12-2012

TREATMENT THRESHOLDS FOR BOLLWORM (*Helicoverpa zea*) IN DUAL-GENE *Bt* COTTON

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TREATMENT THRESHOLDS FOR BOLLWORM (*Helicoverpa zea*) IN DUAL-GENE *Bt* COTTON

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Entomology

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by
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December 2012

Accepted by:
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ABSTRACT

Dual-gene *Bt* cotton has reduced the need for insecticide treatments for bollworm, *Helicoverpa zea* (Boddie), compared with original single-gene *Bt* technology. Bollgard II® (Monsanto, St. Louis, MO) and WideStrike® (Dow AgroSciences, Indianapolis, IN), both produce the Cry1Ac protein and a second protein, Cry2Ab or Cry1F, respectively. These dual-gene *Bt* cottons provide enhanced control of lepidopteran pests, but remain less than 100% effective against bollworm, particularly when population pressure is high. Current recommended treatment thresholds for bollworm on cotton in South Carolina are as follows: treat with insecticides when three or more large larvae are found per 100 plants or when 5% boll damage is detected. Studies were conducted in an area prone to high bollworm pressure near Blackville, South Carolina, in 2010 and 2011 to develop appropriate thresholds in Bollgard II and WideStrike cotton. Plots containing non-*Bt*, WideStrike, and Bollgard II cotton varieties were examined weekly and treated according to treatment threshold protocols for one of the following: bollworm eggs, larvae in white blooms, or boll damage. Although yields increased with insecticide applications in non-*Bt* cotton, statistical differences in yield among thresholds were not evident within the *Bt* technologies. The conclusion drawn from this limited study was that insecticide applications exclusively targeting bollworm were not necessary in dual-gene *Bt* cotton. Higher levels of bollworm infestation and damage occurred in WideStrike cotton, however, WideStrike lint yields in this study did not differ among varying thresholds and so did not support the conclusion that protection strategies be amended for each technology.

ACKNOWLEDGMENTS

I would like to acknowledge the support of my advisor, Dr. Jeremy Greene, and the members of my committee, Drs. Francis Reay-Jones, Mike Jones, and Peter Adler. Thank you also to the other grad students and the members of Edisto REC's bug crew for all of the hard work put in under the hot Carolina sun. A special thanks to Ginger Devinney for your labor in the field, moral support throughout this project, and for your friendship. And finally, I would like to acknowledge the South Carolina Cotton Board for funding this project.

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INTRODUCTION

Historically, the bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), have been major pests of cotton in the southeastern United States. The bollworm/budworm complex was the most damaging and costly of all the cotton insect pests for 13 years between 1979 and 1996 (Diffie et al. 2004). In 2002, the complex was responsible for reducing cotton yields across the US by 613 thousand bales (2.31%) (Williams 2003).

Until the introduction of genetically engineered cotton, the primary means of controlling lepidopteran pests was chemical insecticides. However, resistance to organophosphates and pyrethroids during the 1990s reduced the effectiveness of chemical control (Gore and Adamczyk 2004). In 1996, Monsanto Corporation (St Louis, MO) was the first to commercialize genetically engineered cotton. Bollgard® cotton expressed Cry1Ac proteins from a gene found in the soil bacterium *Bacillus thuringiensis kurstaki* Berliner (*Bt*). The *Bt* gene was introduced into cotton to enable engineered plants to produce their own insecticidal Cry1Ac endotoxin, thus reducing the need for insecticide applications (Perlak et al. 2001, Gore and Adamczyk 2004).

Bollgard cotton was found to be highly effective on *H. virescens* and moderately-to-highly effective against *H. zea*. In most situations, annual applications of insecticide remained necessary to prevent yield loss from bollworm because the species is less susceptible than tobacco budworm and often avoids mortality through larval behavior such as feeding on blooms which contain lower levels of the toxin (Gore et al. 2003).

Because Cry1Ac is variably expressed in the cotton plant, some plant parts (such as the blooms) have lower concentrations of the toxin (Gore and Adamczyk 2004). In addition to differences in titer of toxin by plant structure, crop maturity also affects the level of Cry1Ac expression (Gore et al. 2003). Greenplate et al. (1998) found that expression of the Cry1Ac toxin was non-uniform throughout the plant, was often lower in cotton blooms, and decreased in squares and bolls as the growing season progressed (Greenplate 1999). In diet choice studies, bollworm larvae were able to discriminate between diet containing Cry1Ac and untreated diet and showed preference for the untreated diet (Greenplate et al. 1998). Behavior modification, differential survival on blooms, and overall general reduced susceptibility to *Bt* proteins were cited as reasons why bollworm were able to survive on *Bt* cotton (Gore and Adamczyk 2004).

Action thresholds based on the number of eggs, number and size of larvae, and on observed boll damage were refined because Bollgard was not 100% effective in controlling bollworms (Sullivan et al. 1998). In 2003, Monsanto Company released a dual-*Bt* gene cotton technology called Bollgard II®, which produces the original *Bt* protein (Cry1Ac) and a second protein (Cry2Ab). Two years later, Dow AgroSciences (Indianapolis, IN) released WideStrike® cotton, which also produces the original *Bt* protein (Cry1Ac) combined with a different *Bt* protein (Cry1F). These dual-gene *Bt* cotton varieties provide better control of bollworm than the original, single-gene technology, in Bollgard varieties (Gore et al. 2008). Although dual-*Bt* gene technologies further enhance control of caterpillars and reduce the need for insecticides, Bollgard II

and WideStrike cotton varieties do not offer 100% control of bollworm (Greene and Robinson 2010) and continued refinement of treatment thresholds is warranted.

In laboratory studies conducted by Stewart et al. (2001), the greater toxicity of dual-gene *Bt* cotton on lepidopterans compared with single-gene *Bt* cotton was demonstrated. Survival and growth rate were reduced in multiple species, including bollworm, fall armyworm, *Spodoptera frugiperda* (Smith), and beet armyworm, *Spodoptera exigua* (Hübner). Stewart et al. (2001) concluded that dual-toxin technologies would be more effective and have a wider range of activity than first-generation *Bt* cotton.

Differential toxin expression in the plant between Cry2Ab (Bollgard II) and Cry1F (WideStrike) are issues that exceed the argument whether two endotoxins are more effective controlling lepidopterans than one endotoxin. Results from field cage experiments conducted in Mississippi to determine bollworm impact on Bollgard II and WideStrike cotton suggested that bollworm would rarely cause yield loss in either technology (Gore et al. 2008). In a study in North Carolina, Bollgard II showed greater efficacy than WideStrike or Bollgard when bollworm pressure was high (Bacheler et al. 2006). Under light or moderate pressure, however, the dual-gene *Bt* technologies did not differ in bollworm control (Bacheler et al. 2006).

Greene and Robinson (2010) reported differences between Bollgard II and WideStrike in lint yield potential, sustained boll damage, and compensatory ability from trials conducted in South Carolina. Both technologies benefited from supplemental control of bollworm when exposed to high numbers of bollworm (Greene and Robinson

2010). Because bollworms have the ability to cause economic damage, and neither technology demonstrates 100% control of the species, action thresholds may need to be developed specifically for each dual-gene *Bt* technology.

Studies comparing efficacy between Bollgard, Bollgard II, and WideStrike cotton varieties under naturally occurring high pressure have been conducted (Bacheler et al. 2006, Greene and Robinson 2010), as have trials to investigate injury levels from artificially infested dual-gene *Bt* cotton (Gore et al. 2008). However, current threshold recommendations for dual-*Bt* gene cotton in South Carolina remain similar to thresholds used for bollworm on single-gene *Bt* technologies, less the egg threshold recommendation (Greene and Robinson 2010).

The objective of this study was to refine action thresholds for each dual-gene *Bt* technology in order to better enable growers to manage bollworms. These studies address the development of thresholds for bollworm in Bollgard II and WideStrike cotton in areas prone to historically high natural infestation by the species. Thresholds based on egg density, larvae in blooms, and percent boll damage were investigated during the 2010 and 2011 growing seasons.

LITERATURE REVIEW

Cotton, *Gossypium hirsutum* L., is a perennial plant of tropical origin that is grown as an annual crop in the United States. Cotton has an indeterminate fruiting pattern and produces more fruit each season than can be matured (Guinn 1982). From a study in Louisiana, only 24-36% of flowers produced during a growing season matured to harvestable bolls (Kennedy et al. 1991), and other studies have shown higher and lower rates of boll production from blooms. First position bolls on sympodial branches are the most valuable fruits in terms of yield (Gore et al. 2000) and, under ideal conditions, the first position sympodial locations may produce as much as 35% more harvestable bolls than sites at or beyond the second position (Jenkins et al. 1990, Jones and Snipes 1999).

Cotton's indeterminate growth habit allows it to withstand the loss of fruiting structures without significant reduction in yield. Fruit abscission is a natural occurrence that brings the fruit load into balance with the available nutrient, carbohydrate, and water supply (Guinn 1982). Fruit can be abscised due to abiotic causes such as nutrient deficiency, water stress, temperature, and mechanical injury, as well as from biotic causes such as insects and pathogens (Guinn 1982). Cotton can compensate for abscised bolls; however, if the pressure is at a high enough level, economic damage will occur.

A wide spectrum of insect pests can cause economic damage and yield loss in cotton, such as thrips, plant bugs, stink bugs, fleahoppers, and caterpillars (Gore et al. 2000, Adamczyk and Burris 2004). After eradication of the boll weevil, *Anthonomus grandis grandis* Boheman, from the Southeast and before the release of transgenic cotton varieties containing genes from the bacterium *Bacillus thuringiensis* (*Bt*), some of the

primary pests of the crop in the United States were pink bollworm, *Pectinophora gossypiella* (Saunders), bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.) (Gore et al. 2000). In the 2002 cotton insect losses presented at the Beltwide Cotton Conferences, Williams (2003) reported that the bollworm/budworm complex reduced US cotton yields by 613, 102 bales (2.31%).

On cotton, female bollworm and tobacco budworm moths deposit their eggs on young leaves and points of growth (Guinn 1982). In general, bollworm eggs are deposited on the top third of the cotton plant and most are concentrated near plant terminals (Gore et al. 2002). After the eggs hatch, larvae move down the plant and feed on young tissue, squares, and bolls and progress to feed on more mature bolls as they grow. Caterpillar feeding damage stimulates the plant to produce ethylene which can trigger shedding of damaged squares or bolls (Guinn 1982).

In 1996, Monsanto Corporation (St. Louis, MO) was the first to commercialize genetically engineered *Bt* cotton. *Bacillus thuringiensis* (*Bt*) is a gram-positive soil bacterium that naturally produces a protein crystal structure during sporulation. Insects that ingest this crystalline structure solubilize it with proteases of the midgut where the environment is at the right alkaline pH level: Solubilized proteins release δ-endotoxins which then interact with the midgut epithelium. Membrane integrity is compromised in sensitive individuals and those insects may die from starvation, paralysis, or septicemia (Gill et al. 1992). Several *Bt* genes have been identified, which code for the production of toxic proteins. The primary structure is dependent on the coding gene and the specific endotoxin released is toxic to different insect groups. The Cry1 or Cry2 proteins are toxic

to lepidopterans (Gill et al. 1992). Bollgard® cultivars expressed the Cry1Ac endotoxin which reduced the need for insecticide applications for lepidopteran pests such as the tobacco budworm and bollworm. These *Bt* proteins were found to be safe for human use and target-specific to the insect order Lepidoptera (Perlak et al. 2001, Gore and Adamczyk 2004).

Transgenic *Bt* cotton has demonstrated very good control of *H. virescens* and *P. gossypiella* (Williams 2000). While *Bt* cotton is toxic to both the tobacco budworm and the bollworm, it is more active against tobacco budworm. Soon after the introduction of *Bt* cotton, it was determined that bollworms often required supplemental treatment (Layton et al. 1997). The Cry1Ac *Bt* toxin suppressed bollworm populations, but economic injury still occurred under pressure from large populations (Pitts et al.1999, Gore et al. 2003, Greene and Robinson 2010).

Smith (1997) noted that bollworm numbers peaked twice during the 1996 growing season in Alabama. During mid-to-late July, the peak was attributed to the movement of moths from maturing corn into cotton. In early September, high survival of bollworm on cotton was attributed to elevated numbers early in the season and later to location where eggs were laid on the plants (Smith 1997). This pattern continues to be present in the Southeast. Pheromone trap numbers for bollworm from 2007 to 2009 in Barnwell County, South Carolina, supported the July/August peak in numbers reported previously (Greene and Robinson 2010).

Egg location on the plant affects rates of larval survival because the *Bt* toxin is not uniformly expressed throughout the plant (Adamczyk et al. 2001). Bollworm has a broad

host range and early-season larvae primarily develop on weed hosts (Head et al. 2010). The complex of bollworm and tobacco budworm has been reported to feed collectively on over 130 plant hosts (Diffie et al. 2004). Corn and sorghum are major hosts for the complex from mid-June to mid-July, and movement (called flights) to hosts such as cotton, soybeans, and peanuts occurs later in the season (Head et al. 2010). In the Southeast, the critical flight of bollworm moths generally occurs in mid-July (Sullivan et al. 1993, Smith 1997). Using certain broad-spectrum insecticides just before a large bollworm flight can actually increase crop damage because predaceous arthropod populations are decimated (Turnipseed and Sullivan 1999). Natural enemies of bollworm such as lacewings, lady beetles, geocorids, and other predaceous bugs can reduce bollworm populations and their associated crop injury (Lopez et al. 1976, Hutchinson and Pitre 1983).

