Evaluation of Treatment Thresholds for *Helicoverpa zea* (Lepidoptera: Noctuidae) in Non-*Bt* and Dual-Gene *Bt* Cotton[1]

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ABSTRACT Transgenic cotton, *Gossypium hirsutum* L. (Malvales: Malvaceae), that produces two *Bacillus thuringiensis* Berliner (*Bt*) (Bacillales: Bacillaceae) toxins has reduced the need for insecticide treatments for bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), compared with single-gene *Bt* traits. Field trials were conducted in an area prone to high pressure from *H. zea* in South Carolina in 2010 and 2011 to develop action thresholds for this species in dual-gene *Bt* Bollgard II and WideStrike cotton. Plots containing non-*Bt*, WideStrike, and Bollgard II cotton varieties were examined weekly and treated according to threshold protocols for one of the following: bollworm eggs, larvae in white blooms, or boll damage. Although insecticide applications targeting *H. zea* increased yield in non-*Bt* cotton, differences in yield among the thresholds evaluated were not statistically evident when insecticides were applied within the sets of *Bt* traits. Insecticide applications exclusively targeting *H. zea* were not necessary in dual-gene *Bt* cotton. More *H. zea* larvae and damage occurred in WideStrike cotton compared with Bollgard II; however, lint yields for dual-gene Bt cotton did not differ among thresholds and did not support implementing protection strategies unique to each set of traits.

KEY WORDS *Bacillus thuringiensis*, transgenic crop, cotton bollworm

The bollworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), and tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), have historically been major pests of cotton, *Gossypium hirsutum* L. (Malvales: Malvaceae), in the southeastern United States. Until the introduction of genetically engineered cotton that produces insecticidal toxins, foliar-applied insecticides were the primary means of controlling lepidopteran pests. However, resistance to organophosphates and pyrethroids during the 1990s reduced the effectiveness of chemical control (Gore & Adamczyk 2004).

In 1996, Monsanto Company (St Louis, MO) was the first to commercialize genetically engineered cotton (Bollgard®) expressing Cry1Ac proteins from *Bacillus thuringiensis kurstaki* Berliner (*Bt*) (Bacillales: Bacillaceae). Bollgard cotton was found to be highly effective on *H. virescens* and moderately-to-highly effective against *H. zea* (Stewart et al. 2001). Annual applications of insecticide

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were sometimes necessary to prevent yield loss from *H. zea* (Gore et al. 2003), because this species is less susceptible to Cry1Ac than *H. virescens* and often avoids mortality through larval behavior such as feeding on blooms that contain lower levels of the toxin than other plant parts (Gore et al. 2002). Therefore, action thresholds based on the number of eggs, number and size of larvae, and boll damage were developed for Bollgard cotton because it was not 100% effective in controlling *H. zea* (Sullivan et al. 1998).

In 2003, Monsanto Company released the first offering of dual-gene *Bt* cotton, Bollgard II®, which produces Cry1Ac and Cry2Ab. Two years later, Dow AgroSciences (Indianapolis, IN) released WideStrike® cotton, which produces Cry1Ac and Cry1F. Both sets of dual-gene *Bt* cotton traits provide better control of *H. zea* than single-gene Bollgard varieties (Gore et al. 2008). Results from field cage experiments conducted in Mississippi to determine *H. zea* impact on Bollgard II and WideStrike cotton suggested that *H. zea* would rarely cause yield loss in either set of traits (Gore et al. 2008). In North Carolina, Bollgard II showed greater efficacy than WideStrike or Bollgard when *H. zea* pressure was high (Bacheler et al. 2006). Under light or moderate pressure, however, the dual-gene *Bt* traits did not differ in *H. zea* control (Bacheler et al. 2006). Greene & Robinson (2010) and Greene et al. (2011) reported differences between Bollgard II and WideStrike in lint yield potential, sustained boll damage, and compensatory ability from trials conducted in South Carolina. Both sets of traits benefited from supplemental control of *H. zea* with insecticide when exposed to high numbers of *H. zea* (Greene & Robinson 2010, Greene et al. 2011). Additional research in Mississippi demonstrated that supplemental applications of insecticide that specifically controlled lepidopterans (diamide chemistry) resulted in significantly higher yields when compared with the untreated controls (Adams et al. 2013).

Because *H. zea* is presently capable of causing economic damage in dual-gene Bt cotton, and neither set of dual-gene traits demonstrates 100% control of the species, action thresholds may need to be developed specifically for each offering of *Bt* traits. Current threshold recommendations for dual-*Bt* gene cotton in South Carolina remain similar to thresholds used for *H. zea* on single-gene *Bt* cotton but do not suggest using a threshold based on egg density (Greene & Robinson 2010). The objective of this study was to compared and refine action thresholds for *H. zea* based on egg density, larval density, or boll damage in dual-gene *Bt* cotton.

Materials and Methods

Field trials were conducted at the Edisto Research and Education Center near Blackville, South Carolina, in 2010 and 2011. Populations of *H. zea* and *H. virescens* 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) around row-crop production fields. Pheromone lures (Luretape lures, Hercon Environmental, Philadelphia, PA) for *H. zea* and *H. virescens* 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 near the experiments conducted in this study.

Larvae of *H. zea* and *H. virescens* were collected from plots of non-*Bt,* WideStrike, and Bollgard II cotton on 2, 6, and 16 August 2011, and late instars were identified to species using a dissecting scope to note the presence or absence of a distinguishing character on excised mandibles (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.

