DEVELOPMENT OF TREATMENT THRESHOLDS FOR BOLLWORM [*Helicoverpa zea*] IN SECOND-GENERATION *Bt* COTTON

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Abstract

Dual-gene *Bt* cotton has reduced the need for supplemental insecticide treatments for bollworm, *Helicoverpa zea*, compared with original single-gene technology. Bollgard II® (Monsanto) and WideStrike® (Dow Agrosciences) have the Cry1Ac gene in common but have a different combination of either Cry2Ab or Cry1F, respectively. These second-generation technologies enhance control of lepidopteran pests but remain less than 100% effective against bollworm when population pressure is high. Current threshold recommendations are based on those for single-*Bt*-gene technology, and there are no differences in the recommendations between Bollgard II® and WideStrike®. Treatment thresholds recommended for bollworm in South Carolina are 3 or more large larvae per 100 plants or 5% boll damage. This research aims to explore the quantifiable differences between technologies and develop the best possible thresholds for each. Test plots containing non-*Bt*, WideStrike®, and Bollgard II® varieties were scouted and treated weekly for one of the following: bollworm eggs, larvae in white blooms, or boll damage. Thresholds in each of the three categories were selected, and tests plots were sprayed accordingly. Based on inputs and yields, results from the 2010 season indicated that our currently recommended threshold of 5% boll damage might be adequate. At high lint prices (>$1/lb), our threshold of 75 eggs/100 plants might be higher than it should be under heavy pressure from bollworm. A more aggressive approach seemed to be appropriate in 2010, considering the heavy pressure experienced from bollworm. Widestrike® sustained significantly more boll damage than Bollgard II®. Unusually good growing conditions late into the season during 2010 very likely allowed for significant yield compensation, masking some of the negative impact of bollworm. Versions of these trials will be conducted again in 2011 to account for seasonal variability and to refine recommendations for bollworm in dual-gene *Bt* cotton.

Introduction

In 1996, Monsanto Corporation (St Louis, MO) was the first to commercialize genetically engineered cotton. Bollgard® cotton expressed the Cry1Ac gene from the soil bacterium *Bacillus thuringiensis* (*Bt*). The protein produced (δ-endotoxin) was found to be a safe insecticide that is specific to the insect order Lepidoptera (Perlak et al. 2001, Gore and Adamczyk 2004). Transgenic *Bt* cotton seldom, if ever, needs supplemental insecticide applications to control lepidopteran pests such as the tobacco budworm, *Heliothis virescens*, and pink bollworm, *Pectinophora gossypiella* (Stewart et al. 2001). However, to prevent economic loss from the bollworm, *Helicoverpa zea*, it is often necessary to apply foliar insecticides (Gore and Adamczyk 2004). Because one or two annual insecticide applications are necessary to prevent economic loss, action thresholds have been established in each state (Gore et al. 2008).

In order to forestall resistance development to *Bt* cotton (i.e. Bollgard®), Monsanto released a dual-toxin *Bt* cotton during 2003 called Bollgard II® that has the original Cry1Ac gene as well as the additional gene Cry2Ab. Two years later, Dow Agrosciences (Indianapolis, IN) released a variant of dual-*Bt*-toxin technology called WideStrike®. This cotton contains a different construct of *Bt* genes: Cry1Ac and Cry1F (Gore et al. 2008). These dual-gene technologies further enhanced in-plant control of caterpillar pests and reduced the need for foliar insecticides again. Laboratory studies in 2001 clearly demonstrated the greater toxicity of dual-gene *Bt* cotton over expression of a single insecticidal protein. Survival and growth rate were affected in multiple species, including bollworm, *H. zea*, fall armyworm, *Spodoptera frugiperda*, and beet armyworm, *Spodoptera exigua*. The study concluded that dual-toxin technologies would be more effective and have a wider range of activity than first-generation *Bt* cotton (Stewart et al. 2001). Though improved, Bollgard II® and Widestrike® do not offer 100% control of bollworm (Greene and Robinson 2010). Furthermore, differential expression of toxins in the plant and the differences between

