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# EVALUATIONS OF BOLLGARD®, BOLLGARD II®, AND WIDESTRIKE® TECHNOLOGIES AGAINST BEET AND FALL ARMYWORM LARVAE (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

Transgenic cottons containing the Bollgard®, Bollgard II® and WideStrike® traits were grown in 2005 and 2007 to examine the efficacy against beet armyworm *Spodoptera exigua* (Hübner) and fall armyworms *S. frugiperda* (J. E. Smith). Results suggest that both dual-gene traits are more efficacious against these armyworm species than Bollgard®. In these studies, WideStrike® appears to be more efficacious against fall armyworms than Bollgard II®, while Bollgard II® is more efficacious against beet armyworms than WideStrike®. Possible reasons for these differences in efficacy are discussed.

Key Words: *Spodoptera exigua*, *Spodoptera frugiperda*, armyworms, transgenic cotton

RESUMEN

Clases de algodón transgénicos que tienen las características Bollgard®, Bollgard II® y WideStrike® fueron sembrados durante 2005 y 2007 para examinar la eficacia contra el gusano ejército de la remolacha, *Spodoptera exigua* (Hübner) y el gusano cogollero, *S. frugiperda* (J. E. Smith). Los resultados sugerieron que ambas lineas de los dúos genes son mas eficaz contra estas especies de gusanos que Bollgard®. En estos estudios, WideStrike® aparece ser mas eficaz contra el gusano cogollero que Bollgard II®, mientras que Bollgard II® es mas eficaz contra el gusano ejército de la remolacha que WideStrike®. Se discute las razones que son posibles para estas diferencias en eficacia.

Since the first Cry1Ac *Bacillus thuringiensis* Berliner (Bt) cotton variety was commercialized in 1996 (Bollgard ®, Monsanto Ag. Co., St. Louis, MO), advancements for insect control with transgenic technology has occurred that offer improved efficacy against many lepidopteran pests. Current varieties can contain Cry1Ac alone or they can be stacked with Cry2Ab (Bollgard® II, Monsanto Ag. Co.) or Cry1F (WideStrike®, Dow Agrosciences, Indianapolis, IN).

The beet armyworm, *Spodoptera exigua* (Hübner) is a secondary, but serious migratory pest of various vegetable and certain row crops in the southern part of the United States of America. Although larval feeding on cotton is primarily concentrated on foliage, larvae can cause devastating losses in yield during outbreaks (Hardee & Herzog 1997; Adamczyk et al. 1998). The fall armyworm, *S. frugiperda* (J. E. Smith) also is a destructive migratory pest of many crops in the Western Hemisphere, where it appears to be more common and widespread (Sparks 1979; Young 1979). Like the beet armyworm, this pest has the potential to damage both conventional and Bollgard® cotton bolls (Adamczyk et al. 1998).

Although certain lepidopteran pests of cotton are controlled by Bollgard® cotton (e.g., tobacco budworms, *Heliothis virescens* (F.) and pink bollworms, *Pectinophora gossypiella* (Saunders)), the Cry1Ac δ-endotoxin in Bollgard® cotton is less effective for controlling beet and fall armyworms (MacIntosh et al. 1990; Adamczyk et al. 1998). Consequently, outbreaks of these pests on Bollgard® often need full application rates of foliar insecticide treatments to keep these populations below economic injury levels (Hood 1997; Smith 1997). The addition of other Cry proteins stacked with Cry1Ac (i.e., Bollgard II® and WideStrike®) has improved the efficacy against these armyworms (Adamczyk et al. 2001; Stewart et al. 2001; Adamczyk & Gore 2004). However, differences in survivorship of beet and fall armyworm larvae feeding on Bollgard II® versus WideStrike® cottons have been suggested, but never characterized or explained. The purpose of the study was to examine the efficacy of Bollgard II® and WideStrike® against beet and fall armyworms.

## Materials and Methods

### Field Plots

In May 2005 transgenic cotton varieties containing the Bollgard®, Bollgard II® and WideStrike® traits were planted in research plots in the Mississippi Delta near Stoneville, MS (Table 1). Plots consisted of 2 rows (1.0 m centers) × 10.67 m. All plots were arranged in a randomized complete block design with each variety replicated 4 times (once in each block). Only insecticides not active on Lepidoptera were applied to all plots throughout the season as dictated by local management practices. In Mar 2007 transgenic cotton varieties containing the Bollgard, Bollgard II® and WideStrike® traits were planted in strip plots in the lower Rio Grande Valley of Texas near Weslaco, TX (Table 1).