Pheromone traps are used to monitor moth activity, but trap numbers are often poorly associated with larval densities in the field (Diffie et al. 2004) because moths are extremely mobile and the specific crops within a localized area have little impact on populations of *H. zea* (Jackson et al. 2003). However, corn may impact the total population on a larger scale. Diffie et al. (2004) found a significant correlation between corn acreage and populations of bollworm (Diffie et al. 2004). The wide host range and mobility of *H. zea* make it difficult to characterize what factors in the agroecosystem are contributing to population numbers (Jackson et al. 2003).

The expression of Cry1Ac in Bollgard cotton varied with the structure and maturity of that structure (Gore et al. 2003). Adamczyk et al. (2001) used an ELISA test

to corroborate with earlier studies (Fitt 1998, Holt 1998, Sachs et al. 1998, Greenplate 1999) showing that levels of toxin decreased in many plant parts as the season progressed. The more than 25 different *Bt* varieties expressed dissimilar levels of Cry1Ac δ-endotoxin (Adamczyk et al. 2001). Caterpillars that survived *Bt* toxins (called "escapes") from Bollgard cotton were observed in Alabama (Smith 1997) and were originally thought to be from extremely high bollworm numbers. However, escapes were also observed under moderate pressure, and further investigation showed that eggs laid on dried blooms (bloom tags) led to the increased rate of survival (Smith 1997). The bloom tags did not express a lethal amount of *Bt* toxin, allowing caterpillars to feed and increase in size. Although there were no observed preferences for bloom tags in *Bt* cotton versus conventional varieties (Smith 1997), bollworm larvae were able to discriminate between diet containing Cry1Ac and untreated diet and consequently showed preference for the untreated diet (Greenplate et al. 1998). In field trials conducted by Adamczyk et al. (2001) in Mississippi, it was reported that bollworms were predominantly found feeding on flowers, squares, and bolls as opposed to meristematic tissue where *Bt* protein levels are highest.

Bollworm may preferentially oviposit near flowers in *Bt* cotton. Gore et al. (2002) found more bollworm feeding on white blooms in Bollgard cotton versus conventional, non-*Bt* cotton in Louisiana. Tobacco budworms disperse differently on *Bt* cotton versus conventional varieties, with more caterpillars moving away from the plant terminals and at a faster rate on *Bt* cotton compared with movement on non-*Bt* varieties (Parker and Luttrell 1999). Gore et al. (2002) found that bollworm larvae began to migrate away from

Bollgard terminals within the first hour of eclosion. Larvae of bollworm and tobacco budworm might detect the *Bt* proteins and exhibit an avoidance response. Studies by Greenplate (1999) found decreased levels of Cry1Ac δ-endotoxins in squares and bolls, as well as increased feeding by tobacco budworm as the growing season progressed, supporting the hypothesis that differences in *Bt* expression are based on structure and maturity. Bollworm larvae that feed lower on the plant and on older reproductive components are more likely to survive than those that feed on fresh tissue such as white blooms. The feeding habits and subsequent reduced susceptibility of bollworm to Cry1Ac endotoxins make *H. zea* more likely to survive and damage Bollgard cotton (Gore et al. 2003).

During the first three years of commercial-use of transgenic *Bt* cotton, additional bollworm control, in the form of foliar insecticide applications, was required in order to prevent economic injury (Smith 1997; Layton et al. 1997, 1998; Leonard et al 1997, 1998; Roof and Durant 1997; Gore et al. 2000). In a survey conducted across Mississippi in 1997, it was reported that transgenic cotton was effective in controlling tobacco budworm, but high populations of bollworm still had the capacity to cause excessive damage in some cases (Layton et al. 1998).

Roof and Durant (1997) found that at least one insecticide application was required for *Bt* cotton compared with 4.8 applications in conventional cotton fields in South Carolina. Despite reduced insecticide use, yield increases of 11 and 23% were observed in *Bt* cotton treated with additional insecticide applications in Louisiana (Leonard et al. 1998). Although *Bt* toxins specifically target lepidopteran pests,

supplemental foliar insecticides are also necessary to control bollworm escapes and other insect pests (Leonard et al. 1997, 1998). The targets of insecticide applications in transgenic *Bt* cotton in South Carolina were initially defined as stink bugs and bollworm (Roof and Durant 1997). Across the Cotton Belt, secondary insects such as stink bugs, plant bugs, and armyworms became more prominent pests in late season Bollgard cotton (Pitts et al. 1999).

Mi et al. (1998) reported that monitoring eggs on plants to anticipate feeding damage from caterpillars was no longer useful in transgenic cotton because the *Bt* technology should kill newly hatched larvae. However, Sullivan et al. (1998) recommended an egg threshold of 75 eggs per 100 plants because small larvae feeding underneath bloom tags could survive and were difficult to detect. At two locations in South Carolina (one using disruptive insecticides to decimate natural enemies and the other left undisturbed), insecticide applications using the egg threshold as opposed to the escaped worm threshold (8 large larvae per 100 plants) increased lint yields by 65 and 93 kg/ha (58 and 83 lb/acre), respectively (Sullivan et al. 1998). Transgenic technology and the use of more selective insecticides have made insect pest management decisions more complex. Static thresholds based on the experience of the pest manager or recommendations from the local Cooperative Extension Service do not reflect changes in production costs, crop prices, or physiological susceptibility of cotton varieties (Mi et al. 1998).

Because one or two annual insecticide applications may be necessary to prevent economic loss from bollworm, action thresholds were established in most states (Gore et

al. 2008). Action threshold recommendations for bollworm in single-gene *Bt* technology in South Carolina were: 75 eggs, 30 small (<6.35 mm [0.25 in]) larvae, or 3 large (>6.35 mm [0.25 in]) larvae per 100 plants, or 5% boll damage (Greene 2010). However, single-*Bt* gene technology (Bollgard) was no longer commercially available after the 2010 growing season (Greene and Robinson 2010).

Guidelines were developed to prevent or postpone the development of resistance among target insects to *Bt* cotton. The strategy was to combine the planting of cultivars with high doses of the toxin with refuge plantings that contained no toxin (Gould 1998). Mandating refuge planting of non-*Bt* cotton was intended to produce susceptible individuals to mate with resistant adults and thereby prevent the production of resistant offspring (Caprio 1994). The high fitness costs related to Cry1Ac resistance could, however, delay or inhibit field populations of bollworm from developing resistance to Bollgard cotton (Anilkumar et al. 2008). Dual-toxin cultivars are more toxic and have a wider range of activity on lepidopteran pests (Stewart et al. 2001) and may further delay or inhibit the development of resistance.

In 2003, Monsanto released a dual-toxin *Bt* cotton called Bollgard II® that expresses the original Cry1Ac protein as well as Cry2Ab. In 2005, Dow AgroSciences (Indianapolis, IN) released a dual-toxin technology called WideStrike® that expresses Cry1Ac and Cry1F (Gore et al. 2008). Dual-gene technologies provide enhanced control of lepidopteran pests, but do not offer 100% control of bollworm, and additional insecticide might still be needed (Greene and Robinson 2010).

Laboratory studies conducted by Stewart et al. (2001) clearly demonstrated the greater toxicity of dual-gene *Bt* cotton on lepidopterans over expression of only a single insecticidal protein. In bioassays, larvae fed plant tissues containing both Cry1Ac and Cry2Ab experienced higher mortality than larvae fed on cultivars containing Cry1Ac (Stewart et al. 2001). In another study, the additional gene in Bollgard II that codes for the Cry2Ab protein was also found to increase the mortality of bollworm larvae (Gore et al. 2001). The combination of genes and toxins affected the survival and growth rate of multiple species, including *H. zea, Spodoptera frugiperda* (Smith), and *Spodoptera exigua* (Hübner). Dual-toxin technologies are more effective and have a wider range of activity than first generation *Bt* cotton (Stewart et al. 2001). Second generation *Bt* cotton is generally considered 100% effective against tobacco budworm. It has also enhanced protection against bollworm compared with single *Bt* gene varieties, yet Bollgard II and WideStrike still produce yield gains when there are additional insecticide applications (Greene and Robinson 2010).

Bollgard II and WideStrike cotton vary in efficacy because of the different Cry proteins expressed between technologies. Data from field-cage experiments conducted in Mississippi suggested that bollworm would rarely cause yield loss in either technology (Gore et al. 2008). Bacheler et al. (2006) indicated that Bollgard II had greater efficacy than WideStrike or Bollgard cotton when grown under high bollworm pressure in North Carolina. Dual-*Bt* gene technologies did not differ in controlling light or moderate infestations of bollworm (Bacheler et al. 2006).

Whereas larvae tend to migrate away from terminals in Bollgard and Bollgard II cotton varieties, bollworm are more often found feeding on terminals in WideStrike varieties (Jackson et al. 2010). Bollworm and tobacco budworm have been observed migrating down the plant and away from terminals in *Bt* cotton varieties containing the Cry1Ac gene (Parker and Luttrell 1999, Gore et al. 2002). However, Jackson et al. (2010) concluded that the combination of Cry1Ac and Cry1F proteins in WideStrike did not have any measurable effect on larval movement away from plant terminals as compared with larval movement on a non-*Bt* cotton variety.

Efficacy trials conducted by Greene and Robinson (2010) from 2006 to 2009 in South Carolina found differences in boll damage caused by bollworm between Bollgard II and WideStrike, and both technologies benefited from supplemental control when exposed to extreme bollworm pressure. Although greater losses in lint yield were sustained in some WideStrike varieties than those incurred in Bollgard II varieties, it was speculated that the extended optimal growing conditions allowed the full-season WideStrike variety to compensate for the relatively early and elevated damage caused by bollworm feeding (Greene and Robinson 2010). According to Pitts et al. (1999), the area of South Carolina where this research took place is in the management region, "Savannah River Valley: Eastern Georgia-South Carolina below the lakes" and that "there is no region in the Southeast that has the intensity or predictability of bollworm pressure than this region". Because neither technology demonstrates 100% bollworm control, and these pests have the ability to cause economic damage, action thresholds need to be modified specifically for each technology.

MATERIALS AND METHODS

Species Determination

Populations of bollworm and tobacco budworm were monitored three times per week by counting moths caught in pheromone-baited Hartstack-type traps (Hartstack et al. 1979) placed in undisturbed locations (e.g. near power poles, etc) around row-crop production fields at the Edisto Research and Education Center near Blackville, South Carolina. Pheromone lures (Luretape lures, Hercon Environmental, Philadelphia, PA) for bollworm and tobacco budworm were replaced in each trap (10 traps for each species) every week from May to early October in 2010 and 2011. Trapping data were used to estimate proportions of the two species that were ovipositing and feeding near the location of the trials conducted in this study.

Caterpillars were collected from non-*Bt*, WideStrike, and Bollgard II cotton varieties on 2, 6, and 16 August 2011, and late instars were identified using a dissecting scope based on a distinguishing character of the mandibles. Tobacco budworms have a tooth-like projection on the inner surface of the mandibles, whereas bollworms do not have this projection (Boyer et al. 1977, Jia et al. 2007). Because early instars are difficult to manipulate and mandibular characters are indistinguishable under the dissecting scope, early instars were kept and held on artificial diet until large enough to examine as late instars. The combination of data from pheromone traps and the dissections served to determine abundance of each species.

Voucher specimens of one *Helicoverpa zea* and one *Heliothis virescens* larvae were collected 9 August 2011from cotton at the Edisto REC near Blackville, South

Carolina. Specimens were preserved in 80% ethyl alcohol and deposited in the Clemson University Arthropod Collection.

Overview of Trials

Three separate replicated trials were conducted in 2010 and 2011 at the Edisto Research and Education Center near Blackville, South Carolina. Each trial consisted of non-*Bt* (DP174RF), WideStrike (PHY565WRF), and Bollgard II (DP0949B2RF) cotton varieties planted on 14 May 2010 and 18 May 2011. Plots were eight rows by 12.2 m (40 ft) and treatments were replicated four times using a randomized complete block design. Standard cotton production practices were followed as outlined in the Clemson University Cooperative Extension Service Cotton Production Guide (Jones et al. 2011). Acephate (Orthene 97), a foliar organophosphate, was applied at 1.09 kg (AI)/ha (1 lb/acre) during the first week of bloom to eliminate predaceous arthropods and maximize bollworm pressure. Insecticides ineffective on lepidopterans, but efficacious on hemipterans were applied twice across the entire test area each season to minimize yield impact. In 2010, thiamethoxam (Centric 40 WG) was applied at 0.07 kg (AI)/ha (2.5 oz/acre) on 22 July and dicrotophos (Bidrin 8 EC) was applied at 0.56 kg (AI)/ha (8 oz/acre) on 9 August. In 2011, methyl parathion (Methyl 4 EC) was applied at 0.84 kg (AI)/ha (1.5 pt/acre) on 18 July to both control hemipteran populations and also disrupt beneficial arthropods. Dicrotophos (Bidrin 8 EC) was applied at 0.56 kg (AI)/ha (8 oz/acre) on 4 August. Plots meeting or exceeding targeted action thresholds for bollworm

(Table 1) were sprayed weekly alternating between *beta*-cyfluthrin at 0.023 kg (AI)/ha (2.6 oz/acre) and *lambda*-cyhalothrin at 0.045 kg (AI)/ha (5.12 oz/acre).

