Midsouth Entomologist **4**: 1–13
ISSN: 1936-6019
www.midsouthentomologist.org.msstate.edu

Research Article

Efficacy of Cotton Expressing Pyramided *Bacillus thuringiensis* Insecticidal Proteins Against Lepidopteran Pests

Akin, D.S.*, S.D. Stewart[1], M.B. Layton[2], and J.A. Mills[3]

[1] 605 Airways Boulevard, West Tennessee Experiment Station, Jackson, TN 38301
[2] Department of Entomology and Pathology, Mississippi State University, Mississippi State, MS 39762-9775
[3] Monsanto Company, 1472 Pecan Ridge Drive, Collierville, TN 38017

*Current address: University of Arkansas, Division of Agriculture, 1408 Scogin Dr., Montecello, AR 71656, sakin@uaex.edu

Received: 7-IV-2010 *Accepted: 11-XI-2010*

Abstract: Laboratory, greenhouse, and field experiments were conducted to evaluate the efficacy of cotton (*Gossypium hirsutum* L.) expressing two proteins of *Bacillus thuringiensis* Berliner ('Bollgard II' expressing Cry1Ac + Cry2Ab, Monsanto Company, St. Louis, MO) against economically important lepidopteran pests in Mississippi. Results from leaf assays conducted in the laboratory indicate that Bollgard II cotton caused significantly higher mortality and growth inhibition of *Helicoverpa zea* (Boddie), *Heliothis virescens* (F.), *Spodoptera frugiperda* (J.E. Smith), *S. exigua* (Hubner), *Pseudoplusia includens* (Walker), and *Estigmene acrea* (Drury) than Cry1Ac only and non-Bt cotton. In greenhouse studies, Bollgard II cotton provided significantly enhanced protection against common defoliating pests (i.e., *S. exigua* and *P. includens*) compared with Bollgard and non-Bt cotton. In these studies, non-Bt and Bollgard cotton genotypes sustained nearly identical levels of defoliation by these pests. Overall, these results suggest that the combination of two insecticidal proteins in Bollgard II cotton significantly improved efficacy against lepidopteran pests compared to Bollgard cotton. Under field conditions with high populations of *H. zea* or other occasional lepidopteran pests (e.g., *S. frugiperda*), Bollgard II cotton should provide a much higher level of overall protection compared with that for Bollgard cotton plants.

Keywords: Bt cotton, Lepidoptera, Cry2Ab, Bollgard II

Introduction

Since the introduction of transgenic cotton expressing the Cry1Ac δ-endotoxin of *Bacillus thuringiensis* var. *kurstaki* (Bt) in 1996 (i.e., Bollgard®, Monsanto Company, St. Louis, MO), producers have taken advantage of this technology to reduce the yield-limiting effects of major lepidopteran pests. The effectiveness of Bollgard cotton against tobacco budworm, *Heliothis virescens*, (F.), and bollworm, *Helicoverpa zea* (Boddie), has been well documented (Deville et al. 2002, Roof and DuRant 1998, Mascarenhas et al. 1994, Benedict et al. 1993). Results of economic studies have demonstrated the value of Bollgard cotton in a variety of environments and under a range of insect pest pressures (Mullins and Mills 1999, Reed et al. 1998, Wier et al. 1998). Previously, Bollgard cotton has provided direct economic benefit due to fewer insecticide applications being required for lepidopteran pests (Layton et al. 2003). Also, natural enemies that are normally suppressed by insecticides were preserved, resulting in fewer occurrences of secondary pest outbreaks (Van Tol and Lentz 1998). However, since most insecticide applications in the midsouth at the time of this writing are targeted towards tarnished plant bug (*Lygus lineolaris* Palisot de Beauvois) (Williams 2010), Bt cotton might not provide the economic benefit it once did, nor are beneficial arthropods preserved with the frequency of these applications.

Despite its obvious advantages, Bollgard cotton has some weaknesses in IPM systems. Bollworm control in Bt cotton is less dependable than is control of tobacco budworm due to the lower susceptibility of *H. zea* to Cry1Ac (Stone and Sims 1993, MacIntosh et al. 1990). This is true particularly once anthesis has occurred and insecticides applied to control non-lepidopteran pests (e.g., cotton aphids and tarnished plant bugs) have disrupted populations of beneficial arthropods (Lambert et al. 1996, Turnipseed and Sullivan 1999). Therefore, damaging bollworm infestations can occur in Bt cotton (Mahaffey et al. 1995). Supplemental insecticide applications have been routinely required for control of this pest in Bt cotton across the southern United States (Layton et al. 1997, 1998, Bachelor and Mott 1997, Leonard et al. 1997, Roof and Durant 1997, Smith 1997). In Mississippi, Layton et al. (2003) reported that bollworm sprays applied to Bollgard cotton per season has ranged from 0.33 to 1.22 applications per acre each year since 1996. These applications for bollworms have further increased since 2003 (Williams 2010). Another disadvantage of Bollgard cotton is its low efficacy against armyworms and loopers (Adamczyk et al. 1998, Sumerford and Solomon 2000, Luttrell et al. 1998). Supplemental insecticide applications are occasionally necessary in Bt cotton to control economic infestations of these pests as well (Stewart et al. 2000).

To improve upon the value of Bollgard cotton, the Monsanto Company has developed cotton plants expressing the Cry1Ac protein found in Bollgard cotton as well as the Cry2Ab protein from Bt (Greenplate et al. 2000a). Sims (1997) confirmed that Cry2A protein exhibits biological activity specific for various Lepidoptera (e.g., heliothines and *Spodoptera* spp.). Although this pyramided-protein Bt cotton (i.e., Bollgard II, Monsanto Company) was developed to enhance control of bollworm as well as that for other lepidopteran pests, Cry2 proteins are actually less effective against bollworm than Cry1 proteins (Sims 1997). However, Cry2Ab is expressed at significantly higher levels than Cry1Ac in selected Bollgard II tissues (Greenplate et al. 2000b, Adamczyk et al. 2001). Therefore, additive or synergistic effects of Cry1Ac and Cry2Ab in Bollgard II cotton could result in greater overall bioactivity.