### Insects

All Lepidoptera utilized in these studies were obtained from laboratory colonies maintained at the USDA, ARS located in Stoneville, MS or Weslaco, TX.

### Bioassays with Larvae

In 2005 bioassays were conducted with only fall armyworm larvae. A single neonate was placed into individual 9-cm diameter petri dishes (8) that each contained a moistened filter paper and a single lower leaf obtained from all plots for a total of 32 larvae per variety. Cotton plants were at peak bloom. The plates were covered with corresponding lids. After 5 d, surviving larvae were carefully transferred with a camel-hair brush into new petri dishes containing fresh filter paper and a new leaf. This procedure continued until pupation. At 7 and 10 d, larvae were prodded with a camel-hair brush and considered alive if coordinated movement was observed. Beginning at 15 d, plates were checked daily for the presence of pupae. Percent survival was analyzed by REML-ANOVA, and means were separated according to Fisher's Protected LSD (Littell et al. 1996; PROC MIXED, SAS Institute 2001).

In 2007 leaves obtained from various sections of the plant containing various transgenic traits were assayed for bioactivity against beet and fall armyworms. Individual leaves were placed into a 50 × 9-mm Tight-Fit Lid sealing Petri dish (BD Falcon® #351006, VWR International). Beet armyworms (5) were placed in a dish containing a terminal (upper canopy) leaf or a mid-canopy leaf (10 dishes per variety) for a total of 50 larvae per variety. Fall armyworm bioassays were conducted identically, except only mid-canopy leaves were used. Leaves were also collected from various times during the growing season. At 5 d after ex-

Table 1. Cotton varieties and traits examined in 2005 and 2007.

| Year | Location | Bt Trait | Variety | Bt Endotoxins | Owner of Bt Trait | Owner of Variety |
|---|---|---|---|---|---|---|
| 2005 | Stoneville, MS | Bollgard | St 4691B | CrylAc | Monsanto | Stoneville (Bayer Cropscience) |
| 2005 | Stoneville, MS | Bollgard II | DP 424B11/RR | CrylAc + Cry2Ab | Monsanto | Delta & Pineland (Monsanto) |
| 2005 | Stoneville, MS | Non-Bt | DP 5415 | None | None | Delta & Pineland (Monsanto) |
| 2005 | Stoneville, MS | Non-Bt | SG 521 RR | None | None | Delta & Pineland (Monsanto) |
| 2005 | Stoneville, MS | Non-Bt | St 474 | None | None | Stoneville (Bayer Cropscience) |
| 2005 | Stoneville, MS | WideStrike | Phy 470WR | CrylAc+ CryiF | DowAgrosciences | Phytogen (Dow Agrosciences) |
| 2007 | Weslaco, TX | Bollgard | DPL 444 BG/RR | CrylAc | Monsanto | Delta & Pineland (Monsanto) |
| 2007 | Weslaco, TX | Bollgard II | Americot 1532 BGII/RR | CrylAc + Cry2Ab | Monsanto | Americot |
| 2007 | Weslaco, TX | Bollgard II | St 4357 BGII/RRF | CrylAc + Cry2Ab | Monsanto | Stoneville Seed Co. (Bayer Cropscience) |
| 2007 | Weslaco, TX | Bollgard II | DPL 424 BGII/RR | CrylAc + Cry2Ab | Monsanto | Delta & Pineland (Monsanto) |
| 2007 | Weslaco, TX | Bollgard II | DPL 143 BGII/RR | CrylAc + Cry2Ab | Monsanto | Delta & Pineland (Monsanto) |
| 2007 | Weslaco, TX | Non-Bt | Americot 262R | None | None | Americot |
| 2007 | Weslaco, TX | Non-Bt | Phy 425 RF | None | None | Dow Agrosciences |
| 2007 | Weslaco, TX | Non-Bt | DPL 5415 RR | None | None | Delta & Pineland (Monsanto) |
| 2007 | Weslaco, TX | WideStrike | Phy 485 WRF | CrylAc + CryiF | Dow Agrosciences | Dow Agrosciences |

posure to cotton tissue, larvae were prodded with a camel-hair brush and considered alive if coordinated movement was observed. Percent mortality by trait was analyzed by REML-ANOVA, and means were separated according to Fisher's Protected LSD (Littell et al. 1996; PROC MIXED, SAS Institute 2001).