Table 1. Target action thresholds for bollworm eggs, larvae in blooms, and boll damage in cotton near Blackville, South Carolina, in 2010 and 2011.

Threshold type	Treatments				
Egg density (Test 1)	Untreated control	Sprayed weekly	25 eggs per 100 plants	75 eggs per 100 plants	125 (100)* eggs per 100 plants
Larvae in white blooms (Test 2)	Untreated control	Sprayed weekly	4 or 5 larvae per 100 blooms	15 larvae per 100 blooms	25 larvae per 100 blooms
Boll damage (Test 3)	Untreated control	Sprayed weekly	4 or 5% boll damage	10% boll damage	20% boll damage

*Parentheses indicate modified threshold for 2011

Test 1- Egg Density Threshold

Following first bloom, plots were monitored weekly for bollworm eggs. Because bollworm eggs are deposited on the top third of the cotton plant and most concentrated near the plant terminals (Gore et al. 2002), egg density was determined by visually examining the top 20% of 25 plants per plot. Plants sampled were located in the middle four rows and away from the plot edge. Eggs were counted on leaves, terminals, pre-floral buds (squares), bracts, and stems.

Test 2- Larvae Density Threshold

At bloom initiation, plots were monitored weekly for caterpillars by visually examining 25 blooms (*in situ*) per plot and classifying larvae present as small, <6.35mm (0.25 in), or large, >6.35mm (0.25 in). Blooms were chosen from the middle four rows and away from plot edges. When fewer than 25 white blooms were observed per plot, the

numbers of caterpillars in available blooms were extrapolated. If no blooms were present in a plot, larvae density was assumed to have reached the highest threshold. Larvae were initially categorized as small or large, but numbers of small and large caterpillars were totaled per plot for analysis.

Test 3- Boll Damage Threshold

After the first cohort of bolls reached "dime" size in all varieties, approx. 12.7 mm (0.5 in) in diameter at widest point, plots were examined weekly by visually examining 25 bolls (*in situ*) per plot for bollworm feeding injury. Bolls were chosen from the middle four rows and away from plot edges. Bolls were considered "damaged" when at least one site on the boll wall was compromised or penetrated by lepidopteran feeding injury. When there were fewer than 25 bolls per plot, missing bolls from fruiting positions were considered damaged and those treatments were considered above treatment threshold.

Plant Measurements

In 2010 and 2011, stand counts were taken to monitor stand uniformity and verify that plot yield would not be impacted by non-uniform stands. During 2010, numbers of plants in one meter of row were counted in four locations in each plot (4 m total). In 2011, total number of plants in rows four and five were counted (each row being 12.2 m).

Nodes above white flower (NAWF) counts were taken three times each season to assess plant maturity and determine physiological "cutout", indicating a maturing crop and last cohort of harvestable bolls (Bernhardt et al. 1986). In the Southeast,

physiological cutout is generally thought to have occurred when plants average five or fewer nodes above the highest first position white flower (Bernhardt et al. 1986).

Before the 2011 harvest, plant mapping was done in response to data from 2010 that suggested significant yield compensation in response to bollworm injury occurred. Five plants per plot were measured, examined, and mapped to look for compensatory growth behavior. All bolls were counted and considered harvestable, worm-damaged, unharvestable, or abscised. Node and branch position were also noted. Following plant mapping, cotton was mechanically harvested and plot yields were calculated assuming 40% lint turnout.

Statistical Analysis

Data for each test were subjected to a two-way repeated measures analysis of variance with date and treatment threshold as fixed effects and replication as a random effect (PROC MIXED, SAS Institute Inc. 2011). Data failing the Shapiro-Wilkes test for normal distribution were transformed prior to ANOVA. Egg data were transformed using $\log(x+1)$, larvae data were transformed using $\sqrt{(x+1)}$, and boll damage data transformed using $\arcsin\sqrt{(\text{proportion of damage})}$. Tukey mean separation tests were also performed using SAS 9.3 (SAS Institute Inc. 2011). Node above white flower data were subjected to a one-way repeated measures analysis of variance with date as a fixed effect (SAS Institute Inc. 2011).

RESULTS AND DISCUSSION

Overview

Species Determination

Eggs were estimated to be primarily those of bollworm and not tobacco budworm based on pheromone moth trap data (Figure 1) and caterpillar collection and identification data. Moth populations peaked in late August for both species in 2010 and 2011. However, bollworm and tobacco budworm peak numbers were lower in 2011 than in 2010. Factors such as overwintering conditions for pupae, and other seasonal variation may largely have accounted for this difference. The months of December preceding the 2010 and 2011 cotton seasons were markedly different in temperature and precipitation. In December 2009, the average minimum temperature was 2.5 °C (36.4 °F), with an extreme low of -3.5 °C (25.7 °F). The following December had a sustained period of cold temperatures, with average lows of -2.8 °C (27.0 °F) and an extreme low of -9.9 °C (14.2 °F). December 2009 was both warmer and wetter with recorded rainfall of 24.6 cm (9.7 in) compared to December 2010 and 6.22 cm (2.45 in) of recorded rainfall (National Climatic Data Center). Soil conditions impact bollworm survival because bollworm pupae overwinter in the soil. The harsher winter conditions in December 2010 may have been a significant factor in reducing the overwintering population of bollworm thus reducing the numbers found on cotton during the 2011 growing season.

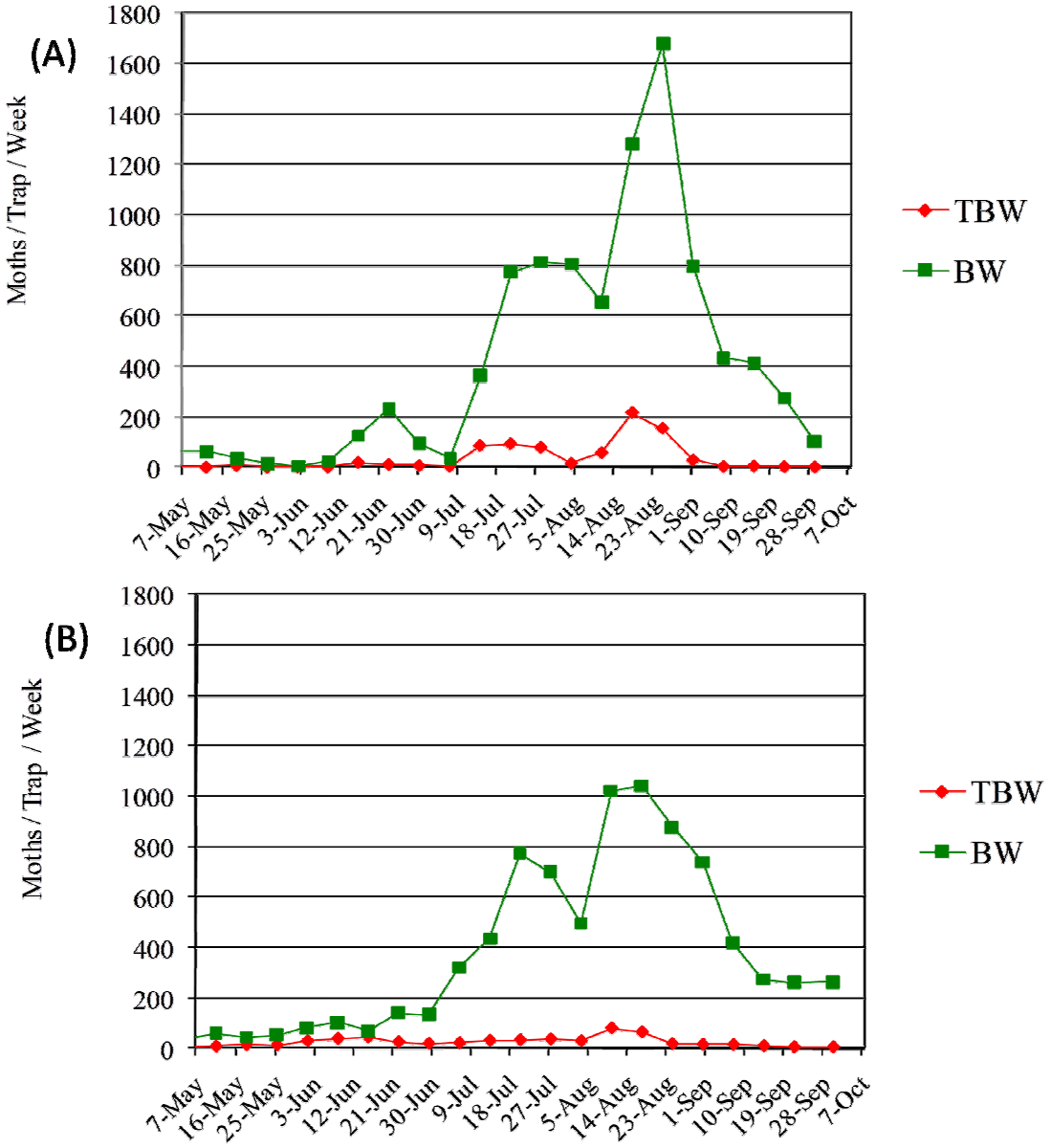

Figure 1. Numbers of bollworm (BW) and tobacco budworm (TBW) adults caught in pheromone traps baited weekly near Blackville, South Carolina, in 2010 (A) and 2011 (B).

Of the caterpillars collected (31) in 2011 from *Bt* cotton varieties, all were bollworm. Only 2 of the 70 caterpillars found in non-*Bt* cotton were tobacco budworm. It was expected that tobacco budworm larvae would not be found on dual-gene *Bt* cotton because *Bt* endotoxins exhibit complete field control of tobacco budworm (Stewart et al. 2001). Tobacco budworm represented only about 3% of the two-species when considering only data from non-*Bt* cotton. The pheromone trap data also showed a larger number of bollworm adults compared with tobacco budworm adults (Figure 1). All eggs, larvae, and plant injury counted were therefore presumed predominantly from bollworm.

Stand Counts

The recommended plant stand for cotton in South Carolina is 6.6-9.8 plants per row meter (2-3 plants per row foot) (Jones et al. 2011). Stand averages per technology in 2010 were as follows: non-*Bt*, 9.28 ± 0.10; WideStrike, 9.01 ± 0.11; and Bollgard II, 8.85 ± 0.12 plants per row meter. In 2011 non-*Bt*, 6.05 ± 0.10; WideStrike, 6.08 ± 0.11; and Bollgard II, 6.40 ±0.14 plants per row meter. Stands in 2011 were thinner than in 2010; however, differences within cotton technologies were not significant ($P > 0.05$). Lint yield differences within each technology were therefore likely attributable to bollworm feeding damage and variable levels of insecticide protection rather than to variations in stand density.

Crop Maturity (NAWF Counts)

Although comparable maturing varieties were chosen for these trials, measurements were taken to detect potential maturity differences due to multiple factors

including insect injury. Unprotected cotton may experience delayed maturity as resources are diverted to vegetative growth because of insect damage. In 2010, some plots had caterpillar feeding damage so severe that first position white flowers were scarce. Many NAWF data in plots of non-*Bt* cotton could not be determined due to the high level of damage and absence of blooms. Because of high damage and missing data in non-*Bt* cotton in 2010, average NAWF calculations are unreliable measures of plant maturity. Node above white flower trends should decrease over the season as cotton plants mature (Gore et al. 2000). In 2011, cotton maturation was observed over the three dates in all varieties (Table 2).

Table 2. Node above white flower counts (±SEM) and statistical comparisons for 2010 and 2011 by technology, averaged across threshold and test in cotton near Blackville, South Carolina, 2010 and 2011.

Year	Date	Cotton technology		
		Non-*Bt*[a]	WideStrike[a]	Bollgard II[a]
2010	26 July	4.25 ± 0.31 A	4.33 ± 0.15 A	3.97 ± 0.12 A
	5 August	4.76 ± 0.57 A	4.22 ± 0.15 A	3.65 ± 0.12 A
	12 August	4.54 ± 0.28 A	3.90 ± 0.15 A	3.09 ± 0.12 B
F		0.49[b]	2.38[c]	16.96[d]
P>F		0.6141	0.0956	<0.0001
2011	15 July	7.01 ± 0.11 A	6.91 ± 0.12 A	6.88 ± 0.11 A
	29 July	4.60 ± 0.11 B	4.65 ± 0.12 B	4.38 ± 0.11 B
	15 Aug	2.52 ± 0.11 C	2.49 ± 0.12 C	2.14 ± 0.11 C
F		486.72[e]	423.39[f]	683.43[g]
P>F		<0.0001	<0.0001	<0.0001

[a] NAWF counts in the same column and year with a different letter are significantly different
[b] df= 2, 46
[c] df= 2, 155
[d] df= 2, 136
[e] df= 2, 175
[f] df= 2, 174
[g] df= 2, 176

Table 3. Statistical comparisons of bollworm egg and larval densities and boll damage in cotton near Blackville, South Carolina, 2010 and 2011.

