EVALUATION OF BOLLGARD II(R) AND WIDESTRIKETM TECHNOLOGIES AGAINST BEET AND FALL ARMYWORMS

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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 and fall armyworms. 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.

Introduction

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 using 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 an secondary, but serious migratory pest of various vegetable and certain row crops in the southern United States of America. Although larval feeding on cotton is primarily concentrated on foliage, larvae can cause devastating losses in yield during outbreaks (Hardee and Herzog 1997; Adamczyk et al. 1998). The fall armyworm, *Spodoptera 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 and pink bollworms, *Pectinophora gossypiella* (Saunders)], the Cry1Ac δ-endotoxin in Bollgard® cotton is less effective for controlling beet and fall armyworms (McIntosh 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 has improved the efficacy (i.e. Bollgard II® and WideStrike™) against these armyworms (Adamczyk et al. 2001, Stewart et al. 2001, Adamczyk and 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. Plots consisted of 2 rows (1.0 m centers) X 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 March 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 using Larvae. In 2005, bioassays were conducted using only fall armyworm larvae. A single larva was placed into individual 9.0 cm 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/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 9.0 cm Petri dishes containing fresh filter paper and 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 using 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 x 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/variety) for a total of 50 larvae/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 day after exposure to cotton tissue (DAE), larvae were prodded with a camel-hair brush and considered alive if coordinated movement was observed. Percent mortality by trait was analyzed using REML-ANOVA, and means were separated according to Fisher's Protected LSD (Littell et al. 1996; PROC MIXED, SAS Institute 2001).

Bioassays using 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 liter cardboard containers). For each inoculation, an egg mass of equal size (ca. 100-300 eggs/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 (Plate 1). Five days after inoculations, the infested leaf and corresponding cages were harvested and transported to the laboratory. Leaf damage (0-5) was estimated using 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 using non-parametric statistics (SAS Institute, 2001).

Plate 1. Cages used to enclose egg masses.

Table 1. Cotton Varieties and Traits Examined in 2005 and 2007.

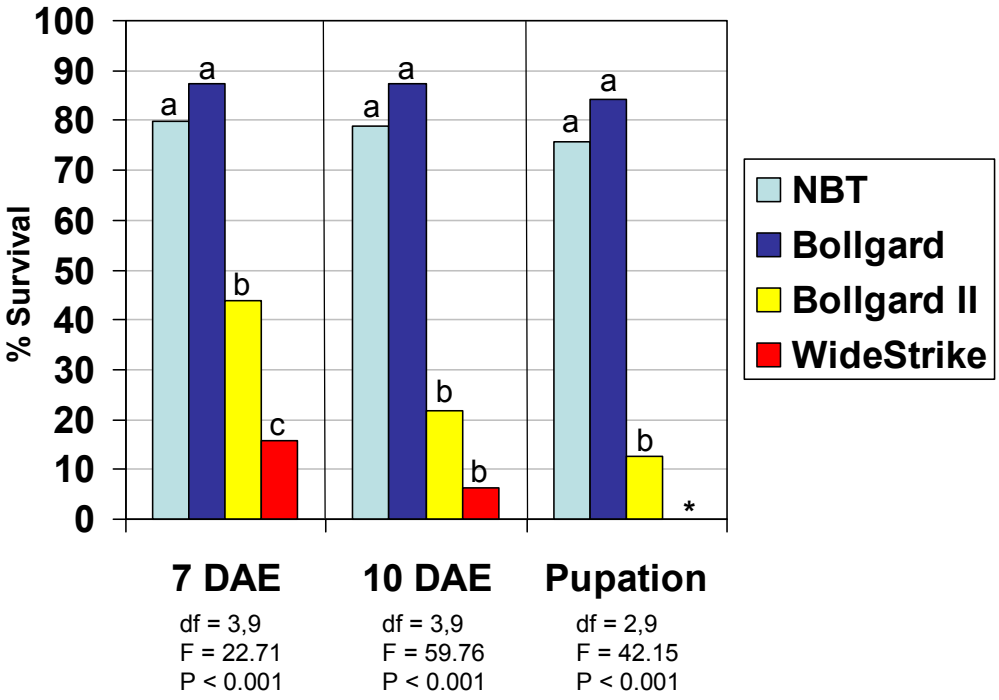
Year	Location	Bt Trait	Variety	Bt Endotoxins	Owner of Bt Trait	Owner of Variety
2005	Stoneville, MS	Bollgard	St 4691B	Cry1Ac	Monsanto	Stoneville (BayerCropscience)
2005	Stoneville, MS	Bollgard II	DP 424BII/RR	Cry1Ac + Cry2Ab	Monsanto	Delta & Pineland (Monsanto)
2005	Stoneville, MS	Non-Bt	DP 5415	None	None	Delta & Pineland (Monsanto)
2005	Stoneville, MS	Non-Bt	SG 521RR	None	None	Delta & Pineland (Monsanto)
2005	Stoneville, MS	Non-Bt	St 474	None	None	Stoneville (BayerCropscience)
2005	Stoneville, MS	WideStrike	Phy 470WR	Cry1Ac + Cry1F	DowAgrosciences	Phytogen (DowAgrosciences)
2007	Weslaco, TX	Bollgard	DPL 444 BG/RR	Cry1Ac	Monsanto	Delta & Pineland (Monsanto)
2007	Weslaco, TX	Bollgard II	Americot 1532 BGII/RR	Cry1Ac + Cry2Ab	Monsanto	Americot
2007	Weslaco, TX	Bollgard II	St 4357 BGII/RRF	Cry1Ac + Cry2Ab	Monsanto	Stoneville Seed Co. (Bayer Cropscience)
2007	Weslaco, TX	Bollgard II	DPL 424 BGII/RR	Cry1Ac + 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	DowAgrosciences
2007	Weslaco, TX	Non-Bt	DPL 5415 RR	None	None	Delta & Pineland (Monsanto)
2007	Weslaco, TX	WideStrike	Phy 485 WRF	Cry1Ac + Cry1F	DowAgrosciences	DowAgrosciences