Cry2Ab (Bollgard II®) and Cry1F (Widestrike™) raised new questions. Field cage experiments conducted to determine bollworm impact on Bollgard II® and Widestrike® cotton during 2003-2006, indicated that bollworm will rarely cause yield loss in either technology (Gore et al. 2008). However, field studies conducted from 2000 to 2003 and in 2005 indicated that Bollgard II® showed greater efficacy than Widestrike® or Bollgard® when bollworm pressure was high, with insignificant differences between second-generation technologies under light or moderate pressure (Bacheler et al. 2006). Additional trials confirmed that extreme bollworm pressure does create measurable differences in control between the available dual-toxin *Bt* technologies. Efficacy trials conducted by Greene and Robinson (2010) during 2006-2009 found that Widestrike® suffered more damage from bollworm than Bollgard II® but that both technologies benefited from supplemental control. Because neither technology demonstrates 100% control of bollworm and these pests have the ability to cause economically significant damage, action thresholds need to be developed specifically for each technology. Current action thresholds for second-generation *Bt* cotton are similar to those recommended for original Bollgard® cotton, without the threshold based on egg density. However, small but quantifiable differences in bollworm control exist between Bollgard II® and Widestrike® cotton varieties. Because levels of feeding injury can be different between technologies, this project aims to measure differences between dual-gene technologies and develop action thresholds specific to each.

Materials and Methods

Experimental plots were established at the Edisto Research and Education Center near Blackville, SC, in an area of historically high bollworm pressure (Pitts et al. 1999, Greene and Robinson 2010). Plots were defined as 8 rows by 12.2 m (40 feet) and replicated 4 times. All plots were oversprayed with an organophosphate at least once before and at least twice after treatment initiation to decimate natural enemies, increasing the chances for significant pressure from bollworm, and to reduce the impact of hemipterans (true bugs), respectively. All insecticide applications at treatment thresholds were with a pyrethroid insecticide at the highest labeled rate. Three tests (Egg Density, Larvae in Blooms, and Boll Damage) containing replicated and randomized plots of non-Bt (DP174RF), Bollgard II® (DP0949B2RF) and Widestrike® (PHY565WRF) cotton were planted on 14 May 2010. Data were analyzed with Agricultural Research Manager (ARM) software (Gylling Data Management, Brookings, SD).

Test 1: Egg Density

Following first bloom, Test 1 was monitored weekly for bollworm eggs. Egg density was estimated by visually examining 25 plants per plot. Because bollworm eggs are generally deposited on the top third of the cotton plant, and most are concentrated near plant terminals (Gore et al. 2002), eggs were counted on leaves, terminals, pre-floral buds (squares), bracts, and stems using a modified whole-plant search where the top 25% of the plant was searched thoroughly, along with a quick inspection of structures lower in the canopy. There were five different treatments based on egg density: untreated control, sprayed weekly from first week of bloom, at 25, 75, and 125 eggs per 100 plants.

Test 2: Larvae in Blooms

At the sign of bloom initiation, Test 2 was monitored weekly for caterpillars by visually examining 25 blooms (*in situ*) per plot for larvae classified as small (< 0.635 cm [0.25 inch]) or large (> 0.635 cm [0.25 inch]). There were five different treatments based on caterpillar density: untreated control, sprayed weekly from the first week of bloom, at 4 or 5, 15, and 25 larvae per 100 blooms.

Test 3: Boll Damage

After the first cohort of bolls reached ~1.27 cm in diameter ("dime" size at ~0.5 inch) in all varieties, Test 3 was examined weekly by visually examining 25 bolls (*in situ*) per plot for feeding injury from bollworm. Bolls were considered "damaged" when at least one site on a boll wall was compromised or penetrated by lepidopteran feeding injury. There were five different treatments based on boll damage: untreated control, sprayed weekly from first week of bloom, at 5, 10, and 20% boll damage.

