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COMPARATIVE EFFICACY OF BT TECHNOLOGIES AGAINST BOLLWORM IN NORTH CAROLINA

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Abstract

The comparative bollworm efficacy of WideStrike™ (Dow AgroSciences, LLC, Indianapolis, IN) and non-Bt cottons were evaluated with either Bollgard® or Bollgard II® (Monsanto Co., St. Louis, MO) under insecticide-sprayed or non-sprayed conditions in two North Carolina locations in 2004. Results from these studies demonstrate that Bollgard II provided better control of bollworms than WideStrike when faced with high populations of bollworms; however, bollworm efficacy between these two technologies was similar under low to moderate bollworm populations. Under these low to moderate infestations, WideStrike was comparable to Bollgard with regard to bollworm control. Seedcotton yields from non-sprayed treatments were jeopardized by hemipteran damage; thus, yield differences among technologies were not discussed. However, yield loss estimates in WideStrike and Bollgard II varieties at the Martin Co. site demonstrate that bug pests can and will devalue these technologies.

Introduction

The 1996 commercialization of transgenic, insecticidal cottons, (Bollgard®, Monsanto Co., St. Louis, MO), that contain a gene from *Bacillus thuringiensis* var. *kurstaki* (Berliner) that encodes for the production of the Cry1Ac δ-endotoxin has transformed heliothine management. The primary targets of these Bt cottons for the majority of the cotton belt were tobacco budworm, *Heliothis virescens* (Fab.), and bollworm, *Helicoverpa zea* (Boddie). To date, Bt cottons have been the solution to tobacco budworm control problems; however, unacceptable control of bollworm has been frequently documented over the 9-year period (Mahaffey et al. 1994, 1995; Lambert et al. 1996-1997; Jackson et al. 2003).

Because of increasing concerns with resistance evolution in heliothines to the single-gene Bollgard varieties, cottons expressing two Bt endotoxins that are active against lepidopteran pests have been introduced commercially. Bollgard II® (Monsanto Co., St. Louis, MO), which produces the Cry1Ac and Cry2Ab endotoxins, has increased bollworm efficacy above that of the single-gene Bollgard varieties, and was registered for commercial use in 2002 (Jackson et al. 2003). Primarily due to the success of Bollgard cottons, Dow AgroSciences, LLC, (Indianapolis, IN) introduced its pyramided-gene technology onto the market in 2004 as WideStrike™. These cottons also produce two Bt endotoxins, Cry1Ac and Cry1F, which are both active against lepidopterans. Although the efficacy of Bollgard II against bollworm has been thoroughly investigated, much less is known about the efficacy of WideStrike against bollworm.

Thus, results from field studies conducted in North Carolina are reported herein where WideStrike genotypes were compared with non-Bt and Bollgard and/or Bollgard II cottons with regard to efficacy against bollworm.

Materials and Methods

Field experiments were established in Martin and Edgecombe Counties, NC, in 2004 to evaluate the comparative efficacy of various transgenic, Bt technologies against bollworm. Technologies evaluated included WideStrike, Bollgard, and Bollgard II, as well as a non-Bt. Four cotton genotypes consisting of PHY470WR (WideStrike), PHY440W (WideStrike), PHY410R (non-Bt), and DP424BGII/RR (Bollgard II) were tested under insecticide-sprayed and non-sprayed conditions at the Martin Co. site. The experiment was designed as a randomized complete split plot with insecticide regime as the whole plot factor and genotype as the subplot factor. Insecticide-treated whole plots were to be maintained free of caterpillar pests. Individual plots measured 4 rows by 40 ft. The Edgecombe Co. test was also comprised of four cotton genotypes: PHY470WR (WideStrike), PHY410R (non-Bt), SG215BR (Bollgard), and DP424BGII/RR (Bollgard II). These genotypes were to be evaluated under insecticide-sprayed and non-sprayed conditions with cotton genotype serving as the whole plot factor and insecticide regime as the subplot factor. Insecticide-treated subplots were sprayed for heliothines based on economic thresholds for each particular genotype. Thus, PHY410R was treated based on thresholds for non-Bt cottons, whereas other genotypes were treated based on thresholds for Bt cottons. Plot size measured 4 rows by 35 ft.