Several experiments have evaluated Bollgard II efficacy against selected lepidopteran pests. In laboratory tests, Stewart et al. (2001) found only 2% of second instar bollworms survived to 15 d on Bollgard II flowers compared with 16% survivorship on Bollgard flowers. A significant reduction in bollworm-damaged fruit was noted in Bollgard II compared with Bollgard genotypes grown in the greenhouse (Jackson et al. 2000). Stewart et al. (2000) reported greater larval stunting and delays in successful pupation for fall and beet armyworms feeding on Bollgard II cotton when compared with Bollgard. Ridge et al. (2000) also found significantly fewer bollworms and soybean loopers in Bollgard II plots compared to Bollgard field plots, although overall infestation levels were low. These results show that Bollgard II cotton provided enhanced control of various lepidopteran pests in those areas.

Bollgard II was approved by the United States Environmental Protection Agency in December 2002 and was made available to growers in a limited number of varieties for the 2003 growing season. Bollgard varieties have been phased out for use across most of the U.S. cotton belt as of 2010. The only insect-protection traits currently available are the pyramided Bt proteins in Bollgard II® (Monsanto) and WideStrike® (Dow AgroSciences). As Bollgard II varieties currently dominate cotton acreage across the midsouth (National Cotton Council 2010), it is important to verify the advantages Bollgard II provides over the original single-protein Bollgard for control of bollworm and occasional lepidopteran pests. Therefore, the overall objective was to compare the relative effectiveness of Bollgard II cotton against selected important lepidopteran pests common to cotton in the southern United States.

Materials and Methods

***Insects*.** All insects for laboratory and greenhouse studies, except for *P. includens* and *E. acrea*, were obtained from laboratory colonies maintained at the USDA-ARS Biological Control Mass Research Rearing Unit, Starkville, MS. All insect species obtained from Starkville were derived from laboratory colonies annually infused with feral moths to maintain genetic diversity. *P. includens* larvae were derived from a laboratory colony collected in Jeanerette, LA the year of experimentation. *E. acrea* larvae were from a single field-collected egg mass in Starkville, MS. All larvae were reared to the second instar on meridic diet (29 °C, 14:10 L/D, 60% RH), except for *E. acrea*, which were reared on cotton leaves of non-Bt cotton (DPL 5415) until the second instar. In greenhouse assays, soybean loopers were reared to third instar on meridic diet at 29 °C, 14:10 L/D, 60% RH.

***Leaf assays*.** A series of experiments evaluated the specific interaction between selected lepidopteran pests and freshly harvested green Bollgard II cotton leaves. Newly unfurled leaves (3rd node from the top of the plant) collected from field-grown cotton were used in Petri dish assays to evaluate the toxicity of non-Bt, Bollgard, and Bollgard II traits against *H. zea*, *H. virescens*, *S. frugiperda*, *S. exigua*, *P. includens*, and *E. acrea*. Leaves were collected in equal numbers from replicated field plots. In 2000, non-Bt (DP50, Delta and Pineland, Scott, MS), Bollgard (DP50B), and Bollgard II (DP50BII; DPLX 9C985EB) were planted on 16 May. On 14 May 2002, cotton varieties planted for *P. includens* bioassays were DP5415 (non-Bt), NuCOTN 33B (Bollgard), and NuCOTN 33BII (Bollgard II, DPLX01T21D). All field plots were grown at the Plant Science Research Station near Starkville, MS.

In 2000, terminal leaves (3rd node from top) were collected 71, 85, 88, 91, and 99 days after planting (DAP) in those experiments evaluating *H. zea*, *H. virescens*, *S. frugiperda*, *E. acrea*, and *S. exigua*, respectively. For *P. includens*, leaf tissue was collected 86 days after planting (DAP) in 2002. For each species, fifty 9-cm Petri dishes per plant genotype were each filled with 15 ml of water agar. One autoclaved paper towel (9-cm diameter) was placed into each dish and moistened with sterile water, followed by one washed leaf and one second instar of the appropriate species. Petri dishes were then placed in an environmental growth chamber at 29 °C (14:10 h L:D, 60% RH) for 3 d, after which time each leaf was replaced with a fresh tissue. At 6 days after infestation (DAI), survival (%), and length (mm) of surviving larvae was recorded.

Insect survivorship was analyzed by using Proc GENMOD (i.e., Chi-square analysis, SAS 1999). Larval size (length) was analyzed by using Proc GLM with Fisher's protected LSD for mean separation at $\alpha = 0.05$ (SAS 1999).

Greenhouse experiments on intact plants. Experiments were performed during 2001 and 2002 to evaluate the efficacy of Bollgard II cotton genotypes against beet armyworm and soybean looper. Cotton varieties evaluated in 2001 were DP 5415 (non-Bt), NuCOTN 33B (Bollgard), and NuCOTN 33BII (Bollgard II, DPLX 01L80D). Identical varieties were used in 2002 except for a different background line of NuCOTN 33BII (DPLX01T21D) had to be substituted. Twenty seeds of non-Bt, Bollgard, and Bollgard II cotton were each planted ca. 2.5 cm deep in individual pots. Ten plants of similar uniformity were selected for testing. All pots were arranged in a completely randomized design and were managed with optimum levels of irrigation and fertilizer. When plants developed to an average of 14 mainstem nodes, one mass each of exactly 100 *S. exigua* eggs were stapled to the third and seventh uppermost sympodial leaf from a plant terminal. Eggs were monitored twice daily to determine the date of larval eclosion. In a separate experiment, non-Bt, Bollgard, and Bollgard II plants, also at 14 nodes, were individually infested with twenty *P. includens* larvae originating from an LSU laboratory colony, (M. Baur, Soybean IPM Laboratory, Louisiana State University Department of Entomology) by using a 000 paint brush. Plant defoliation levels were determined at 5 and 7 days after hatch (DAH) of both species by estimating the average tissue loss on all leaves according to Stewart (1996), where 0% defoliation was no visible feeding and 100% defoliation was all leaf tissue having been consumed. At 7 DAH, cotton plants were removed from the pots by severing the main stem at the soil level and shaking each plant into a container to collect and record the total number of surviving larvae. A cohort of five larvae from each plant were then randomly selected and weighed collectively.

Defoliation, surviving larvae, and larval weights for both species were subjected to analysis of variance procedures by using Fisher's protected LSD for mean separation at $\alpha = 0.05$ (Proc GLM, SAS 1999).