Year	Management factor combination	Egg density			Larvae density			Boll damage		
		df	*F*	*P* > *F*	df	*F*	*P* > *F*	df	*F*	*P* > *F*
2010	NBT Threshold	4, 39.2	0.97	0.4356	4, 44.6	3.92	**0.0082**	4, 16.8	2.75	0.0630
	NBT Date	8, 77.4	11.86	**<0.0001**	6, 67.1	79.32	**<0.0001**	5, 46.6	35.79	**<0.0001**
	NBT Threshold*Date	32, 77.4	0.95	0.5449	24, 67.1	2.54	**0.0015**	20, 46.6	1.95	**0.0310**
	WS Threshold	4, 48.5	1.05	0.3910	4, 42.5	10.08	**<0.0001**	4, 36.3	4.42	**0.0052**
	WS Date	8, 71.1	32.16	**<0.0001**	6, 68	20.06	**<0.0001**	5, 62.4	4.26	**0.0021**
	WS Threshold*Date	32, 71.1	0.96	0.5396	24, 68	3.91	**<0.0001**	20,62.4	0.79	0.7199
	BGII Threshold	4, 34.6	1.45	0.2377	4, 39.1	0.97	0.4374	4, 32.2	3.19	**0.0260**
	BGII Date	8, 71	18.01	**<0.0001**	6, 66.1	4.50	**0.0007**	5, 50.6	1.19	0.3254
	BGII Threshold*Date	32, 71	0.95	0.5509	24, 66.1	0.91	0.5851	20, 50.6	1.53	0.1125
2011	NBT Threshold	4, 29.2	3.02	**0.0337**	4, 23.2	2.04	0.1216	4, 17.3	4.91	**0.0079**
	NBT Date	5, 51.5	16.29	**<0.0001**	4, 45.2	5.52	**0.0011**	4, 45	6.01	**0.0006**
	NBT Threshold*Date	20, 51.5	1.07	0.4066	16, 45.2	2.12	**0.0245**	16, 45	1.21	0.2994
	WS Threshold	4, 15.9	0.29	0.8821	4, 22.2	0.48	0.7524	4, 20.8	0.69	0.6075
	WS Date	5, 57.9	33.56	**<0.0001**	4, 48.7	7.98	**<0.0001**	4, 49.5	5.49	**0.0010**
	WS Threshold*Date	20, 57.9	0.53	0.9421	16, 48.7	0.19	0.9997	16, 49.5	1.24	0.2699
	BGII Threshold	4, 27.6	1.30	0.2929	4, 20.4	1.00	0.4317	4, 32	4.71	**0.0042**
	BGII Date	5, 60.1	19.39	**<0.0001**	4, 42	10.17	**<0.0001**	4, 46.4	1.48	0.2223
	BGII Threshold*Date	20, 60.1	1.05	0.4196	16, 42	1.08	0.4051	16, 46.4	0.78	0.6960

Table 4. Statistical comparisons of cotton lint yield near Blackville, South Carolina, 2010 and 2011.

Year	Management factor	Egg density test			Larvae density test			Boll damage test		
		df	*F*	*P* > *F*	df	*F*	*P* > *F*	df	*F*	*P* > *F*
2010	NBT Threshold	4, 15	26.58	**<0.0001**	4, 14	37.55	**<0.0001**	4, 15	33.57	**<0.0001**
	WS Threshold	4, 14	0.85	0.5189	4, 14	4.73	**0.0126**	4, 14	0.94	0.4695
	BGII Threshold	4, 14	2.73	0.0715	4, 14	1.47	0.2640	4, 14	3.18	**0.0471**
2011	NBT Threshold	4, 14	10.6	**0.0004**	4, 14	2.57	0.0837	4, 15	9.01	**0.0006**
	WS Threshold	4, 14	0.89	0.4958	4, 14	0.93	0.4746	4, 15	0.84	0.5226
	BGII Threshold	4, 14	1.43	0.2757	4, 14	2.37	0.1028	4, 14	0.89	0.4979

Egg Density Threshold

In 2010, the highest threshold of 125 eggs per 100 plants was never reached in any of the varieties (Figure 2A). The threshold of 75 eggs per 100 plants was met or exceeded three times in WideStrike and twice in Bollgard II. The non-*Bt* control never reached 75 eggs per 100 plants, most likely because it suffered high caterpillar feeding damage. Bollworm egg density is not a good predictor of future damage in dual-*Bt* gene cotton because a large number of the larvae do not survive. However, most larvae from eggs on conventional cotton do survive and feed on the cotton plant until pupation. Lower egg numbers on non-*Bt* cotton were likely the result of diminished floral cues (Callahan 1958), increased plant volatiles, reduced leaf area and fruiting structures, or a combination of all which likely discouraged females from ovipositing after initial infestation and damage.

Egg numbers peaked in all three cotton varieties on 21 July (Figure 2A). There was a second, smaller peak between 11 and 18 August. Peaks were similar to those of adult moth numbers in 2010 (Figure 1A). In Alabama, Smith (1997) attributed peak in bollworm numbers during mid-to-late July to moth movement from maturing corn into cotton. The pattern observed during the current study (Figures 1) and in Alabama (Smith 1997) has been consistent over the past several years in the Barnwell County area of South Carolina (Greene and Robinson 2010).

In 2010, egg densities were not significantly affected by threshold nor was there an interaction between threshold and date for each cotton technology. The lack of a significant treatment effect was probably because the insecticide had little ovicidal effect

and did not deter female moths from ovipositing. For these reasons, application decisions were based on egg density numbers averaged across each variety instead of averaged within threshold. Insecticides were not considered to have had a significant effect on the number of eggs on the plants one week after application.

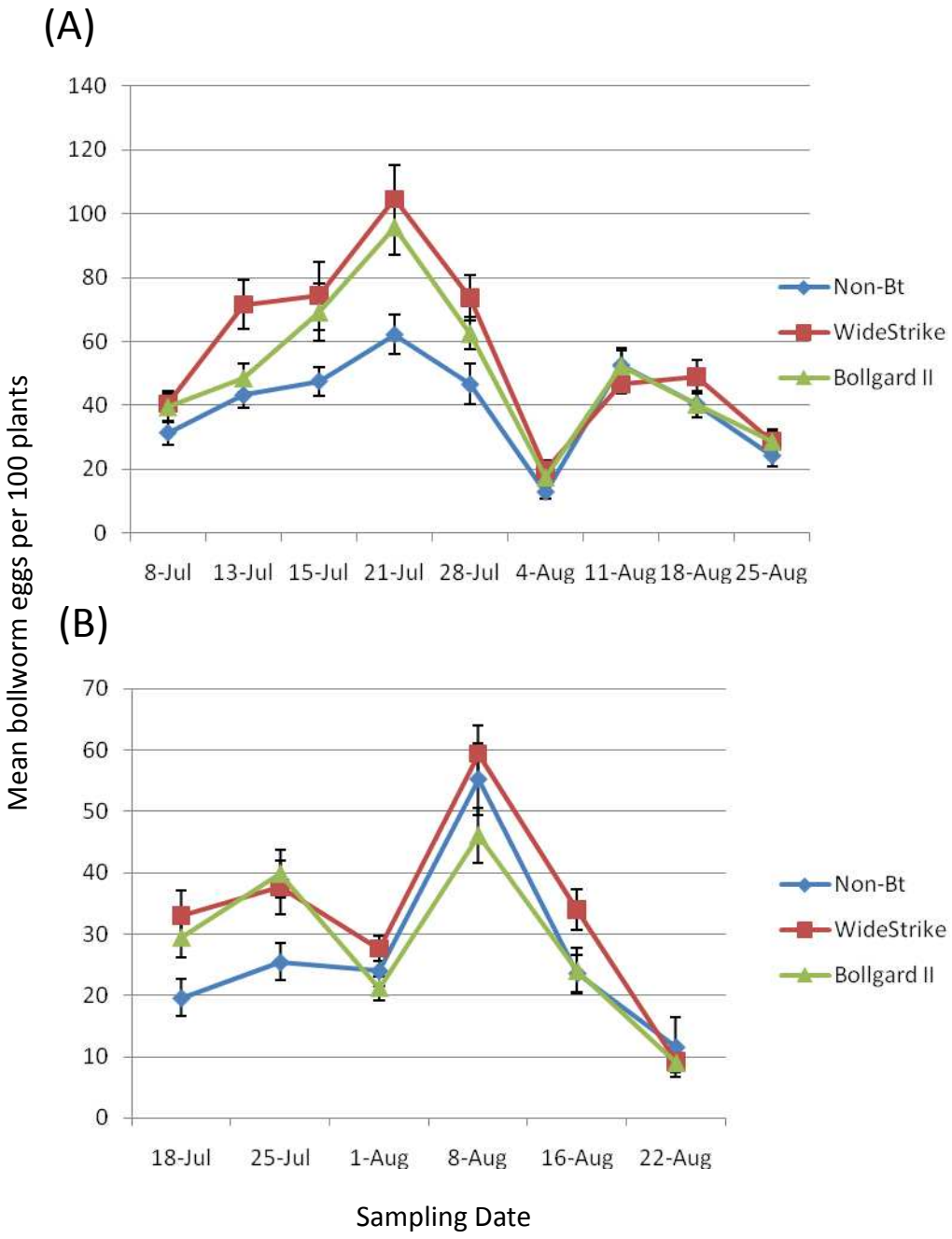

Figure 2. Mean bollworm eggs per 100 plants (±SEM) from egg thresholds by cotton technology near Blackville, South Carolina, in 2010 (A) and 2011 (B).

In 2011, overall egg numbers were lower than in the previous year (Figure 2B). Hot and dry conditions also caused plants to mature faster and shortened the sampling period. Despite lowering the highest egg density threshold from 125 to 100 eggs per 100 plants, the lowered threshold was not reached. Furthermore, the 75 eggs per 100 plants threshold was not met in any variety during 2011. At this same location in 2001, Jenkins et al. (2002) also failed to reach their bollworm thresholds of 75 eggs per 100 plants or four larvae per 100 plants. They concluded that bollworm was not a problem on Bollgard II cotton. A similar conclusion could be drawn from the observations of the current study for both Bollgard II and WideStrike. However, bollworm pressure was considered "moderate" during a 1997 experiment in Blackville, where the 75 eggs per 100 plants threshold was met (Sullivan et al. 1998). The "moderate" pressure in 1997 led to lint yield increases in first generation Bollgard cotton when treated at the 75 egg per 100 plant threshold. Bollworm pressure varies greatly from one location to the next and even in the same location from year to year. Egg density peaked on 25 July and 8 August in 2011. The timing of the peaks was similar to those of 2010; however, in 2010, the larger peak in egg density occurred in July, with a smaller peak in August.

There were no differences in lint yield (kg/ha) between egg threshold treatments within WideStrike and Bollgard technologies in 2010 or 2011. These results suggested that supplemental insecticide for bollworm based on egg density thresholds was unnecessary in WideStrike and Bollgard II. The non-*Bt* control did experience significant yield gains in both years (Figures 3) when treated weekly and following the

aggressive egg threshold (25 eggs per 100 plants) when compared with the untreated control and higher egg thresholds (75 and 125/100 eggs per 100 plants) which were not sprayed for bollworms all season (Figures 3).

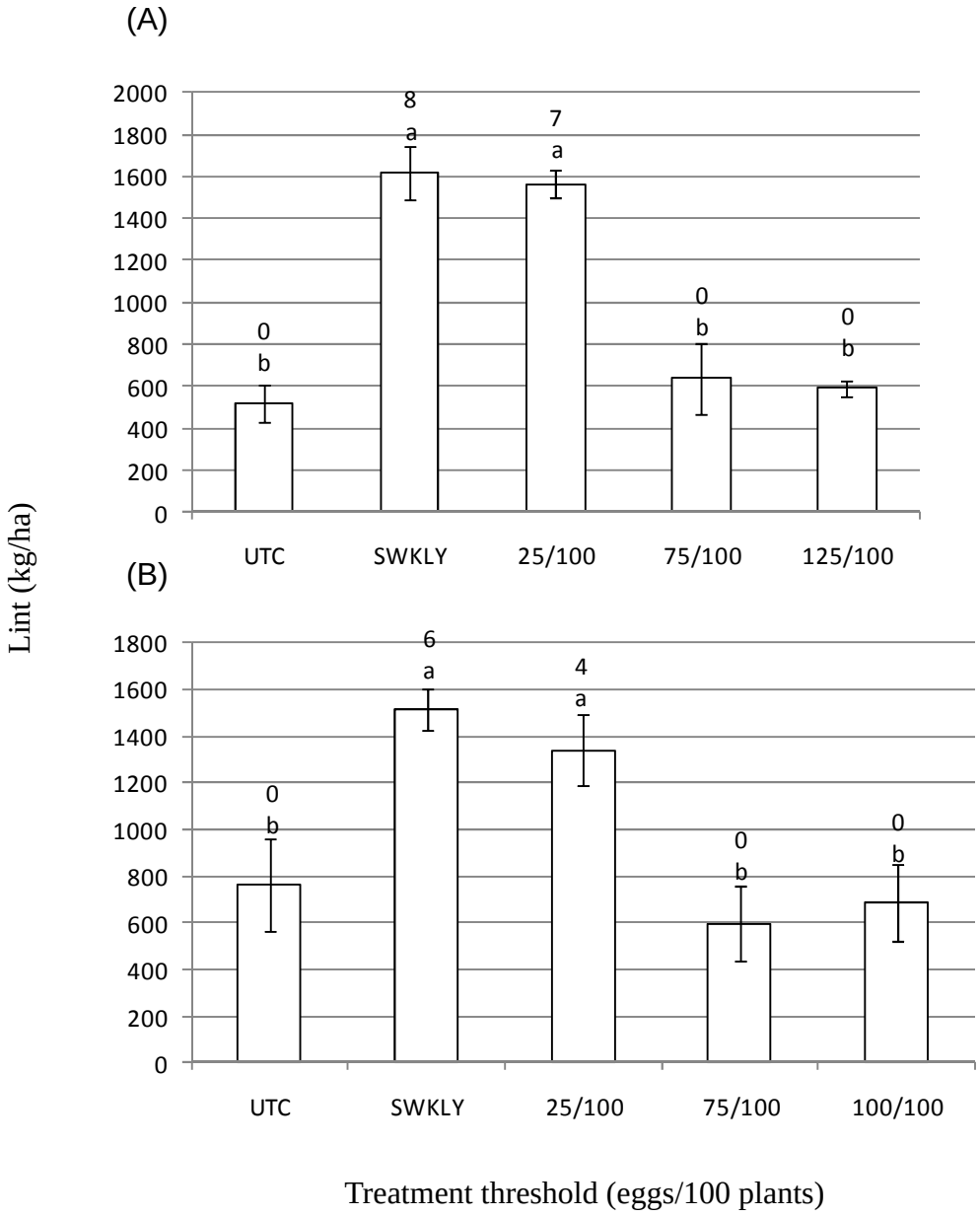

Figure 3. Cotton lint yields from (±SEM) comparing bollworm egg threshold treatments in non-*Bt* cotton near Blackville, South Carolina, in 2010 (A) and 2011 (B). Bars with the same letter are not significantly different. Numbers indicate number of insecticide applications received. UTC, untreated control; SWKLY, sprayed weekly.