Three replicated field experiments were conducted in 2010 and 2011. In each experiment, non-*Bt* (DP174 RF), WideStrike (PHY565 WRF), and Bollgard II (DP0949 B2RF) cotton varieties were planted on 14 May 2010 and 18 May 2011 in plots of eight rows (97 cm spacing) by 12.2 m using a randomized complete block design with four replications per treatment. Standard cotton production practices were followed as outlined in the Clemson University Cooperative Extension Service Cotton Production Guide (Jones et al. 2013). During both years, acephate (Orthene 97) (AMVAC Chemical Corp., Newport Beach, CA) was applied at 1.09 kg (AI)/ha during the first week of bloom to eliminate predaceous arthropods and maximize establishment of *H. zea* in the trial area. Insecticides that were ineffective on lepidopterans but efficacious on hemipterans were applied twice across the entire test area each season to minimize infestations of hemipteran pests such as stink bugs. In 2010, thiamethoxam (Centric 40 WG) (Syngenta Crop Protection, LLC. Greensboro, NC) was applied at 0.07 kg (AI)/ha on 22 July, and dicrotophos (Bidrin 8 EC) (AMVAC Chemical Corp.) was applied at 0.56 kg (AI)/ha on 9 August. In 2011, methyl parathion (Methyl 4 EC) was applied at 0.84 kg (AI)/ha on 18 July to control hemipteran populations and to disrupt beneficial arthropods. Dicrotophos (Bidrin 8 EC) was applied at 0.56 kg (AI)/ha on 4 August 2011. All replicated plots of a treatment were sprayed with alternating applications of *beta*-cyfluthrin (Baythroid XL) (Bayer CropScience, Research Triangle Park, NC) at 0.023 kg (AI)/ha or *lambda*-cyhalothrin (Karate Z) (Syngenta Crop Protection) at 0.045 kg (AI)/ha when average numbers of bollworm or boll damage met or exceeded action thresholds to be tested.

Results from 2010 suggested that there was significant yield compensation by bollworm-damaged cotton. Therefore, before the 2011 harvest, five plants per plot were measured, examined, and mapped to look for compensatory growth behavior. All bolls were counted and subjectively rated as 'harvestable', 'worm-damaged', 'unharvestable', or 'abscised'. Node and branch position were also noted. Cotton was mechanically harvested each year, and plot yields were calculated assuming 40% lint turnout (Jones et al. 2013).

Experiment 1; egg density treatment threshold. Following first bloom, plots were monitored weekly for eggs. Because *H. zea* eggs are deposited on the top third of the cotton plant and are most concentrated near the plant terminals (Gore et al. 2002), egg density was determined by visually examining the upper 20-25% of 25 plants per plot. Plants sampled were located in the middle four rows and away from plot edges. Eggs were counted on leaves, terminals, pre-floral buds (squares), bracts, and stems. Application decisions were based on egg density numbers averaged across each variety (non-Bt, WideStrike, and Bollgard II) instead of being averaged within threshold, and fully protected control plots were sprayed weekly regardless of egg density. Treatment threshold protocols were as follows: untreated control, sprayed weekly (fully protected control), 25 eggs/100 plants, 75 eggs/100 plants, and 125 (2010) or 100 (2011) eggs/100 plants.

Experiment 2; larval density treatment threshold. At bloom initiation, plots were monitored weekly for larvae by visually examining 25 blooms (*in situ*). 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 larvae in available blooms were extrapolated. If no blooms were present in a plot, larval density was assumed to have reached the highest threshold where all pre-floral buds and subsequent flowers were destroyed. Applications of insecticide were made as described previously, and fully protected control plots were sprayed weekly regardless of larval density. Treatment threshold protocols were as follows: untreated control, sprayed weekly (fully protected control), 4 or 5 larvae/100 blooms, 15 larvae/100 blooms, and 25 larvae/100 blooms.

Experiment 3; boll damage treatment threshold. After the first cohort of bolls reached approximately 12.7 mm in diameter at the widest point, plots were examined weekly by visually examining 25 bolls (*in situ*) per plot for *H. zea* 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 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 to be above treatment threshold. Applications of insecticide were made as described previously, and fully protected control plots were sprayed weekly regardless of damage level. Treatment threshold protocols were as follows: untreated control, sprayed weekly (fully protected control), 4 or 5% boll damage, 10% boll damage, and 20% boll damage.

Data analyses. 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 were transformed using arcsine$\sqrt{}$(proportion of bolls damaged). Tukey's mean separation tests were performed using SAS 9.3 (SAS Institute, Inc. 2011).

Results and Discussion

Pheromone trap data from 2010 and 2011 indicated that a larger number of *H. zea* adults were caught than *H. virescens*. Moth populations peaked in late August to early September, and *H. zea* comprised about 90% of captures. In 2011, at the peak of moth capture, 93% of the captured moths were *H. zea*. Of the larvae (31) collected in 2011 from *Bt* cotton varieties, all were *H. zea*. It was expected that *H. virescens* larvae would not be found on dual-gene *Bt* cotton because *Bt* endotoxins have been shown to exhibit complete field control of *H. virescens* (Stewart et al. 2001). Two of the 70 larvae found in non-*Bt* cotton were *H. virescens*. The majority of eggs, larvae, and plant injury incidents counted were, therefore, predominantly *H. zea* or due to their feeding. Moth populations peaked in late August for both species in 2010 and 2011. However, peak numbers of *H. zea* and *H. virescens* for 2010 were almost twice that of 2011. Factors such as overwintering conditions for pupae and other seasonal variation may largely have accounted for this difference between years.