Results and Discussion

Bioassays using Larvae. Both Bollgard II® and WideStrike™ had significantly higher efficacy against fall armyworms than Bollgard® (Figures 1 and 2). 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™. We believe that greater efficacy of WideStrike™ against fall armyworms was observed due to the Cry1F protein (Adamczyk and Gore 2004).

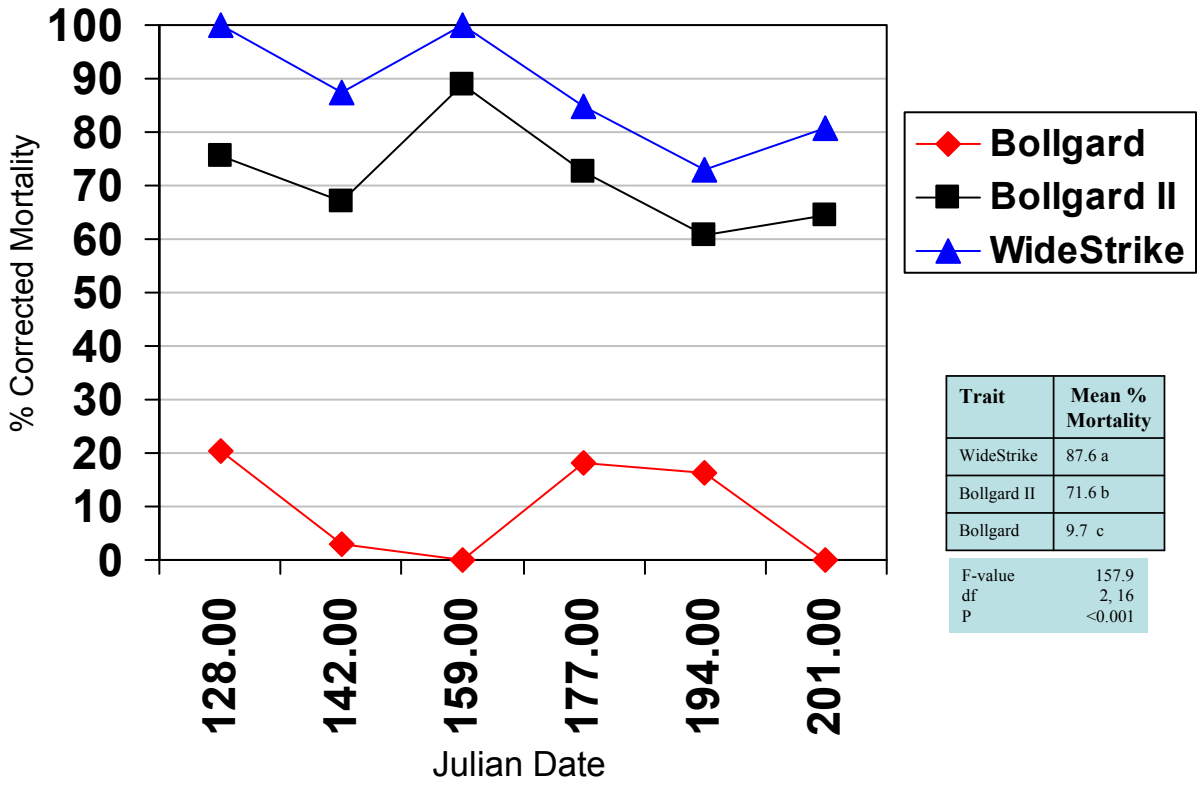
The expression of the Cry1F protein affects beet armyworm survival both temporally and spatially. When using mid-canopy leaves sampled throughout the season, no significant differences were observed between the survival of beet armyworms fed Bollgard II® and WideStrike™ (Figure 3). However, late in the 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) (Plate 3, Figure 4). We believe that low levels of Cry1F reported for new foliage (e.g. young terminal leaves) as compared to mature, fully expanded leaves may partially explain the low mortality observed. Furthermore, 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 provide little efficacy against this pest (Adamczyk and Gore 2004, Adamczyk et al. 1998, Stewart et al. 2001). 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. Results using egg masses and cages support the above observations and conclusions (Figures 5 and 6).