### Bioassays with Egg Masses

Inoculations of beet and fall armyworm egg masses to leaves located in various sections of the plant were conducted in 2007. In the laboratory, egg masses were deposited on nylon cloth placed on the top of adult rearing cages (3.79-L cardboard containers). For each inoculation, an egg mass of equal size (ca. 100-300 eggs per 2.54 $cm^2$ cloth sample) was pinned to the underside of a leaf for all traits and covered with a cage that consisted of a condiment cup (118 mL) (Solo Co., Highland Park, IL) coupled with a hard plastic lid (Fig. 1). Five d after inoculations, the infested leaf and corresponding cages were harvested and transported to the laboratory. Leaf damage (0-5) was estimated with a categorical rating scale where 0% indicated no leaf damage, while 80-100% of leaf consumption was given a value of 5. Damage ratings were analyzed by non-parametric statistics (SAS Institute 2001).

## Results and Discussion

### Bioassays with Larvae

Both Bollgard II® and WideStrike® had significantly higher efficacy against fall armyworms than Bollgard® (Fig. 2 and Fig. 3); however, in both 2005 and 2007, WideStrike® had typically higher efficacy compared to Bollgard II®. It is interesting to note that larval development to pupation was observed for fall armyworms when fed Bollgard® or Bollgard II®, but not WideStrike®. In WideStrike® isolines containing only Cry1F or Cry1Ac, Adamczyk & Gore (2004) showed that the Cry1F protein provided greater efficacy compared to Cry1Ac against this pest. In addition, previous studies have shown little efficacy of Cry1Ac against fall armyworms (Adamczyk et al. 1998). We believe that the greater efficacy of WideStrike® compared to Bollgard II® against fall armyworms is primarily due to the action of the Cry1F protein. Although we did not evaluate isolines containing only Cry1F or Cry2Ab, our data suggests greater efficacy of Cry1F versus Cry2Ab against this pest.

The expression of the Cry1F protein affects beet armyworm survival both temporally and spatially. When using mid-canopy leaves sampled throughout the season, no significant differences ($P > 0.05$) were observed between the survival of beet armyworms fed Bollgard II® and WideStrike® (Fig. 4). However late in the season when beet armyworms were fed terminal leaves located in the upper part of the plant (i.e., upper-canopy leaves), survival of larvae on WideStrike® was very high (>%60) (Fig. 5). Siebert et al. (2009) reported that expression of Cry1F protein is greatest in mature leaves as compared to other structures such as terminal leaves, squares, flowers, and bolls, and that protein levels in mature leaves increases with age. This expression profile for Cry1F is very different than what was reported for Cry2Ab in Bollgard II®, where expression in all tissue appears to remain relatively constant

Fig. 1. Cages used to enclose egg masses.

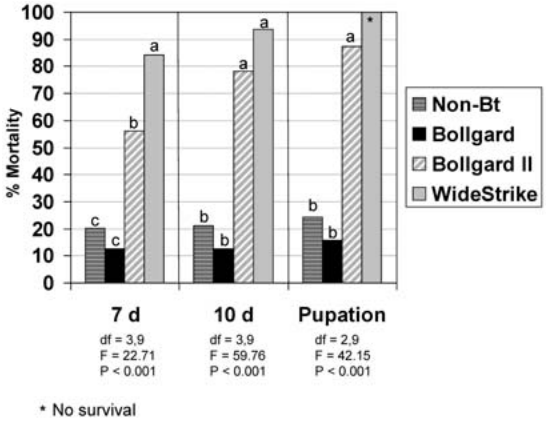


Fig. 2. Mortality of fall armyworms fed lower leaves, Stoneville, MS 2005.