***Small-plot field experiment*.** Field studies against native infestations evaluated the efficacy of non-Bt, Bollgard, and Bollgard II in a system for lepidopteran pest management. Each system was compared for numbers of heliothines, occasional lepidopteran pests, damaged flower buds (squares), damaged fruit (bolls), and seed cotton yield. These trials were conducted at three Mississippi locations in 2000 (Starkville, Brooksville, and Raymond) and one location in 2001 (Starkville). In 2000, varieties used were DP50, DP50B, and DP50BII for non-Bt, single-toxin, and dual-toxin varieties, respectively. Varieties used in 2001 were DP5415 (non-Bt), NuCOTN33B (Bollgard), and NuCOTN33BII (Bollgard II; DPLX01L80D). In 2000, plots were planted on 16 May in Starkville, 22 May in Brooksville, and 25 May in Raymond. Plots were 24 rows × 15 m in Brooksville and 16 rows × 15 m at Raymond and Starkville. In 2001, plot size was 12 rows × 15 m, and was planted on 26 April in Starkville. All experiments were planted on 96-cm row spacing, with treatments replicated three times in a randomized complete block design.

For each variety, insecticide applications were made based on average insect numbers combined across all three replicates by using insect control recommendations for Mississippi (Layton 2000, 2001). When the treatment threshold for a given pest was reached, an insecticide application was made to all plots of that variety. Since there was no set threshold for lepidopteran pests in Bollgard II cotton at the time the experiment was conducted, those plots were treated based upon the recommended threshold for Bollgard. Current guidelines for scouting and managing Bt cotton in Mississippi recommend supplemental foliar treatments for bollworms if the number of larval survivors (≥3 mm [ca. 1/8 in]) exceeds four per 100 plants (Layton 2000, 2001). With exception of the size criterion, this is the same action threshold used to initiate sprays for heliothines in non-Bt cotton.

Insecticide oversprays for lepidopteran pests included either 0.075 kg ai/ha spinosad (Tracer®, Dow AgroSciences), 0.034 kg ai/ha lambda-cyhalothrin (Karate Z®, Syngenta Crop Protection), or 0.037 kg ai/ha cyfluthrin (Baythroid®, Bayer CropScience). In Brooksville (2000), treatments for non-lepidopteran pests included 0.336 kg ai/ha acephate (Orthene®, Valent USA) on 14 June for thrips, and 0.109 kg ai/ha pymetrozine (Fulfill®, Syngenta) on 30 June for cotton aphids. No insecticide applications were made for non-lepidopteran pests in Starkville or Raymond (2000). In the 2001 trial at Starkville, acephate (0.336 kg ai/ha) was applied to control tarnished plant bugs on 14, 19, and 25 June. An additional application of 0.045 kg ai/ha imidacloprid (Provado®, Bayer) was made on 9 July. In Starkville, test plots were furrow irrigated five times in 2000 and twice in 2001. Experiments at Brooksville and Raymond were not irrigated. Native populations of pests were monitored twice weekly by examining 33 plant terminals within each plot for heliothines on each sampling date. Squares and bolls (25 per plot) were also examined for presence of lepidopteran pests (particularly heliothines and fall armyworms) and associated damage.

Seed cotton yields were compared among Bollgard II, Bollgard, and non-Bt cottons by mechanically harvesting the center two rows of each plot in all experiments. Harvest dates in 2000 were 27 September in Starkville, 10 October in Brooksville, and 28 October in Raymond. In 2001, test plots in Starkville were harvested on 28 September.

Insect and damaged structure data were analyzed by using Proc NPAR1WAY [Wilcoxon] (α = 0.05, SAS 1999). Yield data were subjected to analysis of variance procedures and linear contrasts for mean separation (α = 0.05, Proc GLM, SAS 1999).

***Late-planted field experiment*.** In an effort to take advantage of the higher lepidopteran pest populations that normally occur in the latter portion of the cotton production season, a field experiment was planted on 1 July 2002. This is date of planting was much later than the normal window of 15 April to 15 May. Plots of non-Bt (DPL 5415), Bollgard (NuCOTN 33B), and Bollgard II (NuCOTN 33BII, DPLX01T21D) were planted at the Plant Science Research Farm in Starkville, MS. Test plots were planted as a randomized complete block design with three replications. Plots were 23 m long and 16 rows wide with one skip row between plots within each replicate. In addition to the incorporation of an unplanted row between plots, only plants from the inside 14 rows were sampled for insects and associated damage.

After the first sampling date (7 September), scheduled insecticide treatments were initiated to reduce beneficial arthropod populations and enhance Lepidopteran infestations. Treatments were applied once weekly for the duration of sampling, and consisted of oxamyl (Vydate®, DuPont; 0.28 kg ai/ha) or dicrotophos (Bidrin®, Amvac; 0.28 kg ai/ha). Both products lack significant activity on lepidopteran pests at these rates.

Starting at the plant growth stage of 16 mainstem nodes (on 5 September), field plots were sampled by examining various plant structures for lepidopteran larvae or associated damage. Cotton terminal leaves, squares, young bolls, white flowers, pink flowers, old bolls with dried flower (corolla) attached, and old bolls without dried corolla (33 each per plot were inspected in each plots). Drop-cloth sampling procedures were initiated on 5 September and performed five times during the next six weeks. For each sampling date, three drop-cloth subsamples (1.8 m of row each) were conducted in randomly chosen areas of each plot. Infestation levels and diversity of lepidopteran pests were recorded in all samples.

A single, late-season survey of damaged bolls was conducted within each plot on 16 October. These samples were done when cotton plants were well past physiological cutout (NAWF = 5 + ca. 350 HU's). NAWF = 5 was determined as defined by Bourland et al. (1992), where terminal growth had declined to the point that there were less than five main stem nodes above a first-position white flower. In each plot, 333 randomly selected 10–14-day-old bolls on economically important positions of each plant (i.e., first and second sympodial positions) were closely inspected for damage (total of 999 per genotype). Bolls were classified as damaged if boll walls were completely penetrated.

The primary comparison of interest with respect to insect numbers and damage was between Bollgard and Bollgard II. Data from non-Bt cotton was included in the trial to provide an index of the intensity of insect numbers and damage, but these data were not included in the analysis (Tables 8-10). All data for these comparisons were analyzed by using the Wilcoxon rank-sum test (PROC NPAR1WAY, SAS 1999), and subsequently converted to percentage.