Figure 1. Survival of Fall Armyworms Fed Lower Leaves. Stoneville, MS 2005.



* No survival

Figure 2. Mortality of Fall Armyworms at 5 DAE for Bioassays Using Mid-Canopy Leaves Sampled Throughout the Season. Weslaco, TX 2007.

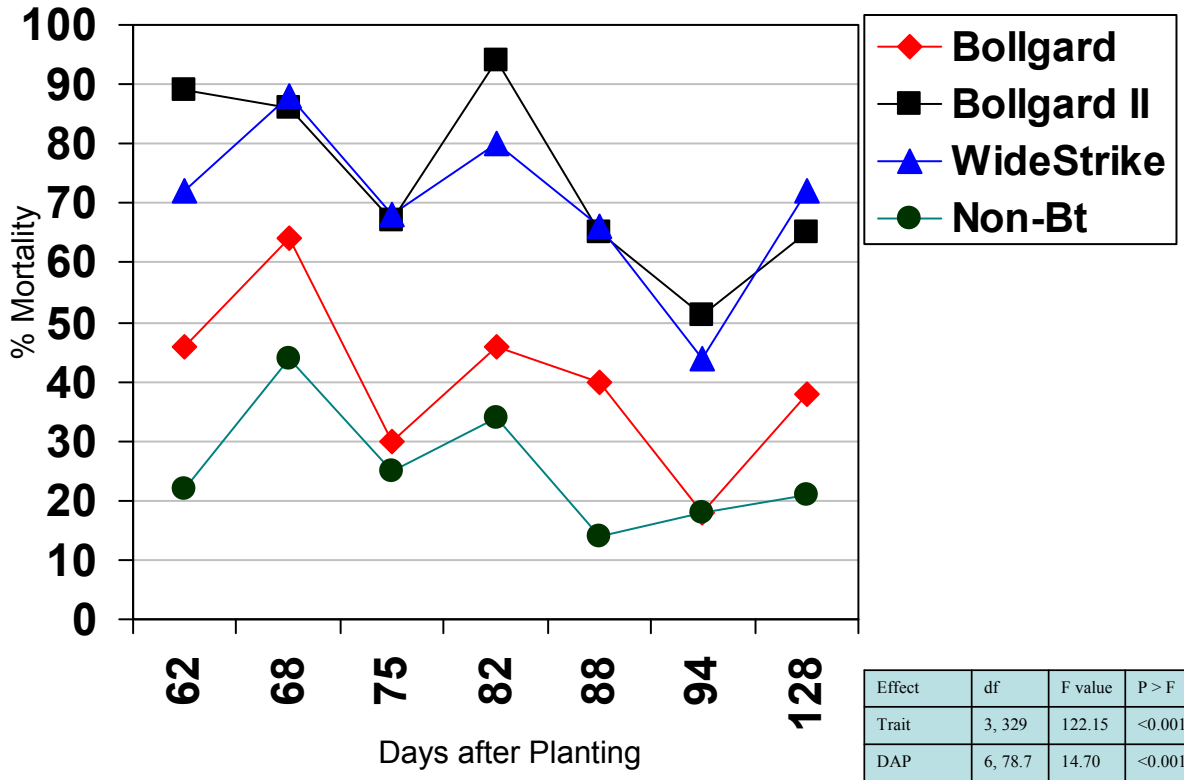


Trait	Mean % Mortality
WideStrike	87.6 a