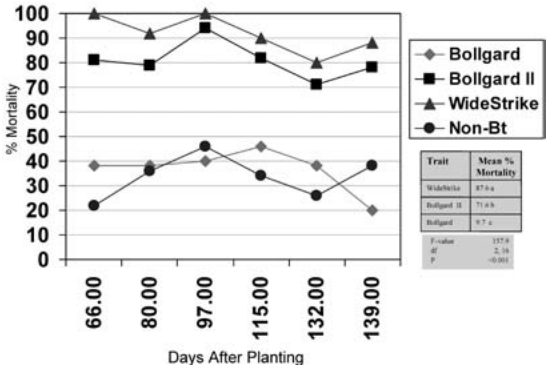


Fig. 3. Mortality of fall armyworms at 5 d after exposure for bioassays with mid-canopy leaves sampled throughout the season, Weslaco, TX 2007.

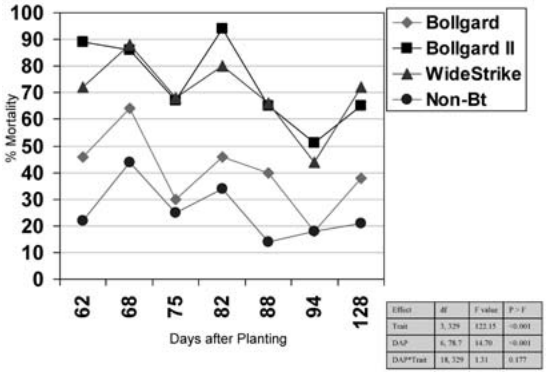


Fig. 4. Mortality of beet armyworms at 5 d after exposure for bioassays with mid-canopy leaves sampled throughout the season, Weslaco, TX 2007.

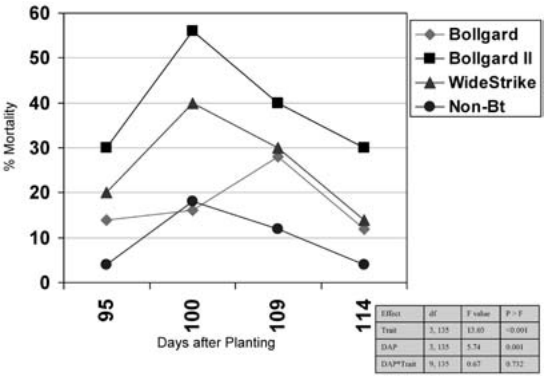


Fig. 5. Mortality of beet armyworms at 5 d after exposure for bioassays with upper-canopy leaves sampled throughout the season, Weslaco, TX 2007.

over time Adamczyk et al. (2001). In addition, mortality at >109 DAP of beet armyworms on WideStrike® terminal leaves was similar to what was observed with Bollgard® which also contains a Cry1Ac-like transgene that provides little efficacy against both beet and fall armyworms (Stewart et al. 2001; Adamczyk et al. 1998; Adamczyk & Gore 2004). We believe that low levels of Cry1F protein reported for new foliage (e.g., young terminal leaves) as compared to mature, fully expanded leaves may partially explain the low mortality observed against beet armyworms. Furthermore, our field studies with leaves collected from different portions of the plant supports these temporal and spatial conclusions. More damage was observed from beet armyworms feeding on WideStrike® leaves compared to Bollgard II® leaves in the upper canopy compared to the middle canopy (Fig. 6). Even with the presumed greater efficacy of Cry1F compared to Cry2Ab against the fall armyworm, this same trend was observed (Fig. 7). Regardless of transgenic trait, it is interesting to note that more damage was observed for both armyworm species caged on leaves located in the upper canopy. This may be partially explained by greater amounts of secondary plant compounds, as well as a thicker leaf cuticle, for leaves located lower in the plant canopy. In some situations, we believe that beet armyworms may need supplemental foliar insecticides for controlling outbreak populations that may be feeding on young tissues especially late in the season where Cry1F levels do not have the time to build to provide adequate control.

Various transgenes that are used to produce cotton with the WideStrike® trait may partially explain the observed protein expression profile of Cry1F. In particular, the *cry1F* trait in WideStrike® is regulated by the (4ocs) DeltaMas 2' Promoter. Previous studies have shown that the dual promoter of *Agrobacterium tumefaciens*