Results and Discussion

***Leaf assays*.** In leaf bioassays comparing toxicity of non-Bt, Bollgard, and Bollgard II cotton, significantly fewer *H. virescens* larvae were found at 6 DAI on Bollgard and Bollgard II than on non-Bt (Table 1). This was due to the inherent susceptibility of *H. virescens* to Cry1Ac in both technologies relative to the size of the larvae (Stone and Sims 1993). All other species survived equally well on non-Bt and Bollgard cotton leaves (>90%). However, the pyramided gene construct of Bollgard II clearly enhanced activity against all species. Significantly lower survivorship of all target pests was observed on Bollgard II tissue than on Bollgard tissue (Table 1). These results are consistent with those reported by Stewart et al. (2001), suggesting that Bollgard II will be more effective and will have a much wider range of activity against lepidopteran pests than the original Bollgard cotton.

Table 1. Survivorship (percentage) of Lepidoptera six days after infestation of second instars on non-Bt, Bollgard, and Bollgard II cotton terminal leaves.

Species	non-Bt	Bollgard	Bollgard II	χ^2	df	p-value
H. virescens	92 ± 4 a	31 ± 7 b	0 c	135.10	2	<0.0001
H. zea	94 ± 3 a	96 ± 3 a	78 ± 6 b	10.06	2	0.0065
S. frugiperda	91 ± 4 a	98 ± 2 a	55 ± 8 b	35.35	2	<0.0001
S. exigua	100 ± 0 a	98 ± 2 a	81 ± 6 b	16.49	2	0.0003
E. acrea	---	100 ± 0 a	8 ± 4 b	185.11	1	<0.0001
P. includens	100 ± 0 a	98 ± 2 a	2 ± 2 b	584.40	2	<0.0001

Means in the same row not followed by a common letter are significantly different (Proc GENMOD; α = 0.05, SAS 1999).

Significant stunting of *H. virescens* and *H. zea* larvae was observed on Bollgard compared to those containing non-Bt cotton (Table 2). Bollgard did not significantly affect larval growth for other target species compared with that on non-Bt cotton. However, larval growth was significantly lower for all species on Bollgard II compared with that on both non-Bt and Bollgard cotton. Gore et al. (2003) indicated that bollworms injured more fruiting structures on non-Bt cotton than on Bollgard or Bollgard II and considered the non-lethal effects of Bt cotton an important contribution to this effect. More importantly, damage to fruiting structures was limited to sites of infestation in Bollgard II cotton (Gore et al. 2003). Even if these larvae were to survive, the extended duration in larval stages might better expose them to attack by predators or pathogens in a field environment.

Table 2. Larval length (mm ± SEM) of Lepidoptera six days after infestation (DAI) of second instars on non-Bt, Bollgard, and Bollgard II cotton terminal leaves.

	Length					
Species	non-Bt	Bollgard	Bollgard II	*F*	df	*p*-value
H. virescens	15.8 ± 0.6 a	8.4 ± 0.9 b	--- [a]	40.7	1, 57	<0.0001
H. zea	21.3 ± 0.6 a	16.0 ± 0.4 b	9.8 ± 0.3 c	147.8	2, 124	<0.0001
S. frugiperda	14.3 ± 0.6 a	14.2 ± 0.5 a	9.1 ± 0.7 b	19.5	2, 105	<0.0001
S. exigua	16.7 ± 0.6 a	14.9 ± 0.4 a	7.7 ± 0.4 b	89.4	2, 125	<0.0001
E. acrea	---	12.9 ± 0.3 a	5.3 ± 0.3 b	63.0	1, 52	<0.0001
P. includens	24.3 ± 0.5 a	19.9 ± 0.4 a	4.0 ± 0.3 b	39.6	2, 96	<0.0001

Means in the same row not followed by a common letter are significantly different (Proc GLM; $\alpha = 0.05$, SAS 1999).
[a] Length data not analyzed due to lack of survival.

All species of lepidopteran targets were second instars at the time of infestation and were allowed to develop in optimum conditions within a growth chamber (i.e., no natural enemies). Therefore, lower survivorship would be expected with neonates feeding on Bt cotton varieties under field conditions. Results of this work suggest that species that normally initiate feeding on leaf tissue are very susceptible to the proteins in Bollgard II.

Greenhouse experiments on intact plants**.** In the 2001 *S. exigua* trials, non-Bt and Bollgard cotton sustained similar levels of defoliation at 5 DAH (ca. 50%), but Bollgard II plants had sustained only 3.5% defoliation (Table 3). At 7 DAH, percent defoliation nearly doubled on both non-Bt and Bollgard, exceeding 90% for both cotton genotypes, but remained very low in Bollgard II (3.6%). *S. exigua* numbers were not significantly different among cotton lines at 7 DAH. However, larvae on Bollgard II plants were severely stunted (>10-fold) compared with those on non-Bt and Bollgard. Although Bollgard II did not reduce the total number of *S. exigua*, the sublethal effects of Bollgard II were still effective in providing protection against this pest.

Table 3. Percent defoliation, number of larvae, and mean weight of *Spodoptera exigua* larvae on non-Bt, Bollgard, and Bollgard II cotton (greenhouse trial, 2001).

	% defoliation		No. Larvae/plant	Mean wt (mg)[a]
	5 DAH	7 DAH	7 DAH	7 DAH
Non-Bt	50.0 ± 2.7 a	92.7 ± 2.6 a	101.0 ± 10.2 a	69.4 ± 8.4 a
Bollgard	48.5 ± 4.6 a	91.2 ± 1.5 a	110.8 ± 10.5 a	51.8 ± 7.2 a
Bollgard II	3.5 ± 0.3 b	3.6 ± 0.3 b	94.4 ± 10.2 a	4.8 ± 0.5 b
F	73.61	873.9	0.64	27.2
df	2, 27	2, 27	2, 12	2, 27
p-value	<0.0001	<0.0001	0.5422	<0.0001

Means in the same column not followed by a common letter are significantly different (Proc GLM, $\alpha =$ 0.05; SAS 1999).
[a] Average weight of five randomly selected larvae per plant.