Experiment 1; egg density treatment threshold. In 2010 and 2011, the highest treatment threshold of 125 or 100 eggs per 100 plants, respectively, was never reached in any variety (Table 1). In 2010, the treatment threshold of 75 eggs per 100 plants was met or exceeded three times in plots of WideStrike and twice in plots of Bollgard II (Table 1). The treatment threshold of 75 eggs per 100 plants was never reached on the non-*Bt* variety. Lower egg numbers on non-*Bt* cotton than in WideStrike or Bollgard II cotton were observed (Figure 1) and 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. The treatment threshold of 25 eggs per 100 plants was reached on seven dates.

In 2011, overall egg numbers were lower than in the previous year (Figure 1). Hot and dry conditions also caused plants to mature faster and shortened the sampling period. Despite lowering the highest egg density treatment threshold from 125 to 100 eggs per 100 plants, this threshold was not reached. The treatment threshold of 75 eggs per 100 plants was also not reached in any variety during 2011.

Egg densities were not significantly affected by the prescribed treatment threshold except for the non-*Bt* cotton in 2011, nor was there an interaction between treatment threshold and date for each cotton variety (Table 2). The lack of a significant treatment effect in this experiment was probably because the insecticide had little ovicidal effects and did not deter oviposition. Thus, insecticides did not have a significant effect on the number of eggs on the plants one week after application.

There were no differences in lint yield between egg threshold treatments for WideStrike or Bollgard traits in 2010 or 2011, which suggested that supplemental insecticide for *H. zea* based on egg density thresholds was unnecessary on dual-gene *Bt* cotton. The yield of non-*Bt* was significantly improved in both years when treated weekly or following the aggressive egg threshold (25 eggs per 100 plants) compared with the untreated control and higher egg thresholds which were not sprayed for *H. zea* all season (Figure 2).

Experiment 2; larval density treatment threshold. In 2010 and 2011, larval density was affected by date in all three varieties (Table 2, Figure 3). In non-*Bt* cotton, there were interactions between the threshold treatment and date on larval densities during both years, as well as in WideStrike cotton in 2010 (Figure 4). These interactions indicate that the effect of threshold on larval densities varied with date. There was high larval pressure during late July (Figure 4), consistent with previous reports for the Blackville area (Greene & Robinson 2010, Greene et al. 2011). Weekly applications of insecticide in WideStrike cotton were effective in maintaining low larval densities, whereas greater variability was observed in other larval thresholds (Figure 4).

A similar trend was observed for WideStrike cotton in 2010. Plots sprayed weekly had lower larval density (Figure 4) and produced higher lint yields than untreated plots (Figure 5). However, there were no significant differences in yield among treatments made based on the prescribed thresholds in WideStrike or Bollgard II cotton in 2010 (Figure 5). In 2011, there was no significant difference in yield among thresholds in any of the three varieties (Table 3), but the yield differences observed among treatments in non-*Bt* cotton approached significance ($P = 0.08$)

Table 1. Insecticide application dates for three experiments using different treatment thresholds and cotton varieties with different *Bt* traits (=technology).

		2010		2011	
Experiment	Technology	Threshold	Application dates	Threshold	Application dates
Egg density	Non-*Bt*	25/100	7/9, 7/16, 7/23, 7/30, 8/13, 8/20, 8/27	25/100	7/28, 8/3, 8/9, 8/18
		75/100	-	75/100	-
		125/100	-	100/100	-
	WideStrike	25/100	7/9, 7/16, 7/23, 7/30, 8/13, 8/20, 8/27	25/100	7/20, 7/28, 8/3, 8/9, 8/18
		75/100	7/16, 7/23, 7/30	75/100	-
		125/100	-	100/100	-
	Bollgard II	25/100	7/9, 7/16, 7/23, 7/30, 8/13, 8/20, 8/27	25/100	7/20, 7/28, 8/3, 8/9, 8/18
		75/100	7/23, 7/30	75/100	-
		125/100	-	100/100	-
Larval density	Non-*Bt*	5/100	7/16, 7/23, 7/30, 8/6, 8/13, 8/20, 8/27	5/100	7/20, 7/28, 8/9, 8/18
		15/100	7/23, 7/30, 8/6, 8/13, 8/27	15/100	7/20, 8/18
		25/100	7/23, 7/30, 8/6, 8/13	25/100	7/28
	WideStrike	5/100	7/16, 7/23, 8/6	5/100	7/20, 8/9, 8/18
		15/100	7/30	15/100	-
		25/100	7/30	25/100	-
	Bollgard II	5/100	7/23, 8/6, 8/13, 8/20	5/100	7/20, 8/18
		15/100	-	15/100	-
		25/100	-	25/100	-

Table 1. Continued

		2010		2011	
Experiment	Technology	Threshold	Application dates	Threshold	Application dates
Boll damage	Non-*Bt*	5%	7/23, 7/30, 8/6, 8/13, 8/20, 8/27	5%	7/28, 8/3, 8/9, 8/24
		10%	7/23, 7/30, 8/6, 8/13, 8/20, 8/27	10%	7/28, 8/3, 8/9
		20%	7/23, 7/30, 8/6, 8/13, 8/20, 8/27	20%	7/28, 8/3, 8/9
	WideStrike	5%	7/23, 7/30, 8/6, 8/20	5%	8/9, 8/24
		10%	7/23, 8/6	10%	-
		20%	7/30, 8/6	20%	-
	Bollgard II	5%	7/23, 7/30, 8/13, 8/20	5%	-
		10%	-	10%	-
		20%	-	20%	-

