. Larvae were checked for mortality by prodding with a camel hair brush 3 and 5 d after infestation. Those that failed to respond to a gentle touch of the brush were counted as moribund. In addition to the fresh tissue bioassays, as many larvae as possible were collected from visual inspection of the different varieties from each replicated plot. Larvae were counted and weighed for a comparison of their status from the different Bt technologies.

Weslaco, Texas Experiments

Cotton varieties Deltapine 444BR (Bollgard®), Deltapine® 143 B2R (Bollgard II®), Stoneville 4357 B2RF (Bollgard II®), PhytoGen 485WR (WideStrike™), and PhytoGen 425RF (Non-Bt) were planted on 5 Mar 2007 in replicated strips, 6 rows wide and approximately 150 m in length. When the cotton was 6 nodes above white flower (3 Aug 2007), the third fully expanded leaf on the terminal was removed from each variety and immediately taken to the laboratory for leaf assays. These assays were identical to those conducted at Stoneville except that only upper canopy leaves were used. However, following the 5-d mortality observation, each leaf was assigned a subjective damage score from 0 to 5, where 0 = no damage; 1 = 1-20% damage; 2 = 20-40% damage; 3 = 40-60% damage; 4 = 60-80% damage; and 5 = 80-100% estimated leaf damage (Adamczyk et al. 2008).

Percentage mortality data for leaf tissue assays were analyzed with the GLIMMIX procedure of mixed model analysis. Degrees of freedom were calculated by the Satterthwaite method (SAS 2003). Means were separated with the LSMEANS statement and adjusted with the Tukey-Kramer test ($\alpha = 0.05$). Weights for the larvae collected from the replicated field plots in Stoneville, MS were analyzed by the PROC FREQ procedure (SAS 2003) and compared by the Kruskal-Wallis type of Chi-square Likelihood ratio test ($\alpha = 0.05$) because large differences in the individual larvae collected and their weights represented a skewed distribution. Means were separated with the LSMEANS statement and adjusted with the Tukey-Kramer test ($\alpha = 0.05$). Leaf damage (0-5) was estimates were assessed with a categorical rating

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scale where 0% represented no leaf damage, while 80 - 100% was assigned a value of 5. Leaf damage ratings were analyzed by a non-parametric (NPAR1WAY) procedure for the one-way classification of damage estimates testing the differences in the Wilcoxon signed- ranked sums with the Kruskal-Wallis test (SAS Institute 2003).

RESULTS

Stoneville, Mississippi Experiments

In the feeding bioassays, no YSAW larvae completed development on WideStrike™ leaves; therefore these data were not used in the analysis. Larvae feeding on Bollgard II® leaves suffered higher mortality than those feeding on leaves from Bollgard® and the Non-Bt cotton (F = 163.7; df = 2, 42; $P < 0.001$) (Fig. 1). Leaves from different canopy positions did not influence larval mortality (F = 0.10; df = 2, 81; P = 0.90). Only 2 live larvae were collected from the Bollgard II® plots, averaging 171.5 ± 51.3 mg. In contrast, there were 101 larvae collected from the Non-Bt cotton weighing 824.25 ± 51.53 mg, and 85 larvae collected from Bollgard® cotton (886 ± 63.2 mg; P = 0.11), indicating that frequent survival of healthy individuals occurred in Non-Bt and Bollgard® cotton.

Weslaco, Texas Experiments

There were significant differences in YSAW mortality among genotypes at 3 d (F = 105.7; df = 4, 36; $P < 0.0001$) and 5 d (F = 166.6; df = 4, 36; $P < 0.0001$) (Fig. 2). Mortality was significantly higher for larvae fed WideStrike™ leaves compared to all other treatments at 3 d (48.1 ± 12) and 5 d (74.5 ± 9.9) after infestation. Mortality on both of the Bollgard II® at 3 d = 32% ± 10 for DP143 and 36% ± 11.1 for ST4357, and at 5 d = 60% ± 7.3% for DP143 and 60% ± 8.9 for ST4357 was significantly higher than for Bollgard® at 3 d = 4%, 5 d = 6% or Non-Bt cotton at 3 d = 4%, 5 d = 8%.