Bollgard II	71.6 b
Bollgard	9.7 c

F-value	157.9
df	2, 16
P	<0.001

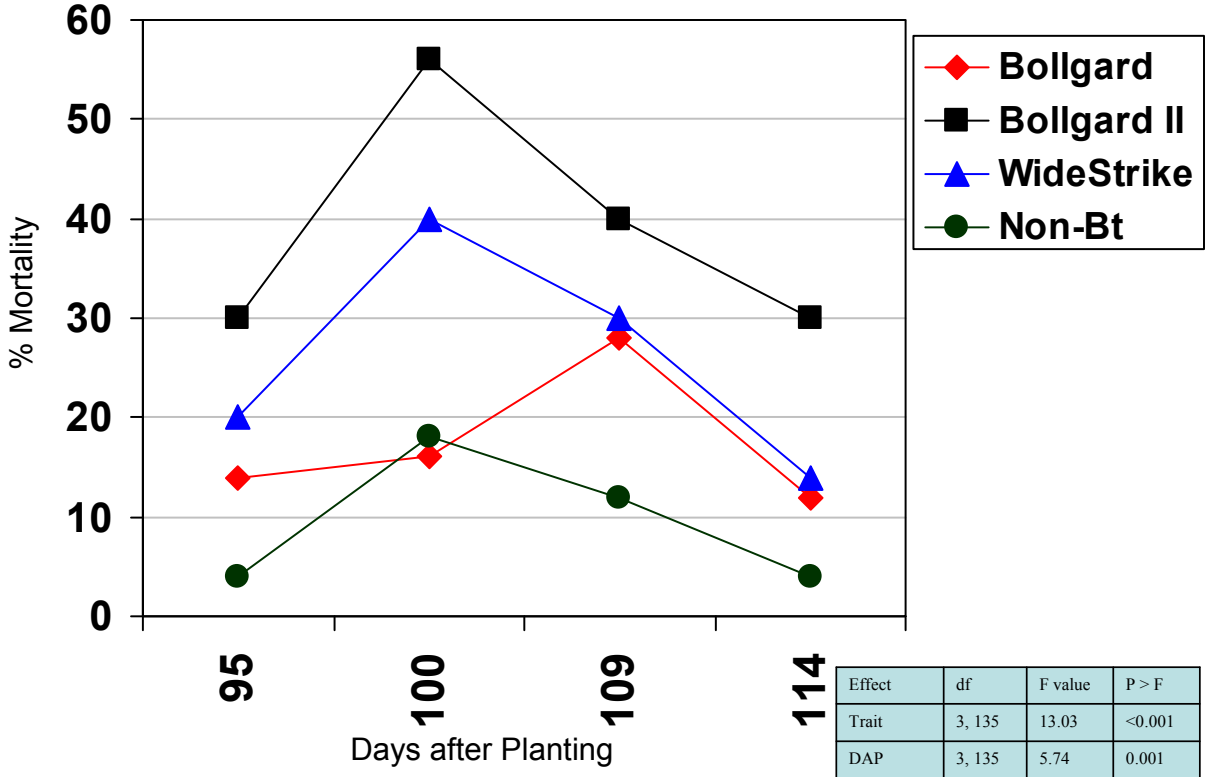
Phy425RR used as a control. Mortality corrected using Abbott's formula

Figure 3. Mortality of Beet Armyworms at 5 DAT for Bioassays Using Mid-Canopy Leaves Sampled Throughout the Season. Weslaco, TX 2007.