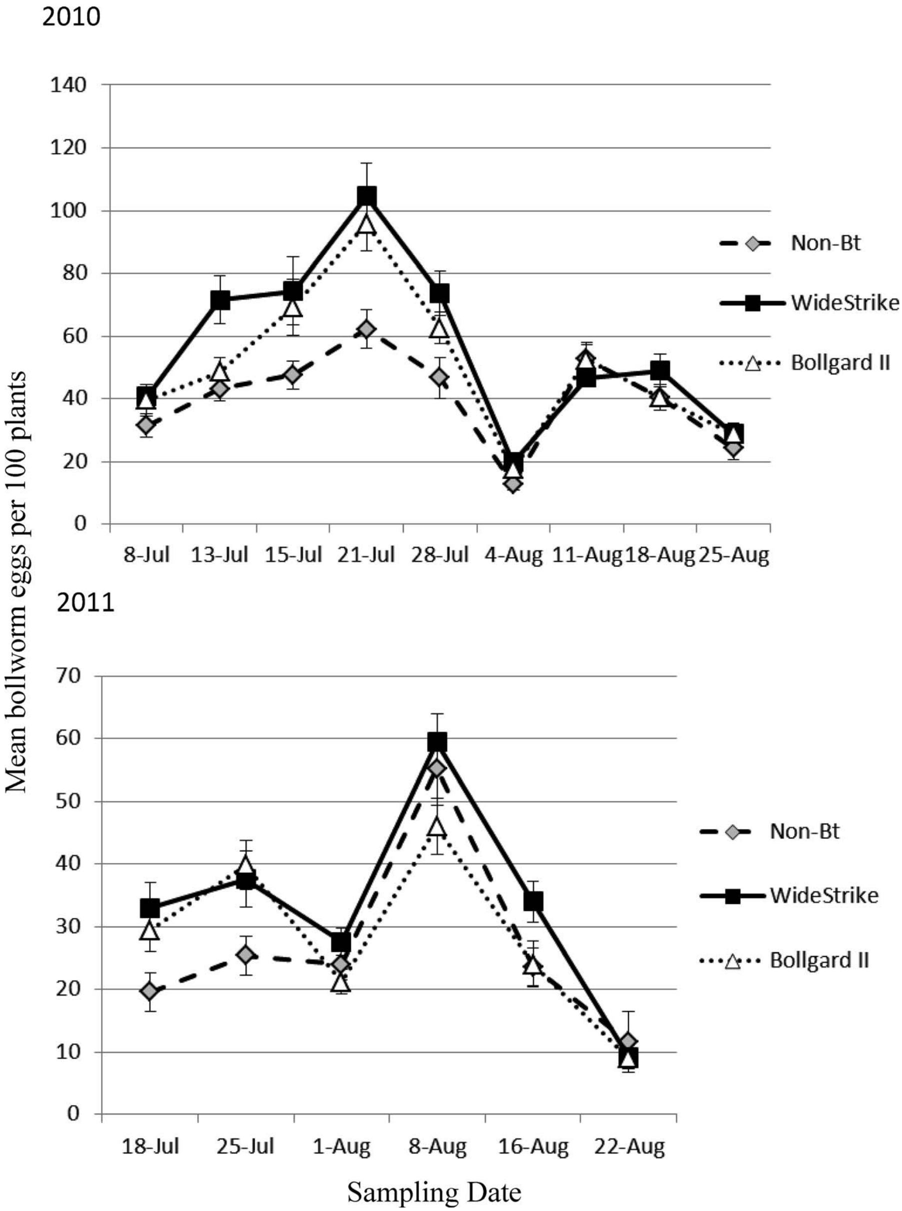

Fig. 1. Mean bollworm eggs per 100 plants (±SEM) averaged across egg thresholds by cotton variety and set of *Bt* traits near Blackville, South Carolina, in 2010 and 2011.

Experiment 3; boll damage treatment threshold. Boll damage in non-*Bt* and WideStrike cotton varied significantly by date (Table 2, Figure 6). Boll damage was elevated following the peak in egg density (Figure 1) during the same period that larvae populations were high (Figure 4). Although boll damage

Table 2. Statistical comparisons of bollworm egg and larval densities and boll damage in cotton near Blackville, South Carolina, 2010 and 2011.

Year	Management factor combination[a]	Egg density			Larval 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

[a]NBT = non Bt cotton, WS = WideStrike cotton, and BGII = Bollgard II cotton.