Larvae Density Threshold

In 2010 and 2011, larval density was significantly affected by date in all three cotton technologies (Figure 4). Bollworm density peaks lagged slightly behind peaks in egg density (Figure 2) and fell between the first and second peaks found from the pheromone trap data (Figure 1).

Larval densities were significantly affected by the interaction of threshold and date in non-*Bt* cotton both years, as well as in WideStrike cotton in 2010 (Figure 5). Weekly applications of insecticide in WideStrike cotton were effective in maintaining low larval densities whereas greater variability was observed in other larval thresholds (Figure 5). The interaction suggests that the timing of insecticide application may be important. The end of July had high larval pressure and cotton may benefit from control at this time in particular. Insecticide applications had a negative impact on larval density (Figure 6). Differences in larval densities correlated with WideStrike lint yield in 2010; lower larval density in the plots sprayed weekly correlated with higher lint yields in these plots than in the untreated plots (Figure 7). Insecticide applications based on treatment thresholds did not impact yield in dual *Bt*-gene cotton in 2010. In 2011, there was no significant difference in yield in any of the three technologies (Table 4). Gore et al. (2008) had similar results from a field cage experiment conducted in Mississippi. White blooms of Bollgard II and WideStrike were infested with bollworm larvae at 0, 50, and 100%. Bollworm infestation had little impact on yield of Bollgard II or WideStrike except when 100% of white flowers were infested for at least one week (Gore et al.

2008). Economic yield loss was projected to occur only with extremely high pressure persisting for more than one week.

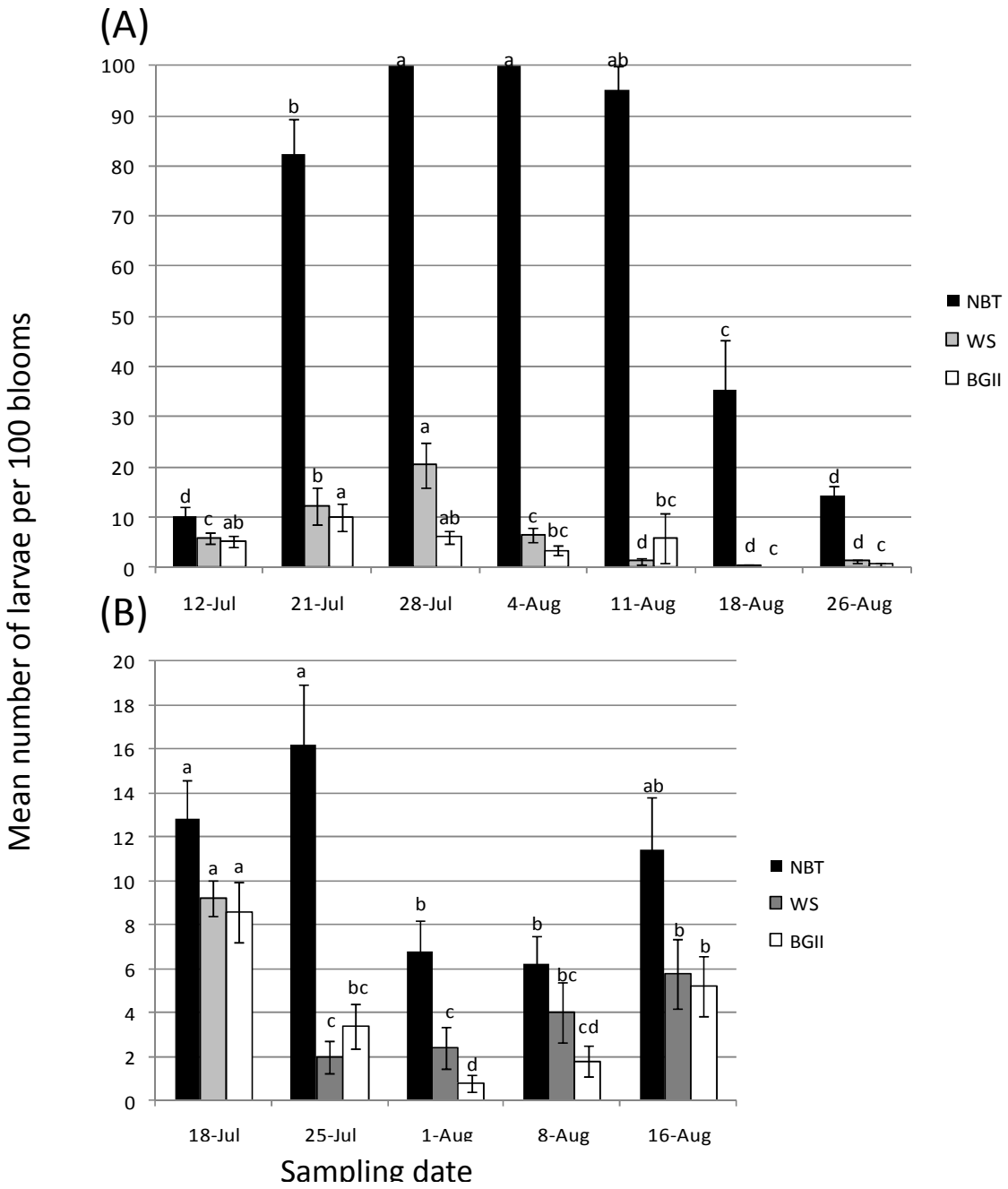

Figure 4. Mean bollworm larvae per 100 blooms (±SEM) by cotton technology and sampling date near Blackville, South Carolina, in 2010 (A) and 2011 (B). Bars of the same cotton technology with the same letter are not significantly different. NBT, non-*Bt*; WS, WideStrike; BGII, Bollgard II.

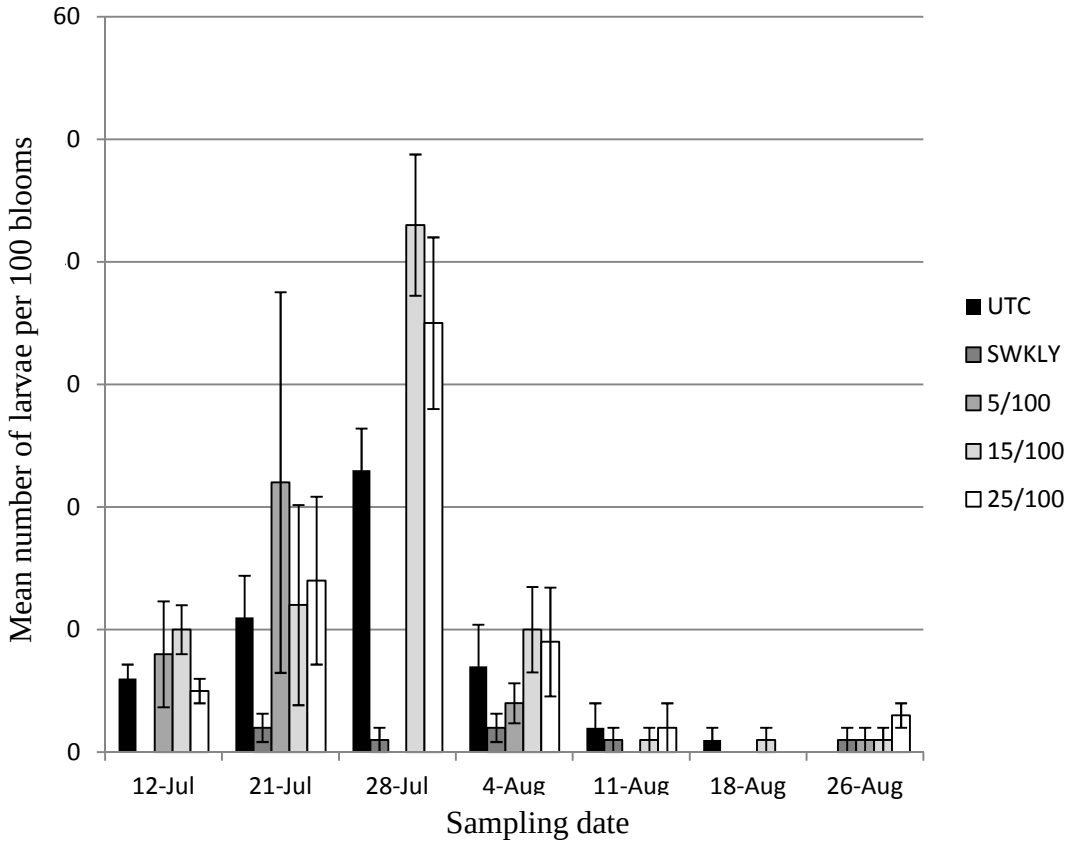

Figure 5. Mean bollworm larvae per 100 blooms (±SEM) in WideStrike cotton by larval treatment threshold and date near Blackville, South Carolina, in 2010. UTC, untreated control; SWKLY, sprayed weekly.

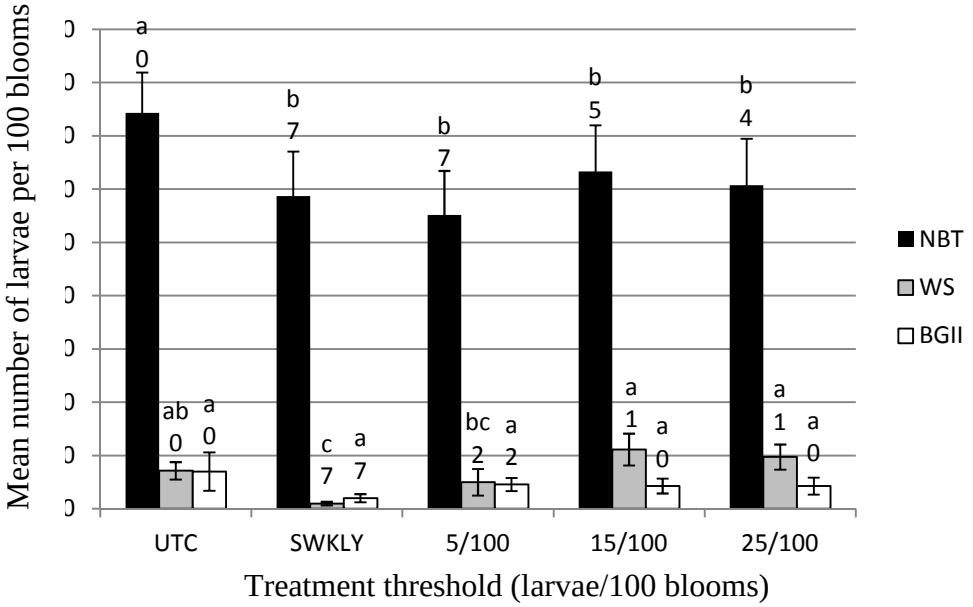

Figure 6. Mean bollworm larvae per 100 blooms (±SEM) by threshold treatment and technology in WideStrike (WS), Bollgard II (BGII), and non-*Bt* (NBT) cotton near Blackville, South Carolina, in 2010. Bars of the same cotton technology with the same letter are not significantly different. Numbers above bars indicate number of insecticide applications received. UTC, untreated control; SWKLY, sprayed weekly.