In 2002, overall defoliation levels were lower than in 2001 (Table 4), but the same general trends were observed. At 5 DAH, defoliation levels of non-Bt and Bollgard cotton were similar, but Bollgard II sustained only 1.2% defoliation. At 7 DAH, defoliation of non-Bt and Bollgard plants were 39.5% and 48.5%, respectively, but Bollgard II plants had sustained only 1.4% defoliation. There were only 0.4 larvae per plant on Bollgard II plants at 7 DAH, compared to 5.7 and 15.1 on non-Bt and Bollgard cotton, respectively. The reduction in *S. exigua* numbers in Bollgard II is consistent with results reported by Adamczyk et al. (2001). The lower numbers of beet armyworm on Bollgard II in this experiment were related either to direct mortality or to avoidance behavior exhibited by larvae abandoning plants. More larvae were observed on Bollgard than on non-Bt cotton, which suggests greater larval mobility from those on non-Bt cotton. Mean larval weights of *S. exigua* for non-Bt cotton were significantly higher than Bollgard genotypes, as in 2001 greenhouse studies (Table 4). On Bollgard II plants, however, larvae were not present in sufficient numbers to make valid statistical comparisons in 2002. The mean weight of the four larvae that were collected was 12.3 ± 4 mg.

Table 4. Percent defoliation, number of larvae, and mean weight of *Spodoptera exigua* larvae on non-Bt, Bollgard, and Bollgard II cotton (greenhouse trial, 2002).

	% defoliation		No. Larvae/plant	Mean wt(mg)[a]
	5 DAH	7 DAH	7 DAH	7 DAH
Non-Bt	24.0 ± 4.4 a	39.5 ± 4.9 a	5.7 ± 0.8 b	75.0 ± 8.0 a
Bollgard	26.0 ± 3.6 a	48.5 ± 5.0 a	15.1 ± 2.5 a	31.3 ± 2.1 b
Bollgard II	1.2+0.3 b	1.4+0.3 b	0.4+0.1 c	--- [b]
F	17.76	38.49	25.05	28.04
df	2, 27	2, 27	2, 27	1, 18
p-value	<0.0001	<0.0001	<0.0001	<0.0001

Means in the same column not followed by a common letter are significantly different (Proc GLM, α = 0.05; SAS 1999).
[a] Average weight of five randomly selected larvae per plant.
[b] Although some larvae were found on Bollgard II plants at 7 DAH, not enough were present for valid comparisons.

In the *P. includens* experiment during 2002, no significant difference in defoliation between non-Bt and Bollgard plots was observed at 5 DAI (Table 5). Bollgard II cotton plants, however, sustained significantly less defoliation (1%) than either non-Bt (12.5%) or Bollgard (8%). At 7 DAI, the same trend was evident in that Bollgard plants provided no protection compared with non-Bt cotton (12.5 vs 15.0%, respectively), but Bollgard II sustained significantly less defoliation (1%). Bollgard cotton had significantly fewer larvae per plant than did non-Bt cotton, but no *P. includens* larvae were found on Bollgard II plants at 7 DAI.

Table 5. Percent defoliation, number of larvae, and mean weight of *Pseudoplusia includens* on non-Bt, Bollgard, and Bollgard II cotton (greenhouse trial, 2002).

	% defoliation		No. Larvae	Mean wt (mg)[a]
	5 DAH	7 DAH	7 DAH	7 DAH
Non-Bt	12.5 ± 1.7 a	15.0 ± 1.8 a	5.0 ± 0.6 a	87.1 ± 5.6 a
Bollgard	8.0 ± 1.1 a	12.5 ± 1.1 a	3.0 ± 0.7 b	31.6 ± 3.2 b
Bollgard II	1.0 ± 0.2 b	1.0 ± 0.2 b	0.0 c	--- [b]
F	24.22	36.31	4.50	81.69
df	2, 27	2, 27	1, 18	1, 16
p-value	<0.0001	<0.0001	0.0480	<0.0001

Means in the same column not followed by a common letter are significantly different (Proc GLM, α = 0.05; SAS 1999).
[a] Average weight of five randomly selected larvae per plant.
[b] No larvae were found on Bollgard II plants.

This might have been due to the greater mobility of larger larvae (L3) compared to those in the *S. exigua* studies (neonates), thereby resulting in escapes. Cry1Ac in Bollgard cotton also reduced larval weights of soybean looper compared with non-Bt cotton. In these greenhouse studies, Bollgard II cotton provided significant protection against heavy infestations of *S. exigua* and *P. includens*.

Small-plot field experiment. In the 2000 and 2001 field experiments, comparing non-Bt, Bollgard, and Bollgard II, the only lepidopteran pests that occurred in sufficient numbers were heliothines. There was no location*variety interaction in 2000 for heliothine numbers (F = 1.13; df = 4, 358; p = 0.34); thus, data from all three locations in 2000 were pooled. More heliothines were found in non-Bt cotton than in either single or dual-toxin cotton, but there was no significant difference in heliothine infestations between Bollgard and Bollgard II cotton (Table 6). Overall insect pressure was generally low in 2000, with non-Bt plots requiring an average of only 1.67 insecticide applications for lepidopteran pests per trial, compared to a seasonal total of four heliothine treatments in non-Bt plots for the 2001 Starkville trial.

Table 6. Number of heliothines, percent damaged structures, and seed cotton yield in Starkville, Raymond, and Brooksville in 2000 (pooled across location).

Genotype	No. heliothines per 100 plants	Mean number damaged terminals, squares, and bolls per plot	Seed cotton yield kg/ha
Non-Bt	1.4 ± 0.2 a	6.0 ± 0.6 a	2130 a
Bollgard	0.1 ± 0.1 b	2.3 ± 0.3 b	2460 a
Bollgard II	0.0 ± 0.0 b	1.0 ± 0.3 c	2147 a
χ^2	17.7	17.9	F = 0.67
df	2	2	df = 2, 12
Pr> χ^2	0.0001	0.0001	p = 0.53

Means in the same column not followed by a common letter are significantly different (Proc NPAR1WAY [Wilcoxon], α = 0.05; Proc GLM, α = 0.05) (SAS 1999).

Table 7. Number of heliothines, percent damaged structures, insecticide applications and costs, and seed cotton yield in Starkville (2001).