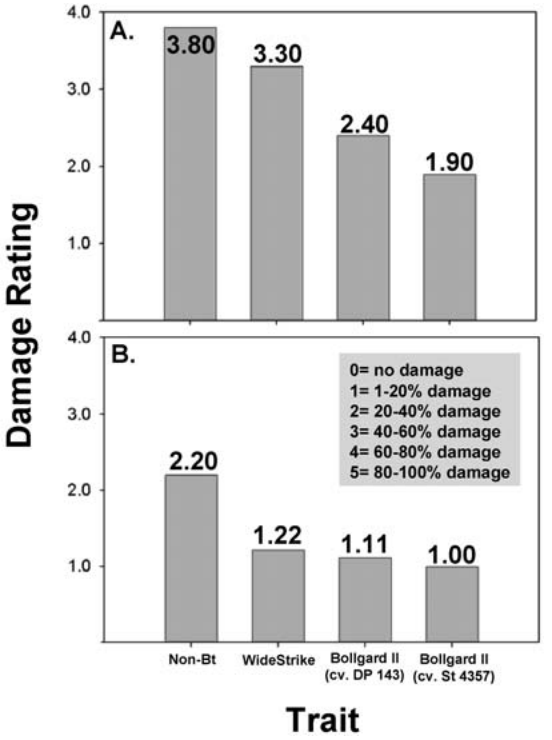


Fig. 6. Damage ratings for beet armyworms caged in the field on various cotton leaves located in the upper (A) and middle (B) canopy, Weslaco, TX 2007. Kiskal-Wallis Test: upper, $P = 0.039$; middle, $P = 0.001$. Cultivars: DP 143 BGII/RR & St 4357BGII/RRF (Bollgard II®), Phy 485 WR (WideStrike®), Phy 425 RR (Non-Bt).

mannopine synthase (*mas*) genes is regulated by plant growth hormones, and that the activity of the *mas* dual promoters increases basipetally in developing plants; it has also been reported that the apical meristem contains a factor that inhibits its stimulation of *mas* promoter activity (Langridge et al. 1989). However, it is important to note that WideStrike® traits contain enhancers that make the promoter more constitutive, whereby losing sensitivity to hormones (US Patent: 5,955,646). Thus, further research is needed to determine the various plant mechanisms that confer protein expression for the WideStrike® traits.

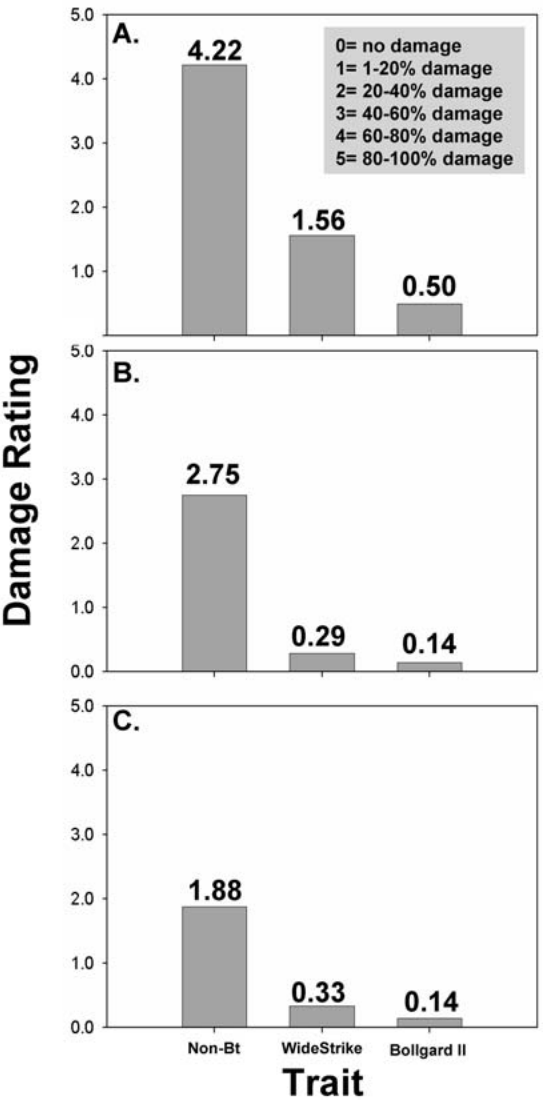


Fig. 7. Damage ratings for fall armyworms caged in the field on various cotton leaves located in the upper (A), middle (B), and lower (C) canopy, Weslaco, TX 2007. Kiskal-Wallis Test: upper, $P < 0.001$; middle, $P = 0.002$; lower, $P = 0.076$. Cultivars: DP 143 BGII/RR (Bollgard II®), Phy 485 WR (WideStrike®), Phy 425 RR (Non-Bt).

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