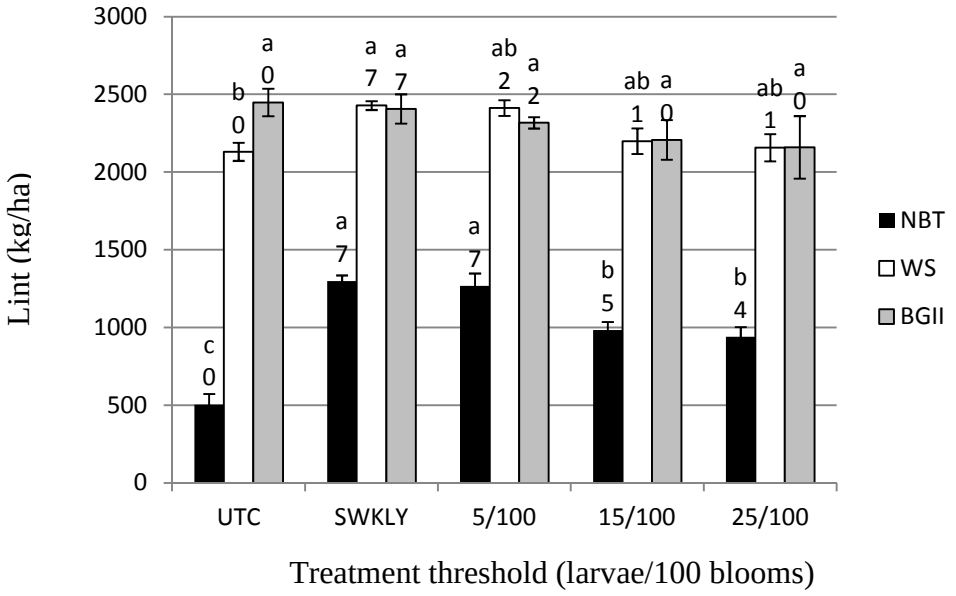

Figure 7. Comparison of 2010 yield (±SEM) by technology in WideStrike (WS), Bollgard II (BGII) and non-*Bt* (NBT) cotton near Blackville, South Carolina. Bars of the same cotton technology with the same letter are not significantly different. Numbers above the bars indicate number of insecticide applications treatment received. UTC, untreated control; SWKLY, sprayed weekly.

Boll Damage Threshold

Boll damage in non-*Bt* and WideStrike cotton varied significantly by date (Figure 8). Boll damage was elevated following the peak in egg density (Figure 2) during the same period that larvae sample numbers were high (Figure 4). This followed the pattern observed with adult moth capture (Figure 1).

Although boll damage in both *Bt* cotton technologies decreased significantly when being aggressively treated for bollworm in 2010 (Figure 9) and Bollgard II alone in 2011 (Figure 10), significant yield impacts based on insecticide treatment were observed only in non-*Bt* cotton (Figure 11). In a study conducted in North Carolina, fewer larvae and reduced boll damage were observed on Bollgard cotton compared with non-*Bt* cotton and likewise on Bollgard II compared with Bollgard cotton (Jackson et al. 2003). Bollgard II experienced 997 damaged bolls per acre with insecticide applications and 9,436 damaged bolls per acre when left untreated (Jackson et al 2003). However, subsequent yield data were not presented in the study. In the current study, boll damage in 2010 varied significantly between thresholds in WideStrike cotton (Figure 9), but the injury did not lead to any significant loss in cotton lint yield.

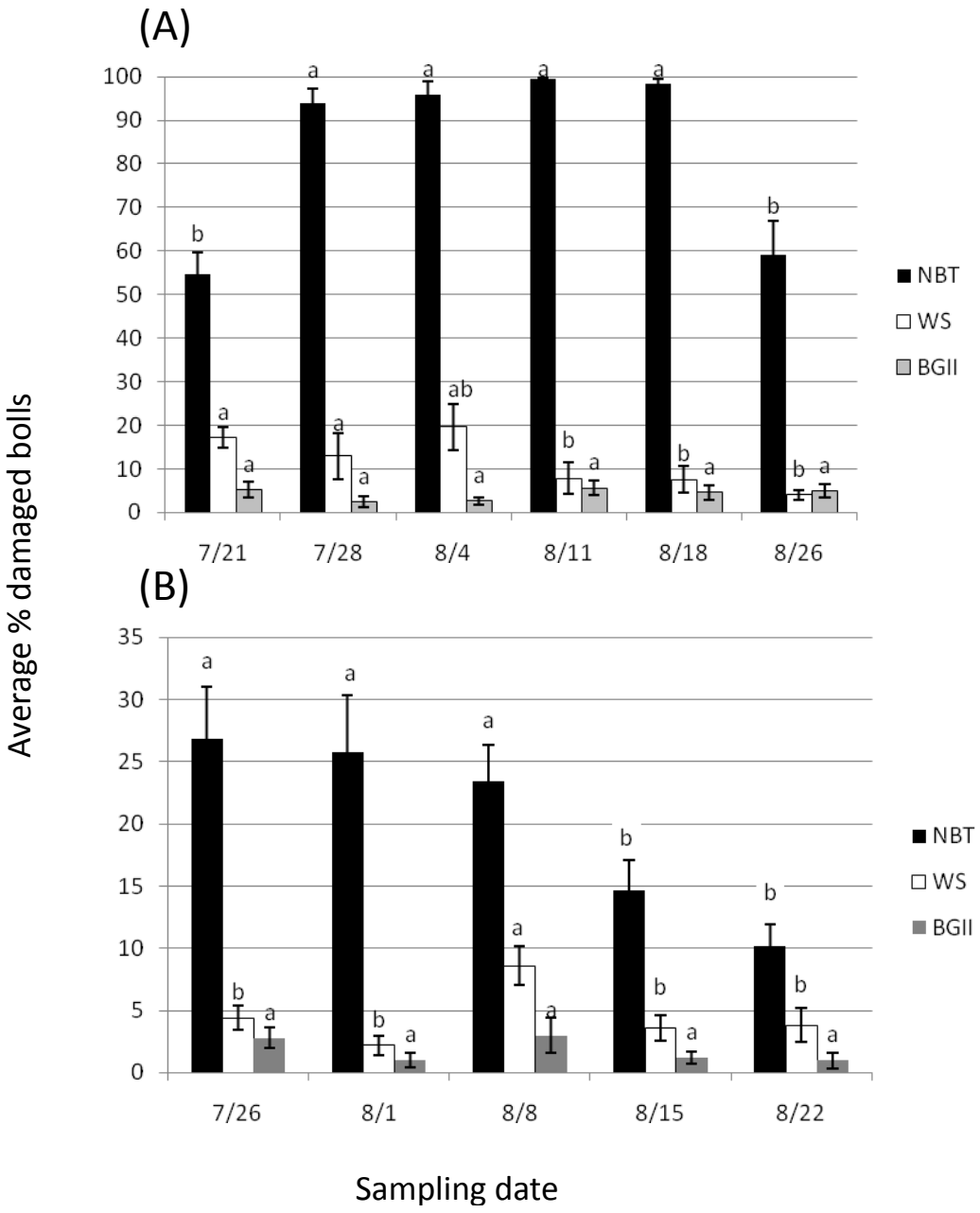

Figure 8. Percent boll damage (±SEM) caused by bollworm to non-*Bt* (NBT), WideStrike (WS), and Bollgard II (BGII) cotton during July and August 2010 (A) and 2011 (B) near Blackville, South Carolina. Bars of the same technology with the same letter are not significantly different.

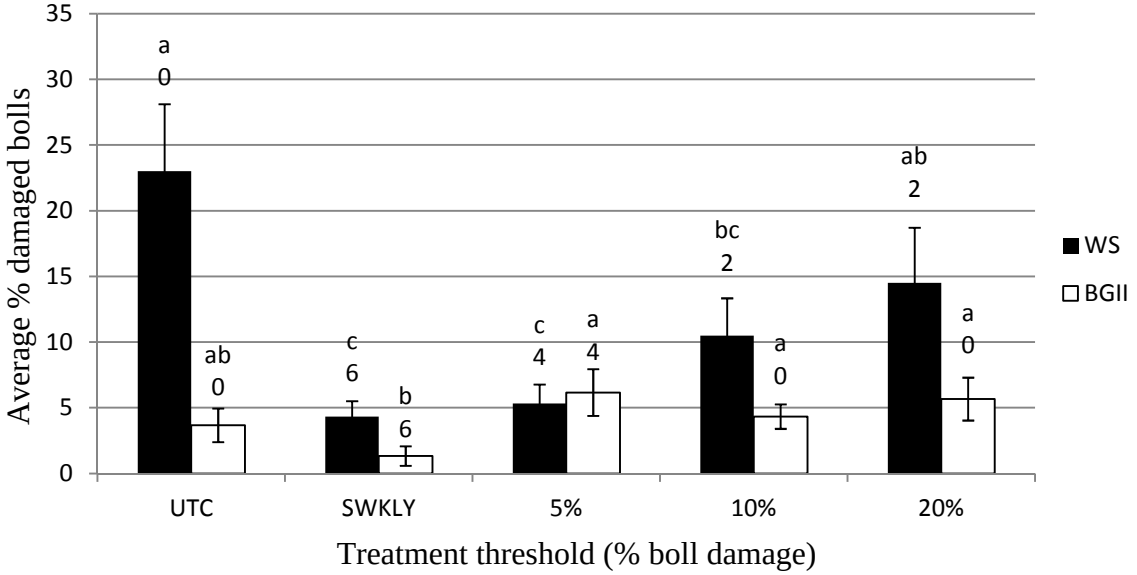

Figure 9. Percent boll damage (±SEM) caused by bollworm averaged across sampling date by boll damage threshold for WideStrike (WS) and Bollgard II (BGII) cotton near Blackville, South Carolina, in 2010. Bars of the same technology with the same letter are not significantly different. Numbers indicate number of insecticide applications received. UTC, untreated control; SWKLY, sprayed weekly.

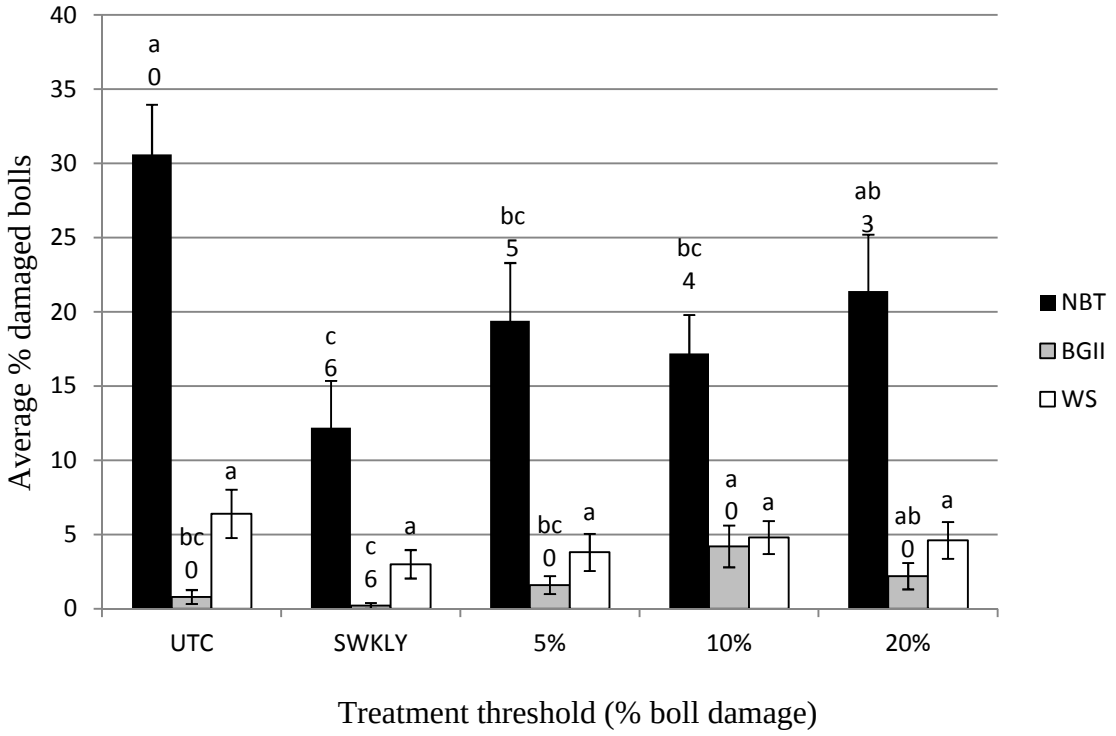

Figure 10. Percent boll damage (±SEM) caused by bollworm averaged across sampling date by boll damage threshold for non-*Bt* (NBT), Bollgard II (BGII), and WideStrike (WS) cotton near Blackville, South Carolina, in 2011. Bars of the same technology with the same letter are not significantly different. Numbers indicate number of insecticide applications received. UTC, untreated control; SWKLY, sprayed weekly.