Genotype	No. heliothines per 100 plants	Mean number damaged terminals, squares, and bolls per plot	Seed cotton yield kg/ha
Non-Bt	3.5 ± 0.7 a	7.2 ± 1.0 a	2725 a
Bollgard	0.7 ± 0.3 b	1.2 ± 0.4 b	2750 a
Bollgard II	0.3 ± 0.1 b	0.4 ± 0.2 b	2554 a
χ^2	39.6	66.7	F = 0.11
df	2	2	df= 2, 6
Pr> χ^2	<0.0001	<0.0001	p = 0.90

Means in the same column not followed by a common letter are significantly different (Proc NPAR1WAY [Wilcoxon], α = 0.05; Proc GLM, α = 0.05) (SAS 1999).

In 2000, insecticide applications for Lepidoptera were not necessary in Bollgard or Bollgard II plots due to light heliothine pressure (Table 6). In non-Bt cotton plots at Starkville, an application of spinosad was made on 12 July and 29 July, and one application of cyfluthrin (Baythroid®, Bayer Crop Sciences) was made on 5 August. In Brooksville, one spinosad application was made for heliothines on 28 July. In Raymond, one application of lambda-cyhalothrin (Karate Z®, Syngenta Company) was applied for heliothines on 10 August. In 2001, the heliothine threshold in non-Bt plots was exceeded on four dates during the season. Insecticide applications included two applications of spinosad (25 June and 9 July), and two applications of cyfluthrin (22 and 29 July). As in 2000, only non-Bt cotton was treated for lepidopteran pests (Table 7).

There was no location*variety interaction for damage in 2000 (F = 2.03; df = 2, 28; p = 0.1498); thus, the data were pooled. Bollgard exhibited less damage in terminals, squares, and bolls than non-Bt cotton (Table 6). There were significantly fewer damaged structures in Bollgard II plots than in those of either non-Bt or Bollgard. In 2001, more damage to terminals, squares, and bolls occurred in non-Bt cotton than in plots containing either of the transgenic varieties (Table 7), but there was no difference between Bollgard or Bollgard II cottons.

There were no significant differences in seed cotton yields between non-Bt, Bollgard, and Bollgard II cotton in either 2000 (F = 0.67; df = 2, 12; p = 0.5298) or 2001 (F = 0.11; df = 2, 6; p = 0.9002). Yields ranged from 2130 to 2460 kg seed cotton/ha in 2000 (Table 6) and between 2554 and 2750 kg seed cotton/ha in 2001 (Table 7).

***Late-planted field experiment*.** Although this experiment was designed to test Bollgard II cotton under conditions of high populations of lepidopteran pests, the only sample dates with significant insect populations and associated damage in Bollgard II occurred on 17 and 24 September (collection dates 3 and 4). The pest species in Bollgard II primarily were comprised of heliothines and fall armyworm on these two dates. Numbers of lepidopteran pests were low in non-Bt plots, and essentially absent in Bollgard or Bollgard II plots during all other sampling periods.

There were no significant differences between Bollgard and Bollgard II on 17 September (Table 8) regardless of plant structure. On 24 September, Bollgard II had fewer damaged terminals, squares, and 10–14-day-old bolls with no dried corolla attached than did Bollgard (Table 9). For heliothine (>3 mm) counts, there was no significant difference between Bollgard and Bollgard II in any of the plant structures inspected for either date. The same observation was noted for fall armyworms in Bollgard and Bollgard II cotton. This is likely due to the inherent efficacy of Bollgard against the heliothine complex and a relatively low population of fall armyworm. However, it should be noted that the only plant structures that contained either larvae or associated damage in all Bollgard II plots were white and pink blooms. In Bollgard cotton, however, larvae and damage were found in other structures such as terminals, squares, young bolls, and old bolls. Therefore, scouting procedures for Bollgard II should be defined to place even greater emphasis on white and pink blooms, as has already been implemented for Bollgard cotton (Layton 2003, 1996).

Table 8. Damage (percentage) to plant structures in late-planted non-Bt, Bollgard, and Bollgard II cotton (17 September 2002).

Genotype	terminals	squares	white blooms	pink blooms	young bolls	old bolls w/o corolla	old bolls w/corolla
Non-Bt[a]	44	24	16	19	7	8	8
Bollgard	1 a	5 a	5 a	6 a	2 a	1 a	1 a
Bollgard II	0 a	0 b	1 a	1 a	0 a	0 a	0 a
χ^2	1.00	4.50	3.33	3.23	2.50	1.00	1.00
df	1	1	1	1	1	1	1
Pr> χ^2	0.3173	0.0339	0.0679	0.0722	0.1138	0.3173	0.3173

Means in the same column not followed by a common letter are significantly different (Proc NPAR1WAY (Wilcoxson), α = 0.05; SAS 1999).
[a]Data for non-Bt genotype is included only to provide a reference of the level of insect pressure in experiments.

In drop cloth samples, significant insect pest infestation was detected on only during the 2 October sample. Pests that occurred in significant numbers on this date were *Trichoplusia ni*, Pseudoplusia *includens*, (cabbage/soybean looper complex), and *S. frugiperda*. There was a significant difference between Bollgard and Bollgard II with respect to looper populations (F = 10; df = 1, 8; p = 0.0133) and fall armyworms (F = 6.4; df = 1, 8; p = 0.0353). Bollgard cotton contained 18.5 loopers per 100 row-feet, while no loopers were found in Bollgard II cotton. There were also no fall armyworms in drop cloth samples of Bollgard II, while Bollgard plots contained an average of 7.4 fall armyworm larvae per 100 row-feet. These data demonstrate that Bollgard II was more efficacious against these pests.

Table 9. Damage (percentage) to plant structures in late-planted non-Bt, Bollgard, and Bollgard II cotton (24 September 2002).

Genotype	terminals	squares	white blooms	pink blooms	young bolls	old bolls w/o corolla	old bolls w/corolla
Non-Bt[a]	54	29	20	16	24	21	16
Bollgard	6 a	3 a	2 a	0 a	1 a	6 a	4 a
Bollgard II	0 b	0 b	2 a	0 a	0 a	0 b	0 a
χ^2	4.35	5.00	0.00	0.00	1.00	4.36	2.40
Df	1	1	1	1	1	1	1
Pr> χ^2	0.0369	0.0253	1.00	1.00	0.3173	0.0369	0.1213

Means in the same column not followed by a common letter are significantly different (Proc NPAR1WAY (Wilcoxson), α = 0.05; SAS 1999).
[a]Data for non-Bt genotype is included only to provide a reference of the level of insect pressure in experiments.