Results and Discussion

Test 1: Egg Density

The fully protected plots of each technology received 8 applications of insecticide (Table 1). The threshold of 25 eggs/100 plants was reached 7 times in all technologies, and WideStrike® and Bollgard II® received 3 and 2 applications, respectively, at 75 eggs/100 plants. Although average numbers of eggs did exceed 100 eggs/100 plants during the season, none of the technologies received insecticide at a threshold of 125 eggs/100 plants (Table 1).

Table 1. Total number of insecticide applications applied to each technology and treatment threshold of varying bollworm egg density during 2010 near Blackville, SC (Test 1 – Density of Bollworm Eggs).

Technology	Threshold (bollworm eggs/100 plants)				
	UTC	Weekly	25	75	125
Non-*Bt*	0	8	7	0	0
Widestrike®	0	8	7	3	0
Bollgard II®	0	8	7	2	0

Significantly fewer eggs were detected in the non-*Bt* control over the season (Figure 1), presumably because non-*Bt* cotton suffered heavy feeding damage, degrading the quality of oviposition sites over time. Because pre-floral buds (squares) and blooms in non-Bt plots were destroyed/consumed by the population of caterpillars, female bollworm moths likely avoided non-Bt plots due to plant volatiles released from the feeding injury and lack of floral cues used to select suitable hosts. More eggs were detected in WideStrike® plots than in Bollgard II® plots over the season (Figure 1). This might be explained by differences in maturity between the two varieties. Data collected concerning maturity (NAWF counts) will be analyzed to determine if there was a difference in the number blooms present across varieties during the time frame of heaviest oviposition. If more blooms were present in WideStrike® plots than in Bollgard II® plots during this extended event, it might help explain the difference in oviposition across the *Bt* technologies.

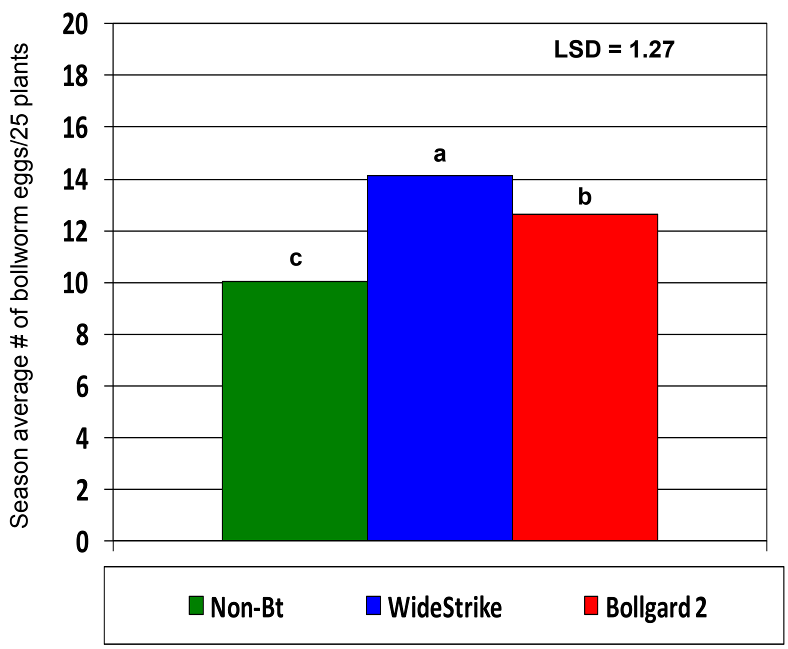

Figure 1. Number of eggs per 25 plants averaged across the season by technology during 2010 near Blackville, SC (Test 1 – Density of Bollworm Eggs).