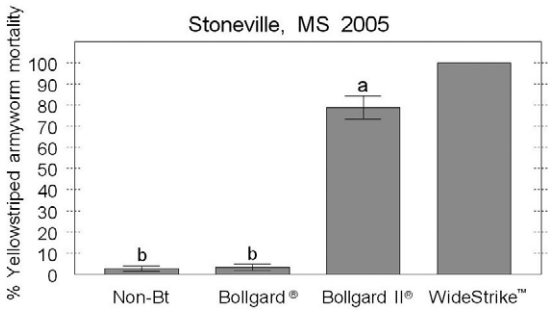


Fig. 1. Mortality of yellowstriped armyworms placed on late-season cotton leaves collected from Deltapine 444BR (Bollgard®), Stoneville 4357 B2RF (Bollgard II®), PhytoGen 485 WRF (WideStrike™), PhytoGen 425 RF (Non-Bt), and Coker 312 (Non-Bt), Stoneville, MS, 2005. Data for WideStrike™ were removed from the statistical analysis because all larvae were dead.

Significant differences in the leaf damage ratings occurred (Kruskal-Wallis = 30.98, df = 4, P = < 0.001) at 5 d from the time larvae were placed on leaf tissue. Damage ratings for Widestrike™ and Bollgard II® ranged from 1.0 to 1.3, respectively, (Fig. 3). Larvae consumed <13% of the leaf tissue in these treatments. The Non-Bt and Bollgard® cotton had noticeably significantly higher damage when compared to the Widestrike™ and Bollgard II®. Overall leaf tissue consumption was 4 times higher for the Bollgard® and non-Bt cotton when compared to the stacked traits The bioassay also revealed the large consumption rate of YSAW larvae, which consumed >80% of the Non-Bt and Bollgard® leaf tissue based on the feeding damage estimates.

DISCUSSION

The fact that WideStrike™ can cause 80 -100% mortality from cotton tissue collected late in the season and from the lower, middle, and upper canopy is advantageous from the standpoint that YSAW infestations generally occur later in the season from other growing regions across the cotton belt. Adamczyk et al. (2008) reported that Bt toxins are active in WideStrike™ lower, middle, and upper canopy leaves from assays conducted with both fall and beet armyworms in south Texas. Furthermore, Siebert et al. (2008) quantified the levels of both Cry1Ac and Cry1F in WideStrike™ cotton grown in several locations in the southern cotton belt and found that Cry1F accumulates in leaves and fruiting forms, with the exception of flowers, while Cry1Ac decreased in terminals and fruiting forms as the season progressed. These findings confirm the results seen in this study where WideStrike™ is highly efficacious against YSAW in late season from cotton grown in Stoneville, Mississippi and the Lower Rio Grande Valley of south Texas. The Cry2Ab trait found in Bollgard II® is also active against YSAW, but not to the same degree as Cry1F in WideStrike™.

The data presented here from the leaf-feeding bioassays indicate that the different forms of stacked Bt traits appear to have similar activity against YSAW as against other armyworm species. Our results indicate, as documented in bioassays with other armyworm species feeding on cotton (Adamczyk & Gore 2004; Stewart et al. 2001; Adamczyk et al. 2008; Siebert et al. 2008) including fall and beet armyworms, that none are susceptible to the Cry1Ac trait, and in some cases are healthier than those reared on non- Bt cotton varieties.