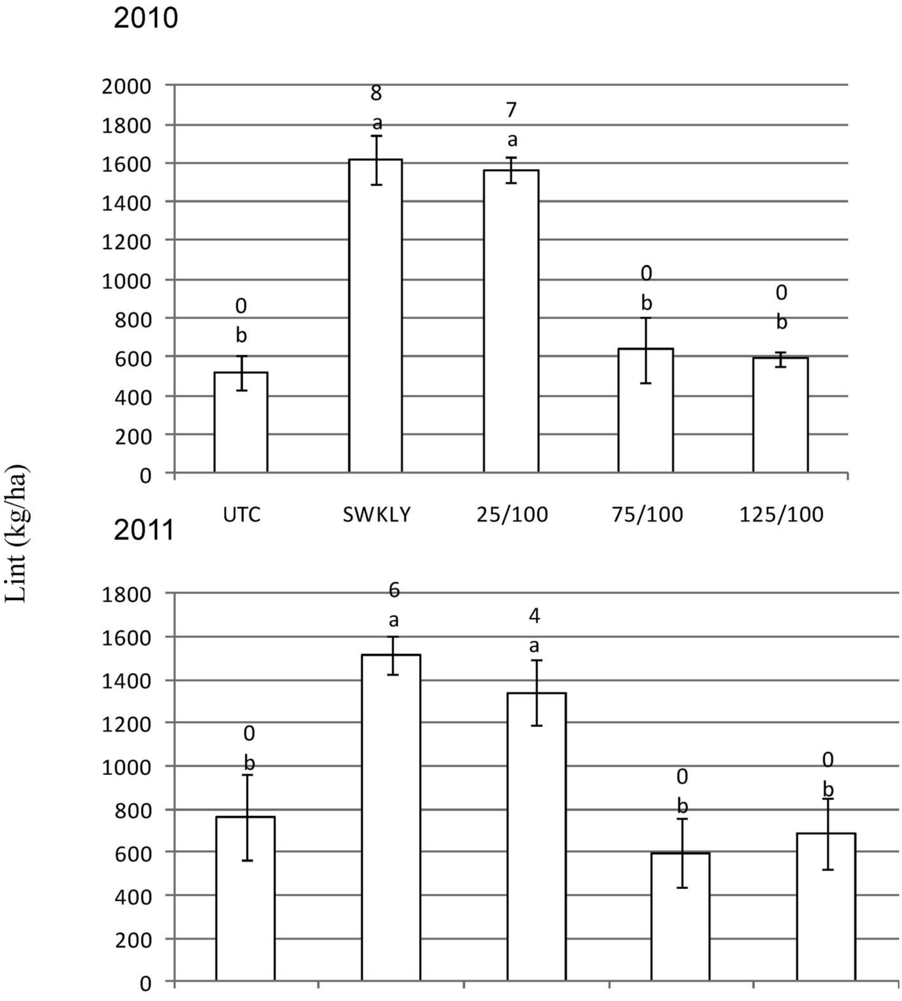

Fig. 2. Cotton lint yields (±SEM) determined by comparing bollworm egg threshold treatments in non-*Bt* cotton near Blackville, South Carolina, in 2010 and 2011. Bars with the same letter are not significantly different; numbers indicate number of insecticide applications received; UTC = untreated control, SWKLY = sprayed weekly.

in both sets of *Bt* traits decreased significantly when aggressively treated for *H. zea* in 2010 (Figure 7), significant yield impacts based on insecticide treatment were observed only in non-*Bt* cotton (Table 3). In 2010, *H. zea* damage was high enough in non-*Bt* cotton that all thresholds were treated weekly after scouting began. Boll damage in 2010 varied significantly between thresholds in Wide-