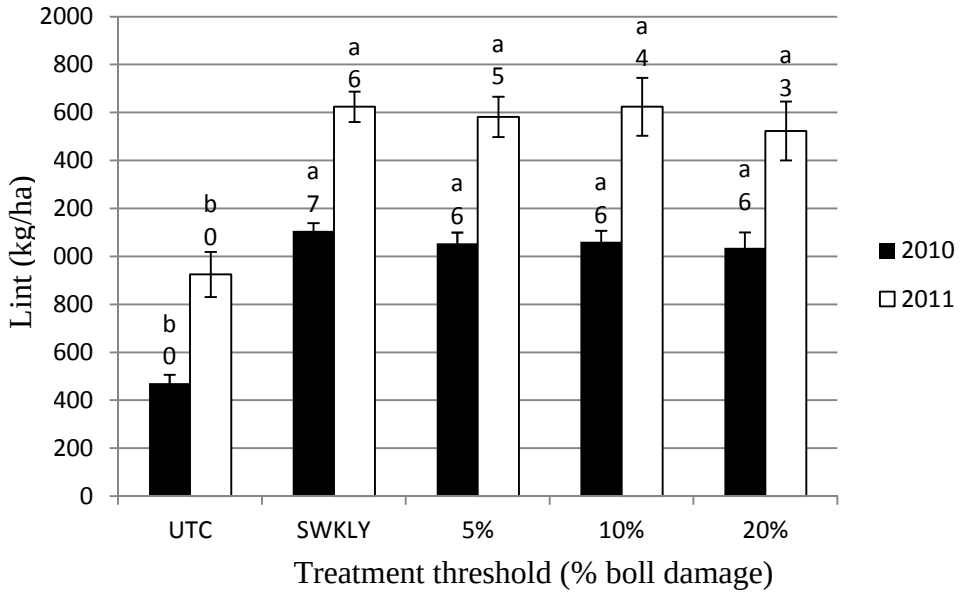

Figure 11. Cotton lint yield (±SEM) averaged by treatment for 2010 and 2011 in non-*Bt* cotton near Blackville, South Carolina. Bars of the same year with the same letter are not significantly different. Numbers indicate number of insecticide applications received. UTC, untreated control; SWKLY, sprayed weekly.

Yields of non-Bt cotton were significantly lower in untreated plots compared with plots in 2010 (Figure 11). Bollworm damage in 2010 was high enough that all thresholds were treated weekly after scouting began. In 2011, treatments receiving 6, 5, 4, or 3 applications experienced no significant differences in yield, only differing significantly with those plots receiving no insecticide applications.

In a study in North Carolina that included replicated tests and surveys of producer-managed fields, minor differences in bollworm control by technology were shown, but the researchers concluded that these differences were less significant than yield and quality differences between varieties (Bacheler et al. 2006). WideStrike and Bollgard II had no significant yield differences between treated and untreated plots. Treatment significantly reduced boll damage, yet it was not seen in corresponding lint

yield differences. As in the current study, Bacheler et al. (2006) observed higher percent boll damage in WideStrike varieties than Bollgard II (15 and 6% boll damage, respectively, in 2003), yet each technology did not appear to benefit from insecticide treatments based on lint yield (Bacheler et al. 2006).

Plant Mapping

Test 1- Egg Density Threshold

Table 5. Statistical comparisons for plant-mapping variables for bollworm egg density threshold trials on cotton near Blackville, South Carolina, 2011.

Variable	Type of Cotton		
	Non-*Bt*	WideStrike	Bollgard II
Ave. Plant Height	F= 2.45[a]; P= 0.0912	F= 0.22[b]; P= 0.9241	F= 0.73[b]; P= 0.5860
Ave. Nodes/Plant	F= 1.94[a]; P= 0.1563	F= 1.03[b]; P= 0.4270	F= 0.06[b]; P= 0.9929
Height/Node Ratio	F= 1.84[a]; P= 0.1744	F= 0.57[a]; P= 0.6890	F= 1.34[a]; P= 0.2994
Ave. Total Bolls/Plant	F= 2.30[b]; P= 0.1099	F= 0.96[b]; P= 0.4585	F= 0.60[b]; P= 0.6719
Total Vegetative Bolls/Plant	F= 1.20[a]; P= 0.3523	F= 0.47[a]; P= 0.7583	F= 2.55[a]; P= 0.0824
Ave. 1st Fruiting Node	F= 0.69[a]; P= 0.6091	F= 2.53[b]; P= 0.0870	F= 0.39[b]; P= 0.8092
Ave. 1st Position Bolls	F= 1.32[b]; P= 0.3098	F= 2.67[a]; P= 0.0730	F= 0.47[b]; P= 0.7576
% Retention at 1st Position	F= 1.34[a]; P= 0.3009	F= 1.57[b]; P= 0.2374	F= 1.97[a]; P= 0.1514
Ave. 2nd Position Bolls	F= 1.08[a]; P= 0.4000	F= 1.24[b]; P= 0.3381	F= 0.18[b]; P= 0.9468
% Retention at 2nd Position	F= 1.41[a]; P= 0.2796	F= 1.17[a]; P= 0.3649	F= 0.52[a]; P= 0.7210
Ave. Worm Damaged Bolls	F= 0.41[a]; P= 0.7983	F= 1.49[b]; P= 0.2583	F= 1.11[a]; P= 0.3894
Ave. % Damaged Bolls	F= 0.26[a]; P= 0.9013	F= 2.52[b]; P= 0.0884	F= 1.13[a]; P= 0.3803

[a] df = 4, 15
[b] df = 4, 14

Test 2- Larvae Density Threshold

Table 6. Statistical comparisons for plant-mapping variables for bollworm larvae in blooms threshold trials on cotton near Blackville, South Carolina, 2011.

Variable	Type of Cotton		
	Non-*Bt*	WideStrike	Bollgard II
Ave. Plant Height	F= 1.44[b]; P= 0.2738	F= 0.40[b]; P= 0.8080	F= 1.03[a]; P= 0.4239
Ave. Nodes/Plant	**F= 3.28[b]; P= 0.0428**	F= 0.57[a]; P= 0.6865	F= 0.81[a]; P= 0.5385
Height/Node Ratio	F= 0.44[b]; P= 0.7772	F= 0.51[b]; P= 0.7299	F= 1.78[a]; P= 0.1858
Ave. Total Bolls/Plant	F= 0.84[b]; P= 0.5226	F= 0.25[a]; P= 0.9069	F= 0.37[a]; P= 0.8244
Total Vegetative Bolls/Plant	F= 2.12[b]; P= 0.1318	F= 0.13[a]; P= 0.9699	F= 0.71[a]; P= 0.5948
Ave. 1st Fruiting Node	F= 1.76[b]; P= 0.1922	F= 0.97[b]; P= 0.4547	F= 2.60[b]; P= 0.0814
Ave. 1st Position Bolls	F= 2.35[a]; P= 0.1010	F= 1.44[a]; P= 0.2682	F= 0.07[b]; P= 0.9908
% Retention at 1st Position	**F= 3.57[a]; P= 0.0310**	F= 2.60[a]; P= 0.0787	F= 0.66[b]; P= 0.6315
Ave. 2nd Position Bolls	F= 1.83[a]; P= 0.1759	F= 0.72[b]; P= 0.5949	F= 0.26[a]; P= 0.9016
% Retention at 2nd Position	**F= 14.40[a]; P< 0.0001**	F= 0.65[c]; P= 0.6351	F= 0.27[a]; P= 0.8904
Ave. Worm Damaged Bolls	F= 1.68[b]; P= 0.2095	F= 1.60[a]; P= 0.2247	F= 1.35[b]; P= 0.2992
Ave. % Damaged Bolls	F= 1.41[b]; P= 0.2814	F= 0.66[a]; P= 0.6292	F= 0.96[b]; P= 0.4601

[a] df = 4, 15
[b] df = 4, 14
[c] df = 4, 13

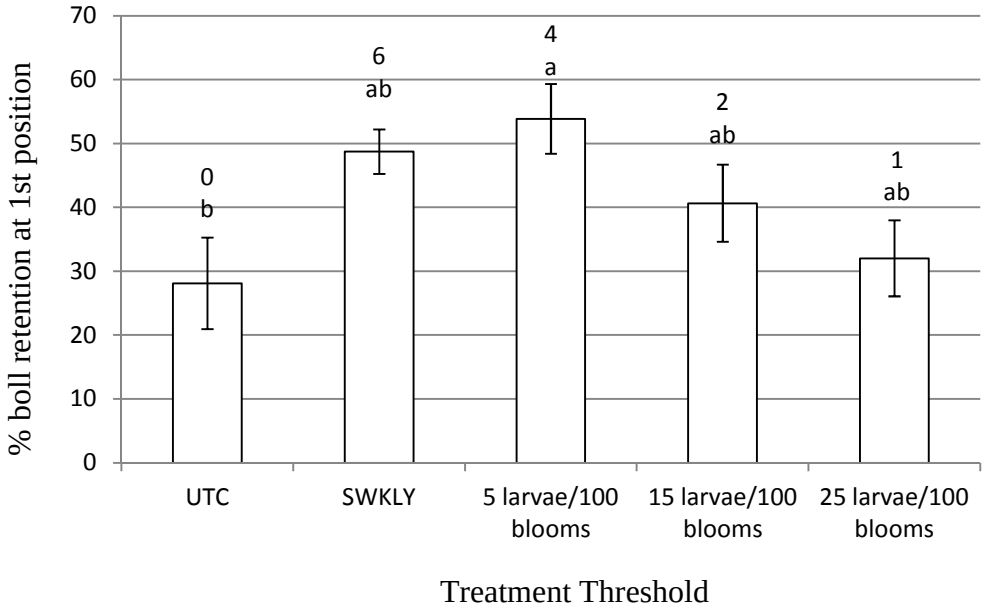

Figure 12. Percent retention at the first position (±SEM) in non-*Bt* cotton from bollworm larvae in blooms test near Blackville, South Carolina, in 2011. UTC, untreated control; SWKLY, sprayed weekly. Numbers indicate number of insecticide applications the treatment received. Bars with the same letter are not significantly different.

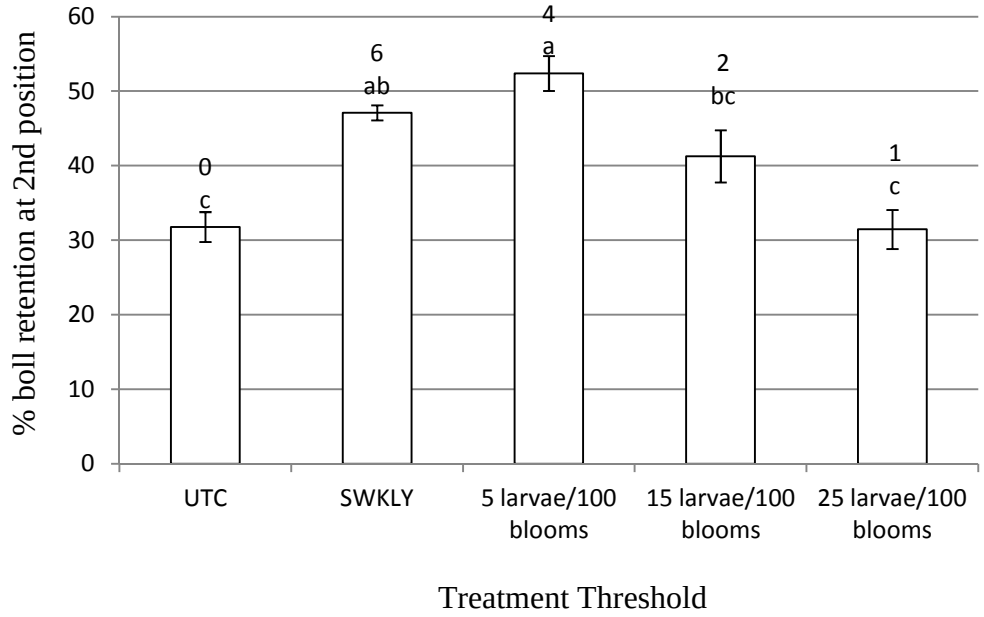

Figure 13. Percent retention at the second position (±SEM) in non-*Bt* cotton from bollworm larvae in blooms test near Blackville, South Carolina, in 2011. UTC, untreated control; SWKLY, sprayed weekly. Numbers indicate number of insecticide applications the treatment received. Bars with the same letter are not significantly different.

Numbers of nodes per plant in non-*Bt* cotton were significantly different among treatments using LSD mean separations, but not using the more conservative Tukey mean separation test (data not shown; Table 6). Cotton plants suffering fruit damage or loss divert resources to vegetative growth, grow taller, and produce more nodes (Guinn 1982). This would help explain the trend of increasing number of nodes with decreasing insecticide protection from bollworm, but the trend was not strong enough to be significant when using conservative measures of statistical difference. Non-*Bt* cotton also had higher percent boll retention of 1st and 2nd position bolls in protected plots than in the untreated control plots (Figures 12 and 13).

Test 3- Boll Damage Threshold

Table 7. Statistical comparisons for plant-mapping variables for bollworm boll damage threshold trials on cotton near Blackville, South Carolina, 2011.