Effect	df	F value	P > F
Trait	3, 329	122.15	<0.001
DAP	6, 78.7	14.70	<0.001
DAP*Trait	18, 329	1.31	0.177

Figure 4. Mortality of Beet Armyworms at 5 DAT for Bioassays Using Upper-Canopy Leaves Sampled Throughout the Season. Weslaco, TX 2007.



Effect	df	F value	P > F
Trait	3, 135	13.03	<0.001
DAP	3, 135	5.74	0.001
DAP*Trait	9, 135	0.67	0.732

Plate 3. Typical Damage Observed from Beet Armyworms Feeding on Terminal Leaves in the Top of the Plant

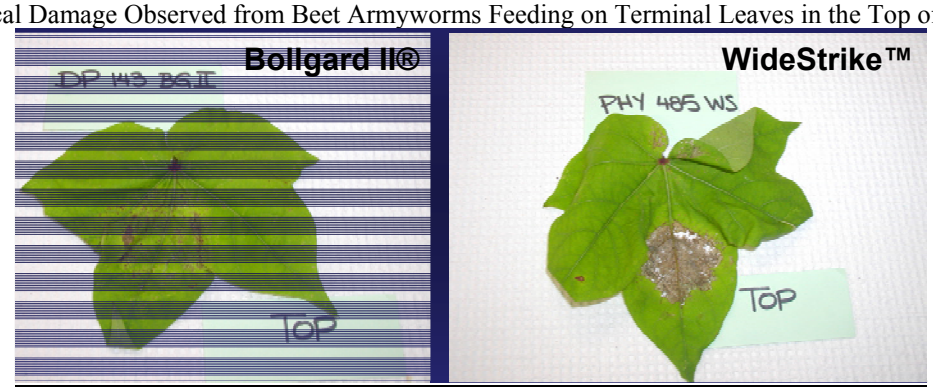


Figure 5. Damage Ratings for Beet Armyworms Caged in the Field on Various Cottons Leaves Located in the Middle and Top Canopy. Weslaco, TX 2007.