In the single survey of damaged bolls on 16 October, non-Bt cotton sustained 14% damaged bolls (Table 10), but damaged bolls in Bollgard cotton comprised only 1.8%. Bollgard II cotton (0.2%) sustained significantly less damage than did Bollgard cotton. Although Bollgard provided a high degree of protection, boll damage from several pests can still occur. These results indicate that even under conditions of moderate pressure from lepidopteran pests, Bollgard II improved levels of pest control relative to Bollgard cotton.

Table 10. Percent damaged bolls percentage in late-planted non-Bt, Bollgard, and Bollgard II cotton (16 October 2002).

Genotype	Percent damaged bolls
Non-Bt[a]	14.0
Bollgard	1.8 ± 0.2 a
Bollgard II	0.2 ± 0.2 b
F	35.67
df	1, 4
p-value	0.0038

Means in the same column not followed by a common letter are significantly different (Proc GLM; α = 0.05; SAS 1999).
[a]Data for non-Bt genotype is included only to provide a reference of the level of insect pressure in experiments.

Although high efficacy was observed, some cotton-growing areas of the United States might not benefit from the additional advantages provided by Bollgard II cotton. For example, in areas with low populations of bollworm (e.g., Texas High Plains [Williams 2010]), the original Bollgard technology would likely suffice. This is true assuming there is an additional fee for Bollgard II over that of Bollgard. In contrast, Bollgard II should be highly advantageous to cotton growers who experience predictably high levels of bollworm pressure, such as those commonly seen in North Carolina (Williams 2010). Additionally, due to the enhanced overall toxicity provided by Bollgard II, coupled with low levels of cross resistance between Cry1Ac and Cry2Ab (Tabashnik et al. 2002, Gould et al. 1995), this second-generation Bt cotton might also be an effective resistance management tool.

Summary

Under controlled conditions of single-leaf (laboratory) and intact-plant defoliation assays (greenhouse), Bollgard II cotton provided significantly better protection from multiple lepidopteran pests over Bollgard cotton. Plant expression levels of Cry1Ac are similar between Bollgard and Bollgard II (Adamczyk et al. 2001), and the enhanced efficacy seen in these experiments is likely attributed to the presence of Cry2Ab. This novel protein expressed in Bollgard II cotton enhances control of occasional lepidopteran pests, and provides enhanced control of *H. zea* and *H. virescens*.

In field experiments managed according to typical cultural practices, Bollgard II did not outperform Bollgard cotton with respect to insect control or yield. This was likely due to the relatively light populations experienced throughout the duration of experiments. These results suggest that the overall value of Bollgard II, relative to Bollgard, will be lower in areas that routinely experience low populations of lepidopteran pests. However, late-planted studies designed to enhance populations of lepidopteran pests using unorthodox practices of cotton production (i.e., 1 July planting plus insecticides applied for suppression of beneficial arthropods) demonstrated that Bollgard II cotton did provide greater boll protection against lepidopteran pests than did Bollgard.

Bollgard II cotton has the potential to provide higher overall efficacy against lepidopteran pests compared to Bollgard cotton. Results from our field experiment corroborated research investigating Bollgard II efficacy in North Carolina (Jackson et al. 2000), Arkansas (Allen et al. 2000), and the Mississippi Delta (Adamczyk et al. 2001), suggesting that Bollgard II might not require supplemental insecticide applications for lepidopteran pests in many areas.

References

Adamczyk, J.J., L.C. Adams, and D.D. Hardee. 2001. Field efficacy and seasonal expression profiles for terminal leaves of single and double *Bacillus thuringiensis* toxin cotton genotypes. J. Econ. Entomol. 94: 1589-1593.

Adamczyk, J.J. Jr., V.J. Mascarenhas, G.E. Church, B.R. Leonard, and J.B. Graves. 1998. Susceptibility of conventional and transgenic cotton bolls expressing *the Bacillus thuringiensis* Cry1Ac delta-endotoxin to fall armyworm (Lepidoptera: Noctuidae) and beet armyworm (Lepidoptera: Noctuidae) injury. J. Agric. Entomol. 15: 163-171.

Allen, C.T., M.S. Kharboutli, C. Capps, and L.D. Earnest. 2000. Effectiveness of Bollgard II cotton varieties against foliage and fruit feeding caterpillars in Arkansas, pp. 132-135. In Proc. 2000 Cotton Res. Meeting.

Bachelor, J.S. and D.W. Mott. 1997. Efficacy of grower-managed Bt cotton in North Carolina, pp. 858-861. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Benedict, J.H., E.S. Sachs, D.W. Altman, D.R. Ring, T.B. Stone, and S.R. Sims. 1993. Impact of delta-endotoxin-producing transgenic cotton on insect-plant interactions with *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). Environ. Entomol. 22: 1-9.

Bourland, F.M., D.M. Oosterhuis, and N.P. Tugwell. 1992. Concept for monitoring cotton plant growth and development using main-stem nodes counts. J. Prod. Agric. 5: 532-538.

Deville, S.F., J.W. Mullins, and J.M. Mills. 2002. Seven Years of Economic Comparisons of Bollgard Cotton. *In* P. Dugger and D. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Gore, J, B.R. Leonard, and R.H. Gable. 2003. Distribution of bollworm, *Helicoverpa zea* (Boddie), injured reproductive structures on genetically engineered *Bacillus thuringiensis* var*. kurstaki* Berliner Cotton. J. Econ Entomol. 96: 699-705.

Gould, F.L., A. Anderson, A. Reynolds, L. Bumgarner, and W.J. Moar. 1995. Selection and genetic analysis of a *Heliothis virescens* strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88: 1545-1559.