Yield data demonstrated significantly reduced yields in plots of non-*Bt* cotton, despite aggressive application of insecticide for bollworm (Figure 2). Yields from plots of WideStrike® and Bollgard II® that were aggressively protected from bollworm (7 or 8 applications of insecticide) were comparable. Across all technologies, yields consistently increased with the number of insecticide applications. There were no significant differences among yields from untreated plots and those treated weekly or at 25 or 75 eggs/100 plants with WideStrike® technology (Figure 2). Plots of Bollgard II® treated weekly or at 25 eggs/100 plants yielded statistically higher yields than those treated at 75 eggs/100 plants or those left untreated. With the differences in yield observed in this test and at lint prices above $1/lb, net returns will likely favor aggressive control of bollworm in dual-*Bt*-gene cotton. If the value of lint continues to remain high, an aggressive treatment threshold using bollworm eggs as a trigger might be a valid approach.

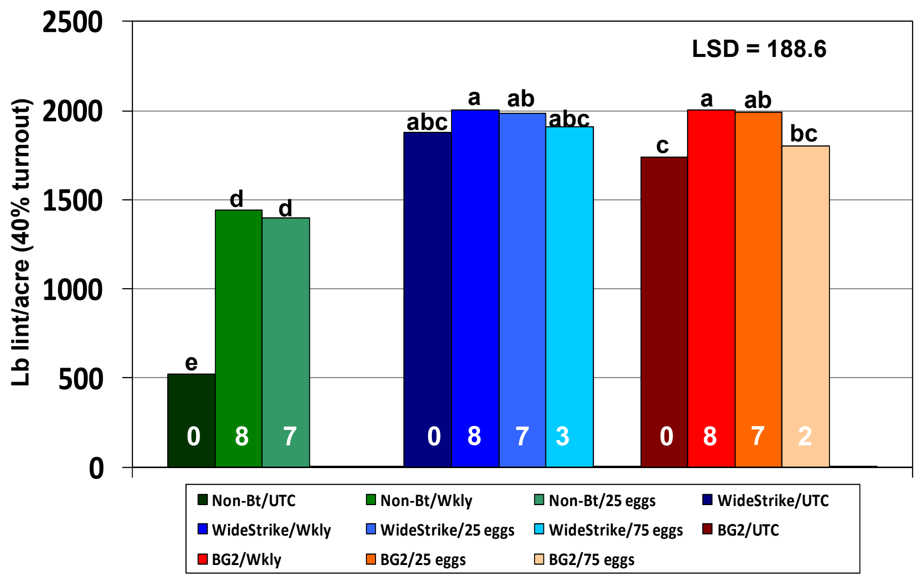

Figure 2. Lint yield per acre by technology and treatment threshold of varying bollworm egg density during 2010 near Blackville, SC (Test 1 – Density of Bollworm Eggs). Number in bar represents seasonal total number of insecticide applications to that treatment.

Test 2: Larvae in Blooms

The fully protected plots of each technology received 8 applications of insecticide (Table 2). The threshold of 5 larvae/100 blooms was reached 7, 3, and 2 times in plots of non-*Bt*, WideStrike® and Bollgard II®, respectively. Plots of non-Bt cotton were treated 5 or 4 times at 15 or 25 larvae/100 blooms, respectively. High bollworm pressure was observed in the untreated non-*Bt* plots, leading to considerable damage by caterpillars. There were often no white blooms located in non-*Bt* plots after extensive searching. When no blooms were located, all blooms were considered damaged in those plots. No more than 4-5 bollworm larvae/100 blooms were found in plots of Widestrike® or Bollgard II®.

Table 2. Total number of insecticide applications applied to each technology and treatment threshold of varying bollworm larval density during 2010 near Blackville, SC (Test 2 – Density of Bollworm Larvae in Blooms).