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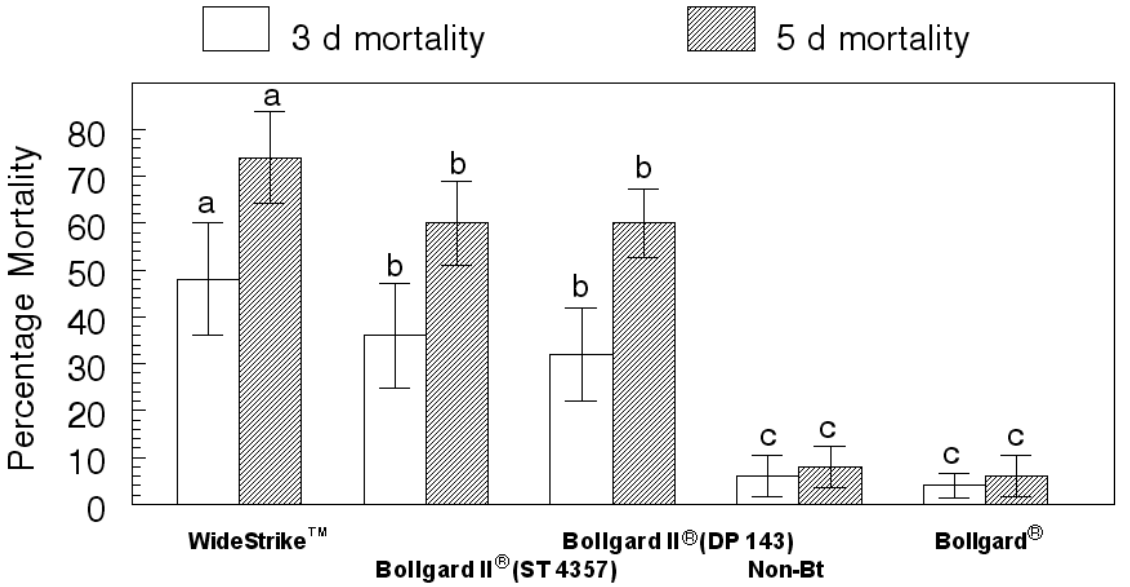


Fig. 2. Mean (±SE) mortality of yellowstriped armyworms after 3 d (solid bars) and 5 d (cross hatch) feeding on cotton leaves collected from the upper terminal of Deltapine 444 BR (Bollgard®), Stoneville 4357 B2RF (Bollgard II®), Deltapine 143 B2 (Bollgard® II), PhytoGen 485 WRF (WideStrike™), and PhytoGen 425 RF (Non-Bt), Weslaco, TX, 2007. Significant differences among cotton traits with 3 and 5 d are indicated by small letters.

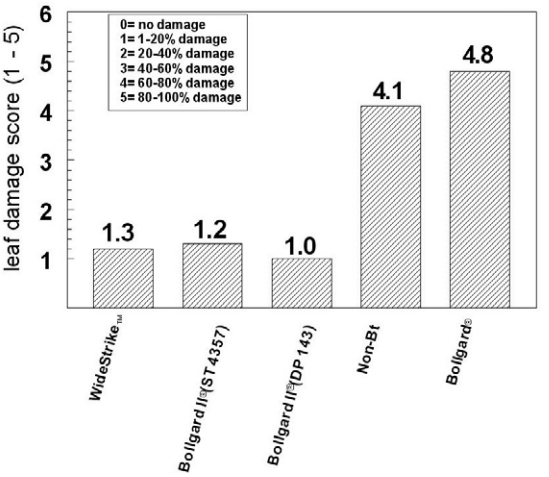


Fig. 3. Damage ratings for leaf damage feeding estimates from yellowstriped armyworms after 5 d of feeding on cotton leaves collected from field plots of late-season cotton with Deltapine 444BR (Bollgard®), Stoneville 4357 B2RF (Bollgard II®), Deltapine 143 BG II (Bollgard II®), PhytoGen 485 WRF (WideStrike™), and PhytoGen 425 RF (Non-Bt), Weslaco, TX, 2007.

Our leaf dish assays indicated that from 60% (Weslaco) to 80% (Stoneville) control of YSAW could be expected for the stacked Bollgard II® in controlling young YSAW larvae on cotton that has terminated fruit growth and development and is maturing. This is a positive and acceptable level given that YSAW are primarily leaf feeders. Our observations in the field are that they may consume an occasional floret (square), but not maturing bolls.

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