Greenplate, J.T., S.R. Penn, J.W. Mullins, and M. Oppenhuizen. 2000a. Seasonal Cry1Ac levels in DP50B: The ‘Bollgard basis’ for Bollgard II, pp. 1039-1040. *In* P. Dugger and D. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Greenplate, J.T., S.R. Penn, Z. Shappley, M. Oppenhuizen, J. Mann, B. Reich, and J. Osborn. 2000b. Bollgard II efficacy: quantification of total lepidopteran activity in a 2-gene product, pp. 1041-1043. *In* P. Dugger and D. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Jackson, R.E., J.R. Bradley, Jr., A.D. Burd, and J.W. Van Duyn. 2000. Field and greenhouse performance of bollworm on Bollgard II cotton genotypes, pp. 1048-1052. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Lambert, A.L., J.R.Bradley, and J. Van Duyn. 1996. Effects of natural enemy conservation and planting date on the susceptibility of Bt cotton to Helicoverpa zea in North Carolina, pp. 931-932. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Layton, M.B. 1996. *Insect Scouting and Management in Bt-transgenic cotton*. Publication 2108. Mississippi State Cooperative Extension Service.

Layton, M.B. 2000. *2000 Cotton Insect Control Guide*. Publication 343. Mississippi State Cooperative Extension Service.

Layton, M.B. 2001. *2001 Cotton Insect Control Guide*. Publication 343. Mississippi State Cooperative Extension Service.

Layton, M.B. 2003. *2003 Cotton Insect Control Guide*. Publication 343. Mississippi State Cooperative Extension Service.

Layton, B., S.D. Stewart, and M.R. Williams. 1998. Performance of Bt cotton in Mississippi, 1997, pp. 970-973. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Layton, M.B., M.R. Williams, and S.D. Stewart. 1997. Bt cotton in Mississippi: The first year, pp. 861-863. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Layton, M.B., M.R. Williams, and J.L. Long. 2003. Performance of Bt cotton in Mississippi: A 7-year summary, pp. 1527-1531. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Leonard, B.R., H. Fife, K. Torrey, J.B. Graves, and J. Holloway. 1997. *Helicoverpa/Heliothis* management in NUCOTN and conventional cotton cultivars in Louisiana, pp. 863-867. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Luttrell, R.G., A. Ali, S.Y. Young, and K.S. Knighten. 1998. Relative activity of commercial formulations of *Bacillus thuringiensis* against selected noctuid larvae (Lepidoptera: Noctuidae). J. Entomol. Sci. 33: 365-377.

MacIntosh, S.C., T.B. Stone, S.R. Sims, P.L. Hunst, J.T. Greenplate, P.G. Marone, F.J. Perlak, D.A. Fischoff, and R.L. Fuchs. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. J. Invertebr. Pathol. 56: 258-266.

Mahaffey, J.S., J.R. Bradley, Jr., and J.W. Van Duyn. 1995. Bt. cotton: Field performance in North Carolina under conditions of unusually high bollworm populations, pp. 795-798. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Mascarenhas, V.J., R.G. Luttrell and J.C. Schneider. 1994. Activity of transgenic cotton expressing delta-endotoxin against tobacco budworm, pp. 1064-1067. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Mullins, J.W. and J.M. Mills. 1999. Economics of Bollgard versus non-Bt cotton in 1998, pp. 958-961. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

National Cotton Council 2010. 2009-2010 Varieties Planted: Mid-South. http://www.cotton.org/econ/cropinfo/varieties/midsouth.cfm

Reed, J.T., R.G. Luttrell, S.D. Stewart, F.A. Harris, R.O. Bowden, R.E. Furr, and C.D. Parker, Jr. 1998. Development of sustainable, cost-efficient strategies for managing cotton insects—an interim report. Miss. Agric. Forestry Exp. Sta. Bull. 1077.

Ridge, R.L., S.G. Turnipseed, and M.J. Sullivan. 2000. Field comparison of genetically modified cottons containing one strain (Bollgard) and two strains (Bollgard II) of *Bacillus thuringiensis kurstaki*. pp. 1057-1058. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Roof, M.E. and J.A. DuRant. 1997. On-farm experiences with Bt cotton in South Carolina, p. 861. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Roof, M.E. and J.A. DuRant. 1998. Experiences with Bt cotton under light to medium bollworm infestations in South Carolina, pp. 964-965. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

SAS. 1999. SAS/STAT User's Guide, Version 8.0. SAS Institute, Inc. Cary, NC.

Sims, S.R. 1997. Host activity spectrum of the CryIIA *Bacillus thuringiensis* subsp. *kurstaki* protein: Effects on Lepidoptera, Diptera, and non-target arthropods. Southwest. Entomol. 22: 395-404.

Smith, R.H. 1997. An extension entomologist's 1996 observation of Bollgard (Bt) technology, pp. 856-858. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Stewart, S.D. 1996. Soybean Insect Management. Publication 883. Mississippi State Cooperative Extension Service.

Stewart, S.D., J.J. Adamczyk Jr., K.S. Knighten, and F.M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival of Noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94: 752-760.

Stewart, S.D., K.S. Knighten, and F.M. Davis. 2000. Efficacy of Bt cotton expressing two insecticidal proteins of *Bacillus thuringiensis* Berliner on selected caterpillar pests, pp. 1043-1048. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Stone, T.B. and S.R. Sims. 1993. Geographic susceptibility of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis*. J. Econ. Entomol. 86: 989-994.

Sumerford, D.V. and W.L. Solomon. 2000. Growth of wild *Pseudoplusia includens* (Lepidoptera:Noctuidae) larvae collected from Bt and Non-Bt cotton. Florida Entomol. 83: 354-357.

Tabashnik, B.E., T.J. Dennehy, M.A. Sims, K. Larkin, G.P. Head, W.J. Moar, and Y. Carriere. 2002. Control of resistant pink bollworm (*Pectinophora gossypiella*) by transgenic cotton that produces *Bacillus thuringiensis* toxin Cry2Ab. Appl. Environ. Microbiol. 68: 3790-3794.

Turnipseed, S.G. and M.J. Sullivan. 1999. Consequences of natural enemy disruption with applications of "hard" insectocodes prior to the bollworm flight in conventional and Bt cotton, pp. 1110-1112. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Van Tol, N.B. and G.L. Lentz. 1998. Influence of Bt cotton in beneficial arthropod populations, pp. 1052-1054. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Wier, A.T., J.W. Mullins, and J.M. Mills. 1998. BOLLGARD cotton—Update and Economic comparisons including new varieties, pp. 1039-1040. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Williams, M.R. 2010. Cotton insect losses—2009, pp 1029-1073. *In* P. Dugger and D. A. Richter [eds.], Proc. Beltwide Cotton Conf. National Cotton Council, Memphis, TN.