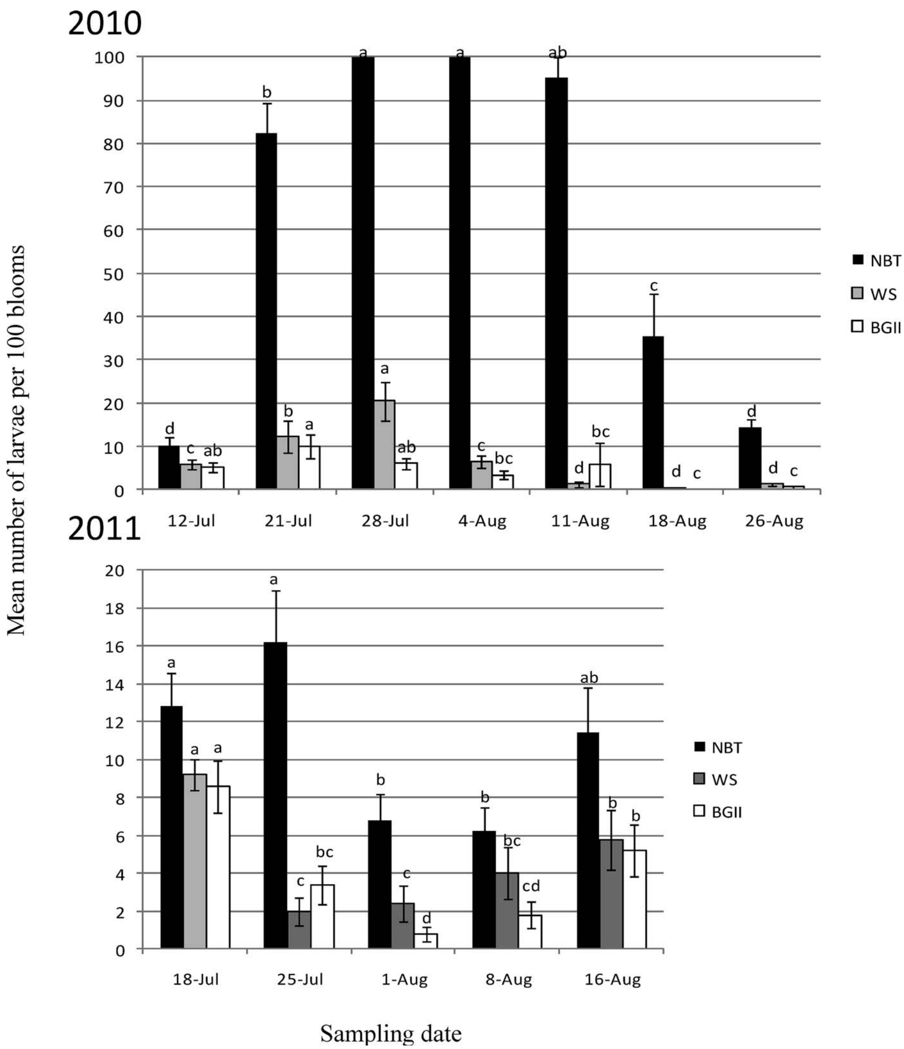

Fig. 3. Mean bollworm larvae per 100 blooms (±SEM), across treatment threshold by variety and *Bt* cotton traits and sampling date near Blackville, South Carolina, in 2010 and 2011. Bars of the same cotton variety with the same letter are not significantly different; NBT = non-*Bt,* WS = WideStrike, BGII = Bollgard II.

Strike cotton (Table 2), but the injury did not lead to any significant loss in lint yield (Table 3). Bacheler et al. (2006) also observed higher percent boll damage in WideStrike varieties than Bollgard II (15 and 6% boll damage, respectively, in 2003), yet yield was not significantly improved in either set of traits by insecticide treatment.

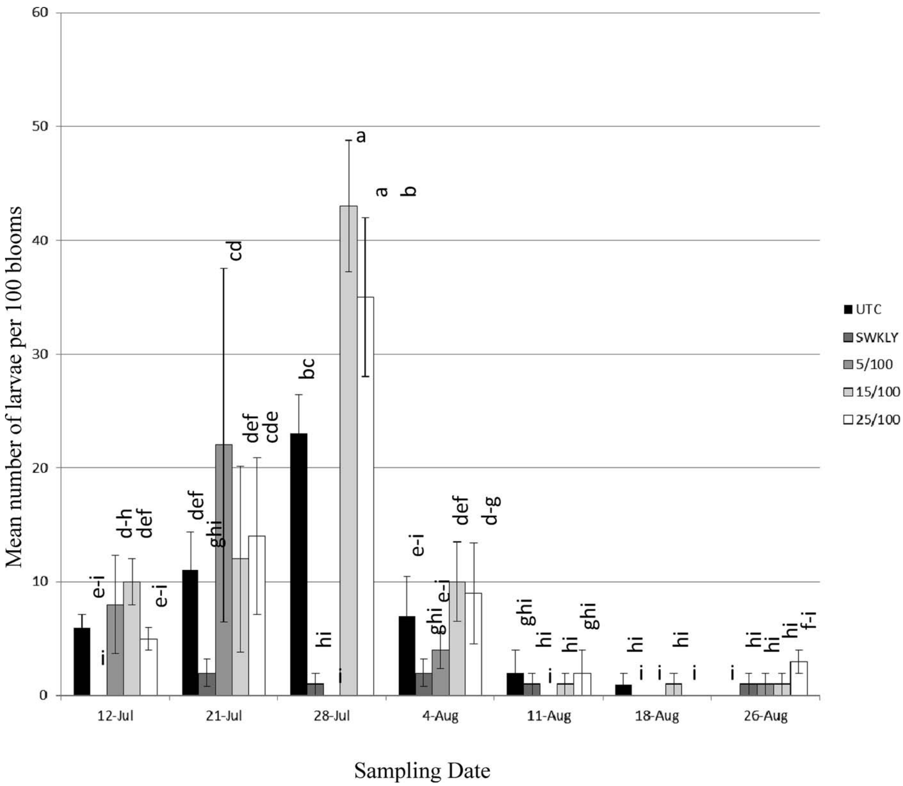

Fig. 4. Mean bollworm larvae per 100 blooms (±SEM) in WideStrike cotton by larval treatment threshold and date near Blackville, South Carolina, in 2010. UTC = untreated control, SWKLY = sprayed weekly.

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 *H. zea* populations were higher. During 2011, plots of non-*Bt* cotton protected weekly had higher incidence of 1st ($F = 4.66$; df = 4, 15; $P = 0.0409$) and 2nd position boll retention ($F = 6.17$; df = 4, 14; $P = 0.0044$), showing that bolls were protected by weekly insecticide application. However, this was only seen in the non-*Bt* control, with no differences in retention patterns between insecticide thresholds on dual *Bt*-gene cotton (data not shown).

Measurable differences in *H. zea* density and damage levels were observed between sets of *Bt* traits. WideStrike cotton regularly supported higher populations of *H. zea* and suffered 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. This difference between efficacy of WideStrike and Bollgard II traits has been noted across the Cotton Belt (Jackson et al. 2006, Greene et al. 2011). However, in this study, as with Bollgard II, lint yields from WideStrike plots were not different among treatments triggered on different thresholds for *H. zea*, although yields were