Technology	Threshold (bollworm larvae/100 blooms)				
	UTC	Weekly	4-5	15	25
Non-*Bt*	0	8	7	5	4
Widestrike®	0	8	3	0	0
Bollgard II®	0	8	2	0	0

Significantly more bollworm larvae were detected in blooms from plots of non-*Bt* cotton than in WideStrike® or Bollgard II® (Figure 3). Numbers of larvae in blooms in untreated and fully protected plots of WideStrike® and Bollgard II® were similar. Numerically more larvae were observed in WideStrike® at thresholds of 15 and 25 larvae/100 blooms than in Bollgard II®, which remained untreated during the season (Figure 3).

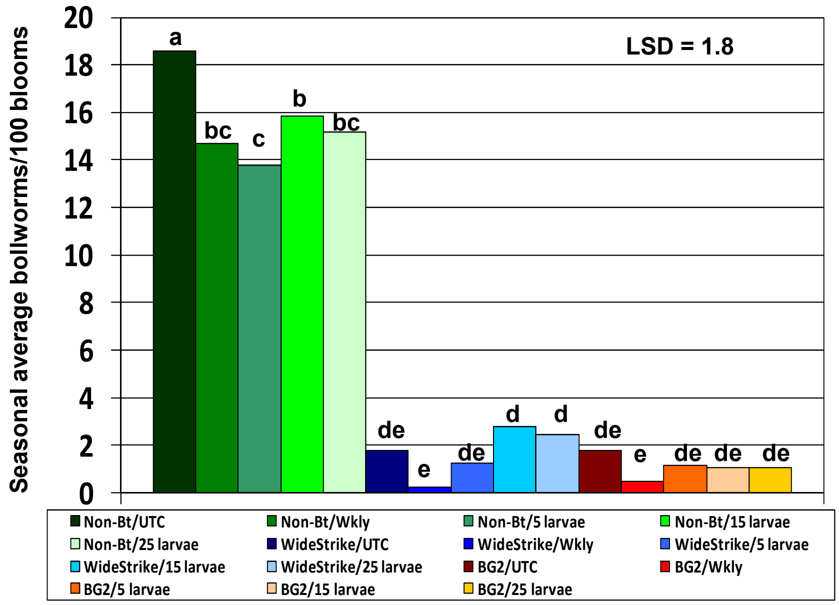

Figure 3. Season average bollworm larvae/25 blooms/plot by technology and treatment threshold of varying bollworm larval density during 2010 near Blackville, SC (Test 2 – Density of Bollworm Larvae in Blooms).

Yield data demonstrated significantly reduced yields in plots of non-*Bt* cotton, despite aggressive application of insecticide for bollworm (Figure 4). Yields from plots of WideStrike® and Bollgard II® that were aggressively protected from bollworm (8 applications of insecticide) were comparable. Yields in WideStrike® and Bollgard II® increased with full protection or with insecticide use at 4-5 larvae/100 plants, and the increases were statistically significant with WideStrike®. With the differences in yield observed in this test and at lint prices above $1/lb, net returns will likely favor aggressive control of bollworm in dual-*Bt*-gene cotton. If the value of lint continues to remain high, an aggressive treatment threshold using larvae in blooms as a trigger might be a valid approach.