Variable	Type of Cotton		
	Non-*Bt*	WideStrike	Bollgard II
Ave. Plant Height	F= 0.74[b]; P= 0.5806	F= 0.28[b]; P= 0.8857	F= 0.52[a]; P= 0.7193
Ave. Nodes/Plant	F= 1.73[a]; P= 0.1963	F= 0.45[b]; P= 0.7705	F= 0.34[b]; P= 0.8460
Height/Node Ratio	F= 1.05[a]; P= 0.4152	F= 0.16[a]; P= 0.9529	F= 0.33[b]; P= 0.8529
Ave. Total Bolls/Plant	F= 0.90[a]; P= 0.4869	F= 0.36[b]; P= 0.8337	F= 0.34[a]; P= 0.8497
Total Vegetative Bolls/Plant	F= 0.44[a]; P= 0.7768	F= 1.19[a]; P= 0.3564	F= 0.29[a]; P= 0.8816
Ave. 1st Fruiting Node	F= 1.98[b]; P= 0.1529	F= 0.43[a]; P= 0.7825	F= 1.24[b]; P= 0.3402
Ave. 1st Position Bolls	**F= 3.27[a]; P= 0.0409**	F= 0.73[b]; P= 0.5847	F= 0.21[a]; P= 0.9269
% Retention at 1st Position	**F=4.66[a]; P= 0.0120**	F= 0.91[a]; P= 0.4808	F= 0.60[a]; P= 0.6672
Ave. 2nd Position Bolls	**F= 6.17[b]; P= 0.0044**	F= 0.59[b]; P= 0.6731	F= 1.01[b]; P= 0.4355
% Retention at 2nd Position	**F= 4.10[c]; P= 0.0252**	F= 0.87[a]; P= 0.5029	F= 1.01[a]; P= 0.4328
Ave. Worm Damaged Bolls	F= 0.71[b]; P= 0.6014	F= 0.60[a]; P= 0.6671	F= 0.37[a]; P= 0.8277
Ave. % Damaged Bolls	F= 0.83[b]; P= 0.5287	F= 0.90[a]; P= 0.4904	F= 0.55[a]; P= 0.7015

[a] df = 4, 15
[b] df = 4, 14
[c] df = 4, 12.1

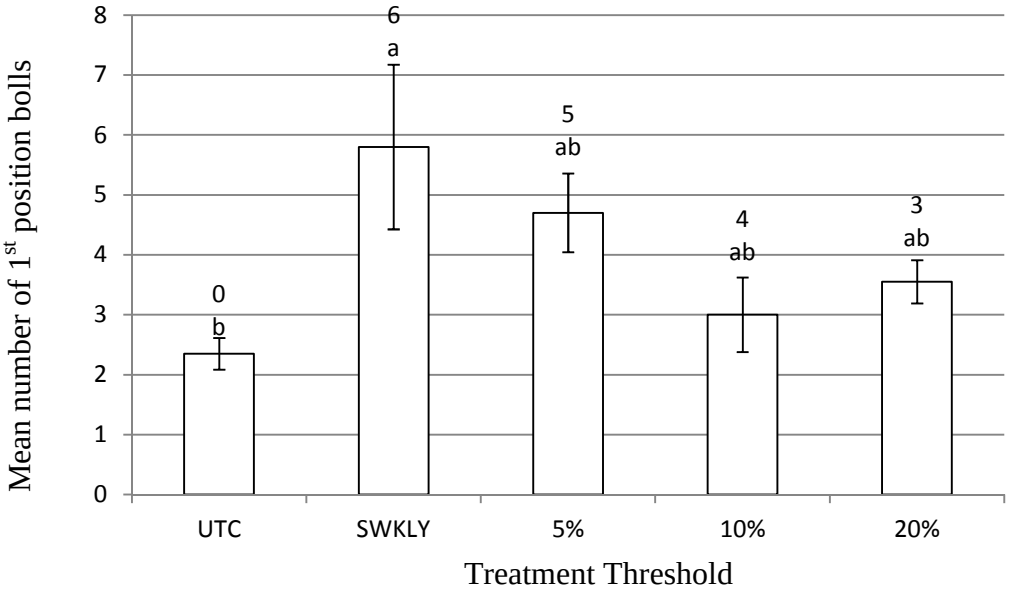

Figure 14. Mean number of first position bolls (±SEM) in non-*Bt* cotton from bollworm boll damage test near Blackville, South Carolina, in 2011. UTC, untreated control; SWKLY, sprayed weekly. Numbers indicate number of insecticide applications the treatment received. Bars with the same letter are not significantly different.

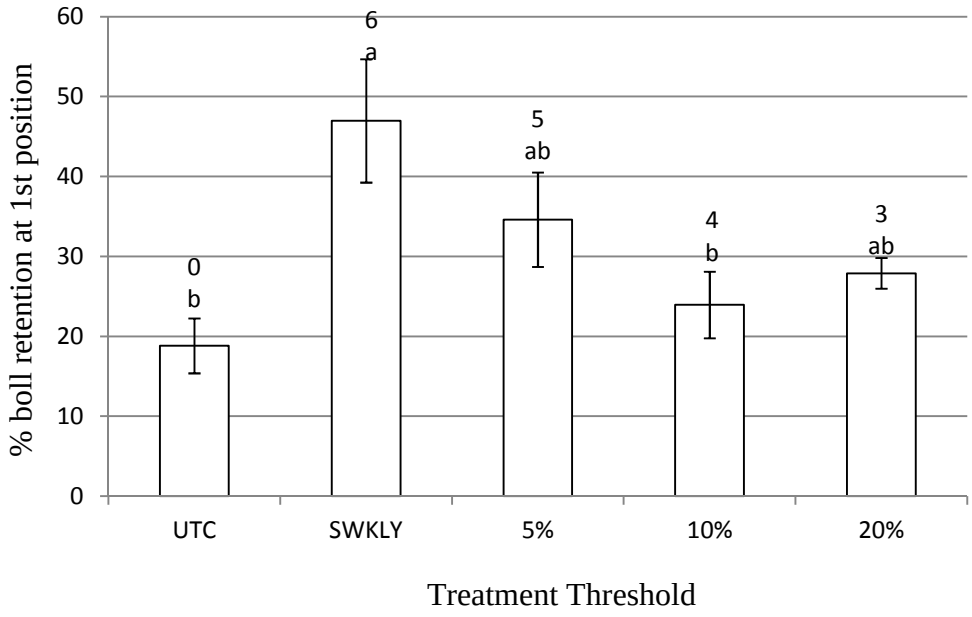

Figure 15. Percent retention at the first position (±SEM) in non-*Bt* cotton from bollworm boll damage test near Blackville, South Carolina, in 2011. UTC, untreated control; SWKLY, sprayed weekly. Numbers indicate number of insecticide applications the treatment received. Bars with the same letter are not significantly different.

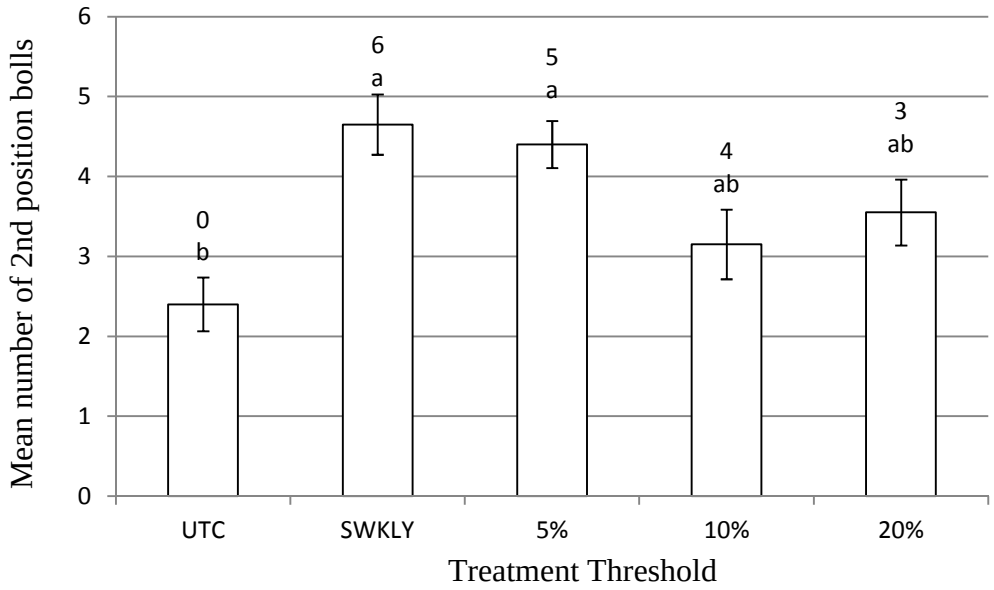

Figure 16. Mean number of second position bolls (±SEM) in non-*Bt* cotton from bollworm boll damage test near Blackville, South Carolina, in 2011. UTC, untreated control; SWKLY, sprayed weekly. Numbers indicate number of insecticide applications the treatment received. Bars with the same letter are not significantly different.

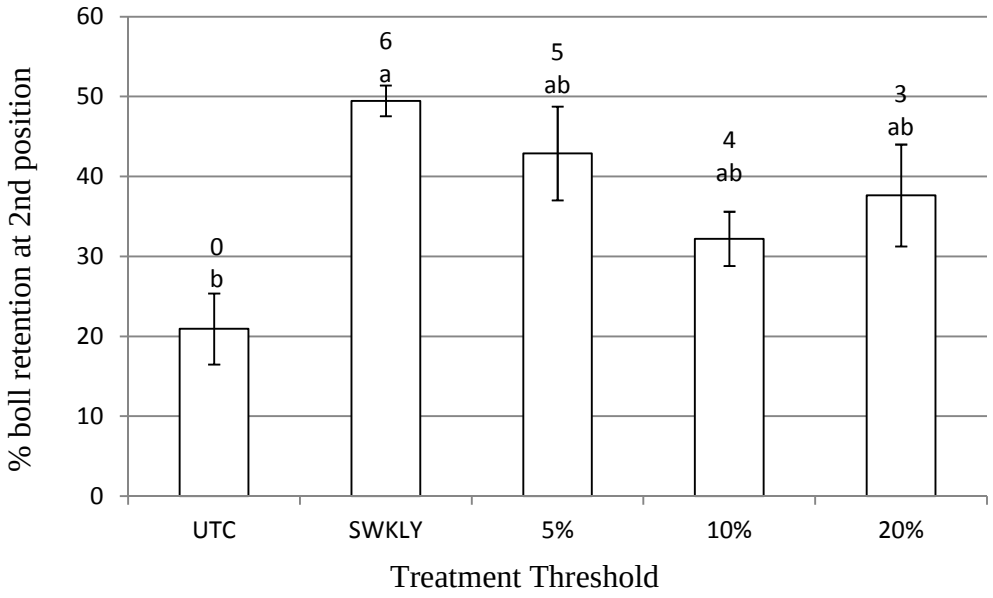

Figure 17. Percent retention at the second position (±SEM) in non-*Bt* cotton from bollworm boll damage test near Blackville, South Carolina, in 2011. UTC, untreated control; SWKLY, sprayed weekly. Numbers indicate number of insecticide applications the treatment received. Bars with the same letter are not significantly different.

Data from the boll damage threshold test were similar to data observed in the test with larval density in blooms. Weekly protected plots had greater numbers of 1st and 2nd position bolls than unprotected plots (Figures 14 and 16) which correlated with higher percent retention at these two positions (Figures 15 and 17). Weekly insecticide applications reduced the number of bollworms and other pests and allowed the valuable 1st and 2nd position bolls to survive undamaged to maturity.

CONCLUSIONS

Despite high bollworm pressure in 2010, there were no significant differences in lint yield among thresholds in the dual *Bt*-gene technologies, except between WideStrike untreated and sprayed weekly plots in the larval density threshold test. During 2011, no significant yield differences among thresholds in the dual *Bt*-gene technologies occurred. Extended growing seasons conducive for plant compensation were experienced each year, though 2011 had lower bollworm pressure than 2010. Plant mapping data, taken only in 2011, did not indicate compensatory growth for that season, but it is uncertain if compensation occurred in 2010 when bollworm pressure was extremely high. During 2011, plots of non-*Bt* cotton protected weekly had higher incidence of 1st and 2nd position boll retention; yet, this was only seen in the non-*Bt* control, with no differences between insecticide thresholds on dual *Bt*-gene cotton. Even if compensation likely occurred in 2010, conditions favorable for yield compensation do not occur perennially. Such end-of-season conditions should not be expected when making insect control decisions.

No differences in lint yield were found among thresholds within the *Bt* technologies, indicating that insecticide applications exclusively targeting bollworm were unnecessary in dual *Bt*-gene cotton. However, results from this study only span two growing seasons at one location and are not sufficient to warrant modification to South Carolina's current action threshold recommendations for dual-gene *Bt* cotton: three or more large larvae per 100 plants or 5% boll damage. Growers adhering to these recommendations for bollworm might apply one or two insecticide applications for

bollworm in dual-gene *Bt* technologies, as opposed to near weekly dedicated applications required for bollworm control on non-*Bt* cotton in this study.

The impact of secondary pests will also influence control strategies for bollworm. This study was set up to reduce the influence of non-bollworm pests. Further work is necessary to explore the interactions and impacts of secondary pests with bollworm in dual-gene *Bt* cotton. Stink bugs are regularly controlled with insecticides during periods of bollworm infestation, so concomitant control of any bollworms surviving on *Bt* cotton can be expected under most scenarios, thus negating dedicated applications for bollworm.

Measurable differences in bollworm density and damage levels were observed between technologies. WideStrike cotton regularly supported more bollworms and suffered consistently higher boll damage than Bollgard II cotton, which initially suggested that it would be necessary to take a more proactive approach in protecting WideStrike cotton than Bollgard II. However, in this study, lint yields from WideStrike plots did not differ among varying thresholds for bollworm and so did not support the conclusion that protection strategies be amended for each technology. Further research comparing technologies would need to be conducted in order to make such a recommendation.

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