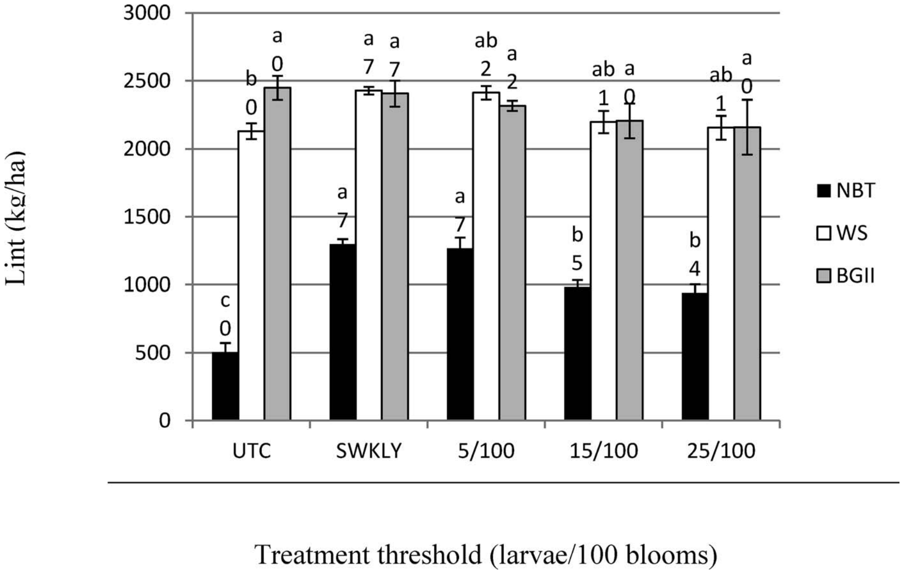

Fig. 5. Comparison of 2010 yield (±SEM) by threshold in WideStrike (WS), Bollgard II (BGII), and non-*Bt* (NBT) cotton near Blackville, South Carolina. Bars of the same cotton variety with the same letter are not significantly different; numbers above the bars indicate number of insecticide applications treatment received; UTC = untreated control, SWKLY = sprayed weekly.

significantly higher in WideStrike plots treated weekly compared with untreated plots in 2010. Therefore, these data do not support a recommendation that *H. zea* thresholds be amended for each package of *Bt* traits. Further research comparing trait combinations would need to be conducted in order to make such a recommendation. Because no differences in lint yield were found among thresholds within the sets of *Bt* traits tested in this study, insecticide applications exclusively targeting *H. zea* were deemed unnecessary in dual *Bt*-gene cotton. However, results from this study were limited to two growing seasons and one location. In contrast, other recent research has demonstrated significant increases in yield with foliar over-sprays to WideStrike and Bollgard II varieties (Lorenz et al. 2012, Orellana et al. 2014). Despite conflicting data, the results generated from this study should be considered in any future modification of South Carolina's current action threshold recommendations of three or more large larvae per 100 plants or 5% boll damage for dual-gene *Bt* cotton. Growers adhering to these extant recommendations for *H. zea* might unnecessarily apply one or two insecticide applications for bollworm in dual-gene *Bt* cotton, or the treatments might be necessary. The dynamic nature of various factors, such as pressure from bollworm, growing conditions (temperature, rainfall, etc.), varietal performance, length of growing season (i.e. delayed autumn) allowing yield compensation, and many others, undoubtedly influence the result of any scenario with or without foliar over-sprays for bollworm. When the literature is examined over time, the effectiveness of insecticide over-sprays for bollworm has seemingly

Table 3. Statistical comparisons of cotton lint yield near Blackville, South Carolina, 2010 and 2011.

Year	Management factor[a]	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

[a]NBT = non Bt cotton, WS = WideStrike cotton, and BGII = Bollgard II cotton.