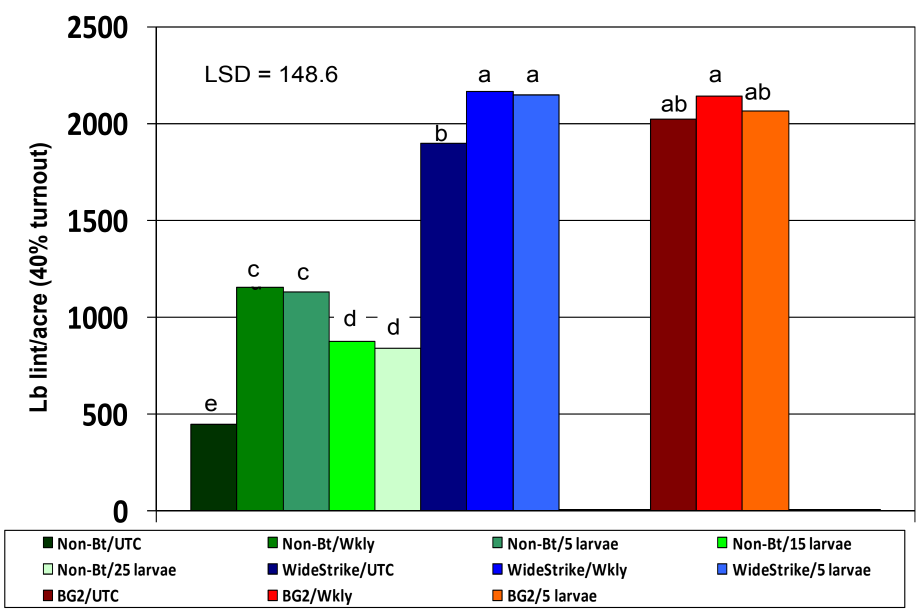

Figure 4. Lint yield per acre by technology and treatment threshold of varying bollworm larval density during 2010 near Blackville, SC (Test 1 – Density of Bollworm Larvae in Blooms).

Test 3: Boll Damage

The fully protected plots of each technology received 8 applications of insecticide (Table 3). The threshold of 5, 10, and 20% boll damage were reached 6 times in plots of non-*Bt* cotton. Plots of WideStrike® and Bollgard II® reached 5% boll damage 4 times, but only WideStrike® reached 10 and 20% boll damage levels (Table 5).

Table 3. Total number of insecticide applications applied to each technology and treatment threshold of varying boll damage during 2010 near Blackville, SC (Test 3 – Boll Damage).

Technology	Threshold (boll damage)				
	UTC	Weekly	5%	10%	20%
Non-*Bt*	0	8	6	6	6
Widestrike®	0	8	4	2	2
Bollgard II®	0	8	4	0	0

Seasonal means of boll damage by technology demonstrated significant feeding damage by bollworm in non-*Bt* cotton (Figure 5). Damage levels in WideStrike® were statistically higher (2.7 fold) than those observed in Bollgard II®.

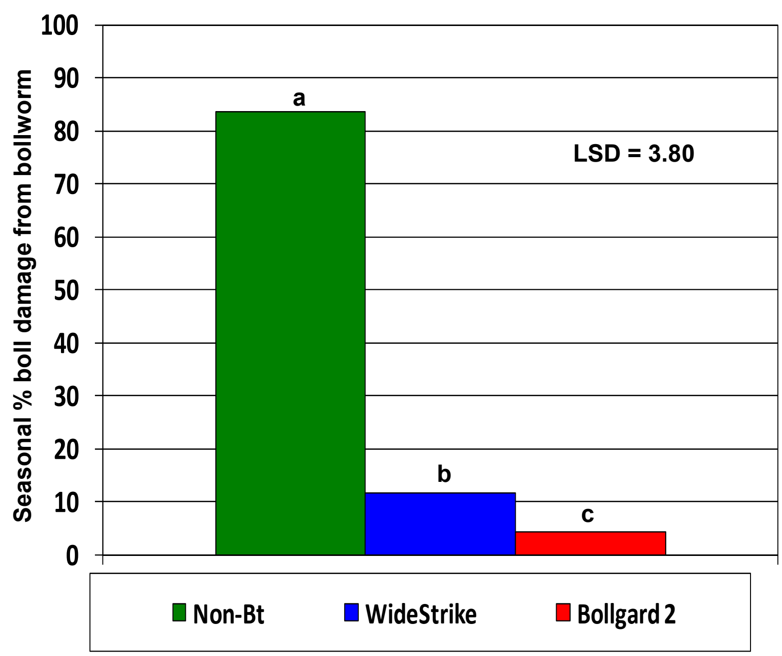

Figure 5. Seasonal boll damage from bollworm by technology during 2010 near Blackville, SC (Test 3 – Boll Damage).