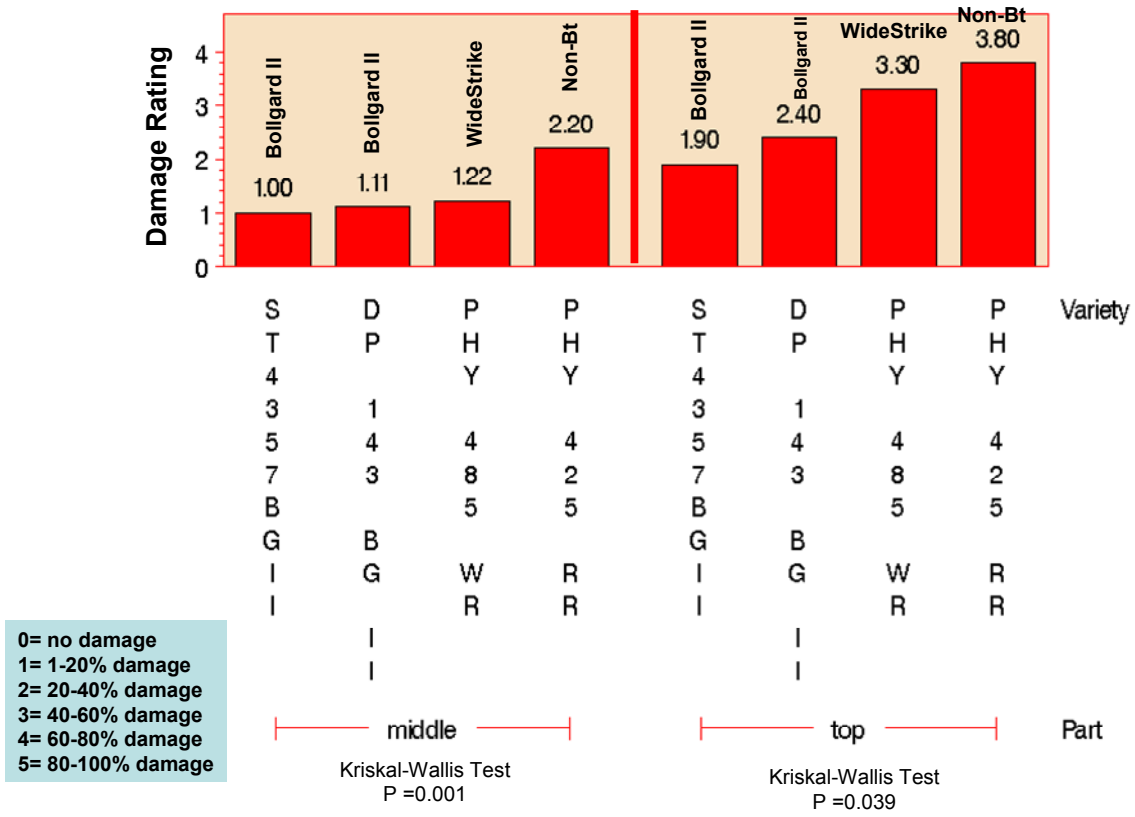
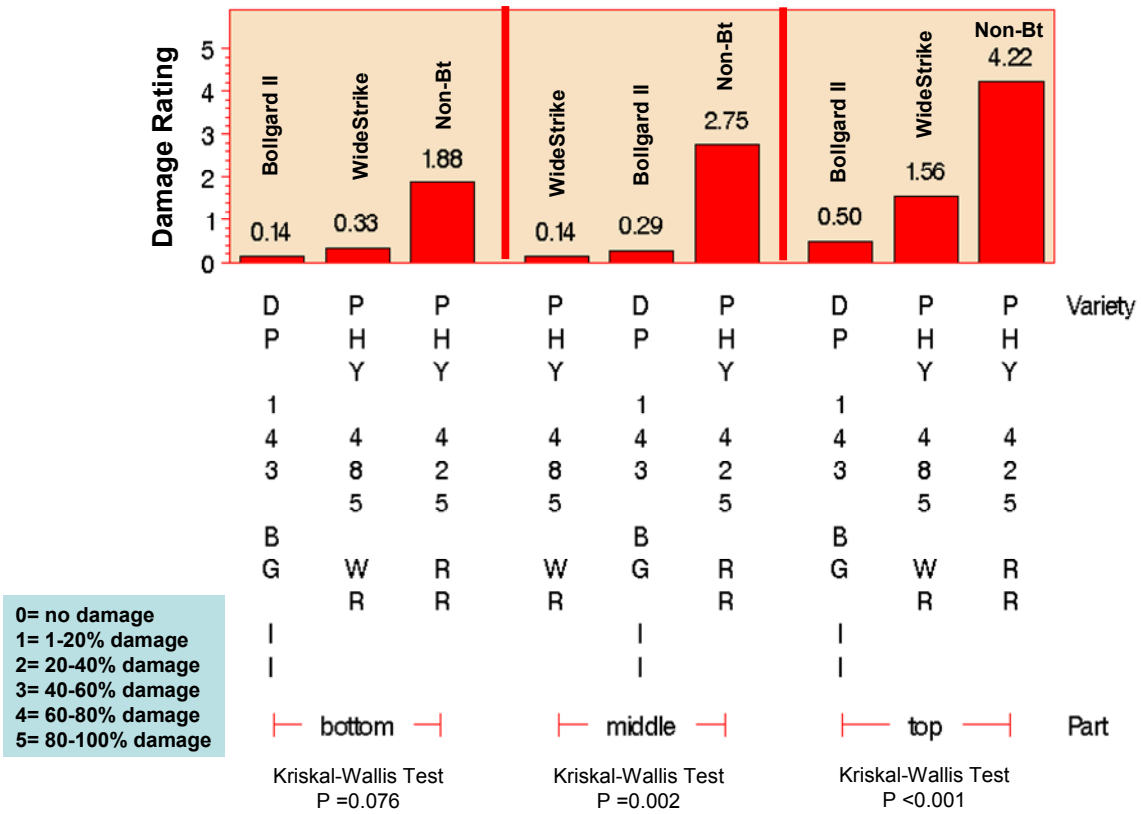


Figure 6. Damage Ratings for Fall Armyworms Caged in the Field on Various Cottons Leaves Located in the Bottom, Middle, and Upper Canopy. Weslaco, TX 2007.



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