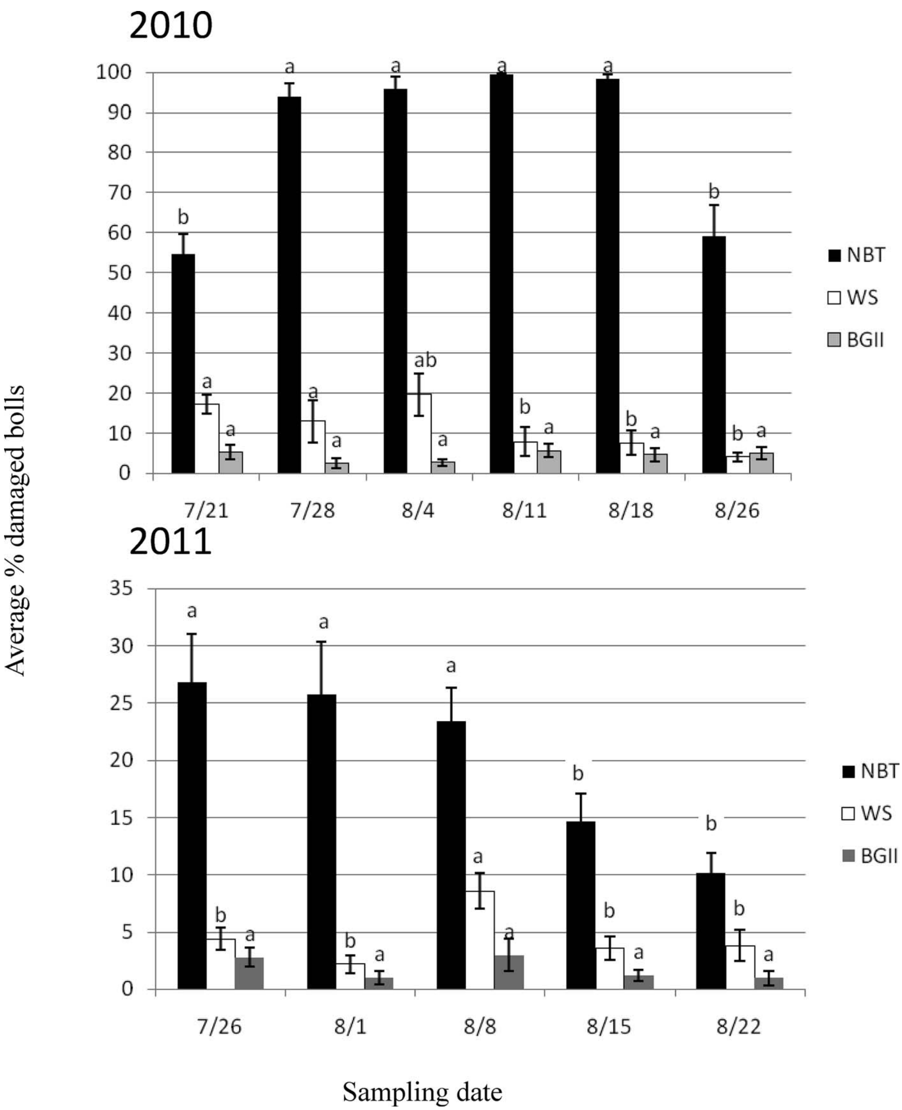

Fig. 6. Percent boll damage (±SEM), averaged across boll damage thresholds, caused by bollworm to non-*Bt* (NBT), WideStrike (WS), and Bollgard II (BGII) cotton during July and August 2010 and 2011 near Blackville, South Carolina. Bars of the same variety with the same letter are not significantly different.

increased in recent years, presumably because of gradual tolerance of *H. zea* to Cry proteins and increased survivability through years of exposure. Finally, it must be noted that, in the southeastern United States, stink bugs are regularly controlled with insecticides during periods of bollworm infestation, so concom-

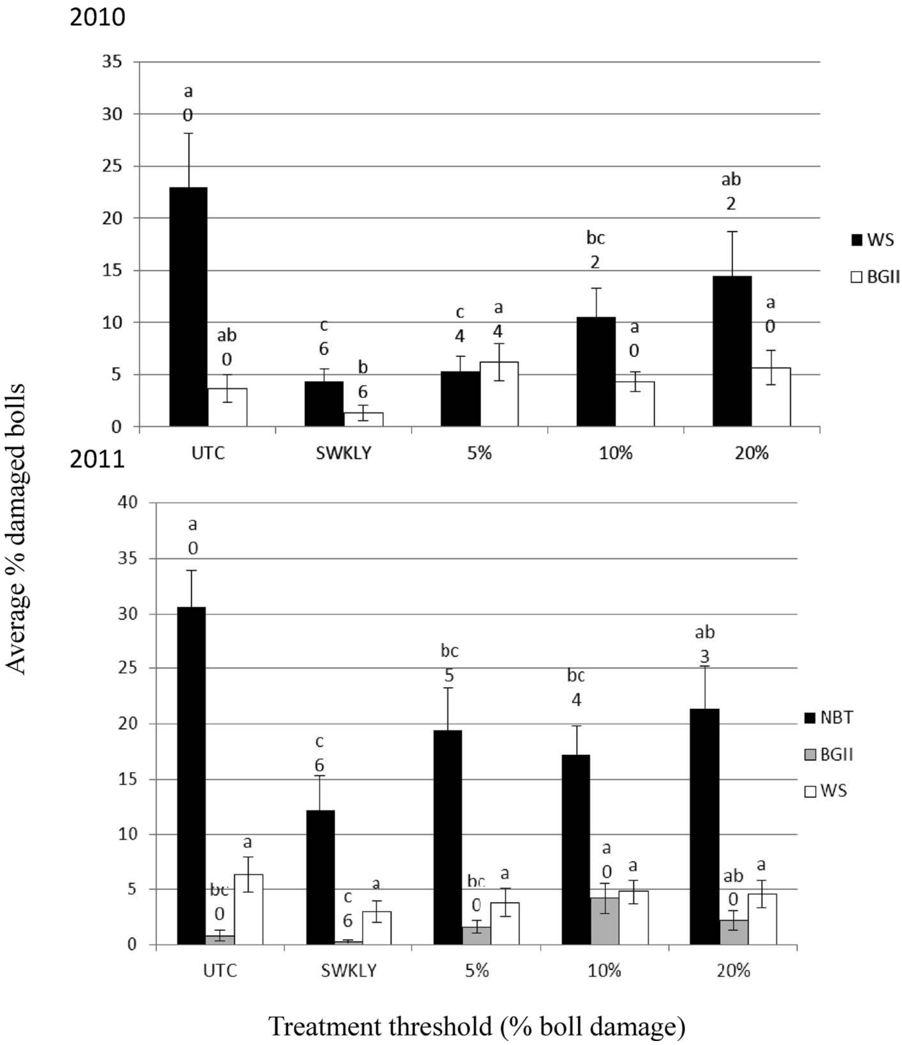

Fig. 7. Percent boll damage (±SEM) caused by bollworm averaged across sampling dates by boll damage threshold for WideStrike (WS) and Bollgard II (BGII) cotton in 2010 and for WideStrike (WS) and Bollgard II (BGII) and non-*Bt* (NBT) cotton in 2011 near Blackville, South Carolina. Bars of the same variety with the same letter are not significantly different; numbers indicate number of insecticide applications received; UTC = untreated control, SWKLY = sprayed weekly.

itant control of any *H. zea* surviving on *Bt* cotton can be expected under most scenarios (as long as the insecticide for stink bugs is active on *H. zea*) (Jackson et al. 2006), thus negating the need for dedicated applications for *H. zea*. In all regions of the Cotton Belt, further work is necessary to explore the interactions and impacts of other pests with *H. zea* in multiple-gene *Bt* cotton.

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