Yield data demonstrated significantly reduced yields in plots of non-*Bt* cotton, despite aggressive application of insecticide for bollworm (Figure 6). Yields from plots of WideStrike® and Bollgard II® that were aggressively protected from bollworm (8 applications of insecticide) were comparable. There were no significant differences among yields from plots treated weekly or at 5% boll damage with both WideStrike® and Bollgard II technologies (Figure 6). As with the previous threshold assessments (egg density and larvae in blooms), the differences in yield observed in this test will likely favor aggressive control of bollworm in dual-*Bt*-gene cotton at lint prices above $1/lb. If the value of lint continues to remain high, an aggressive treatment threshold using boll damage as a trigger might be a valid approach.

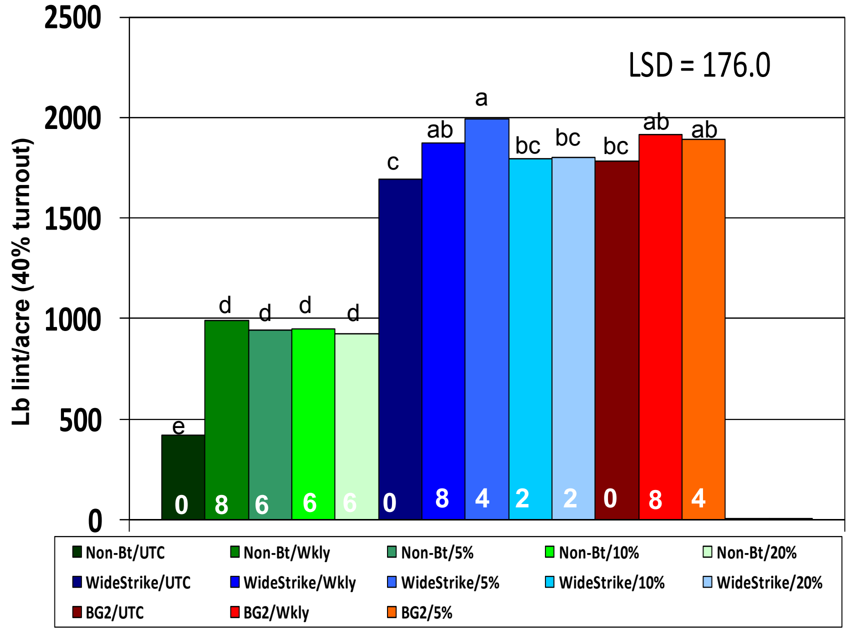

Figure 6. Lint yield per acre by technology and treatment threshold of varying boll damage during 2010 near Blackville, SC (Test 3 – Boll Damage). Number in bar represents seasonal total number of insecticide applications to that treatment.

Summary

In general, yield was related to the number of applications made during the growing season. Although the ultimate goal of this research is to find optimum thresholds specific to each dual-*Bt*-gene technology, differences were noted between the technologies. Observed differences supported the need for developing specific thresholds. Non-*Bt* cotton sustained significantly more boll damage than either dual-gene technology, and Widestrike® suffered statistically more boll damage than Bollgard II®. Yields did not suffer proportionately, perhaps because of the unusually good growing conditions during late season in 2010. Widestrike® may have been able to compensate for lost and damaged bolls at the end of the season due to the excellent late-season growing conditions. Continued testing in 2011 will help account for this seasonal variability. Based on inputs and yields, results from the 2010 season indicated that our currently recommended threshold of 5% boll damage might be adequate. At high lint prices (>$1/lb), our threshold of 75 eggs/100 plants might be higher than it should be under heavy pressure from bollworm. A more aggressive approach seemed to be appropriate in 2010. Further work is needed to analyze the economic implications of these data. Input costs and profits must be considered to determine the practical implications of this work.

Acknowledgments

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