Cotton genotypes were planted on 19-May in Martin Co. and 18-May in Edgecombe Co. Aldicarb (Temik® 15G, Bayer CropScience, Kansas City, MO) was applied in-furrow at planting at 0.75 lb. a. i./acre for control of early season insect pests. Acephate (Orthene® 97PE, Valent USA Corp., Walnut Creek, CA) was applied at 0.75 lb. a. i./acre as a mid-season overspray for control of plant bugs and stink bugs, as well as to eliminate arthropod natural enemies of heliothines. Thiamethoxam (Centric® 70WG, Syngenta Crop Protection, Inc., Greensboro, NC) was also applied in August as an overspray to each test for plant bug and stink bug control. Three applications of lambda-cyhalothrin (Karate Z 2.08CS, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.04 lb. a. i./acre plus spinosad (Tracer 4SC, Dow AgroSciences, LLC, Indianapolis, IN) at 0.89 lb. a. i./acre were made to insecticide-treated whole plots at the Martin Co. site for supplemental heliothine control on 26-July, 4-August, and 10-August. Gamma-cyhalothrin (Prolex™ 1.25 CS, Dow AgroSciences, LLC, Indianapolis, IN) at 0.015 lb. a. i./acre replaced lambda-cyhalothrin at the Edgecombe Co. site, where 1 and 3 applications were made to the SG215BR and PHY410R genotypes, respectively. PHY410R received oversprays on 26-July, 2-August, and 9-August, whereas SG215BR was treated only once on 2-August. A CO2-powered backpack sprayer fitted with a single TX-10 nozzle per row and calibrated to deliver 9.8 gpa at 58 psi was used to apply foliar insecticides. Weed control, fertilization, plant growth regulation, and defoliation were achieved as recommended by North Carolina State University.

Numbers of live heliothine larvae (L3-L5) and associated damage were recorded for 40 squares per plot on 4-Aug, 10-Aug, and 17-Aug in the Martin Co. test. Evaluations of 25 squares per plot were made on 6-Aug in the Edgecombe Co. test. Evaluations consisting of larval numbers and damage on 25-50 bolls per plot were begun at one week after initial bollworm infestation and continued at weekly intervals for each test. Boll samples were made during 3 and 4 weeks in the Edgecombe and Martin Co. sites, respectively. Yields were determined by picking the entire lengths of the two middle rows of each subplot using a mechanical cotton picker at the Edgecombe Co. site, whereas seedcotton yields were estimated by hand-harvesting 20 row ft. of each subplot at the Martin Co. site. Yields were converted to lbs. seed cotton/acre prior to analysis.

Numbers of live heliothine larvae and damaged fruit were converted to percentages and subjected to arcsine square root transformation prior to analysis. Results presented herein consist of the peak percent live bollworm larvae and damage on squares and bolls for each genotype*insecticide regime combination. Seedcotton yields were subjected to square root transformation prior to analysis. These data were then subjected to ANOVA using PROC GLM (SAS Institute 1990).

Results and Discussion

High heliothine populations were encountered in the Martin Co. test, with a significant proportion of the population being tobacco budworm (~15%). Heliothine larval counts differed among the genotype*insecticide regime combinations with non-sprayed non-Bt possessing the highest percentage of infested squares (30%) ($P=0.0001$). All other treatment combinations reduced larval infestation of squares to 2.5% or lower (Table 1). No difference was observed in infested squares between the non-sprayed WideStrike genotypes. All insecticide-sprayed Bt genotypes, along with non-treated Bollgard II, reduced the percentage of infested squares below that of PHY440W, but did not differ from PHY470WR. Differences among genotype*insecticide regime combinations were also observed with regard to the percentage of squares sustaining heliothine damage ($P<0.0001$). The highest level of square damage reached 65.8%, which was recorded in the non-sprayed, non-Bt genotype. Non-sprayed PHY440W reduced square damage to 11.3%, which did not differ from the 7% sustained by non-sprayed PHY470WR. All insecticide-sprayed treatment combinations, as well as non-sprayed Bollgard II, possessed significantly less square damage than the two non-sprayed WideStrike genotypes. Similar results on larval-infested squares and associated damage have been documented in previous studies with insecticide-sprayed and non-sprayed WideStrike or Bollgard II (Langston et al. 2004; Huckaba et al. 2003; Jackson et al. 2003).

The percentage of bolls infested by heliothine larvae followed the same trend as that of square damage with significant differences being observed among the treatment combinations ($P<0.0001$). Thirty percent of the bolls in the non-sprayed, non-Bt genotype were infested with a heliothine larva (Table 2). No differences were observed between the non-sprayed PHY470WR and PHY440W genotypes, which reduced the percentage of infested bolls to

5.0 and 3.8%, respectively. As with square damage, all insecticide-sprayed treatment combinations, along with the non-sprayed Bollgard II, possessed fewer infested bolls than the non-sprayed WideStrike genotypes. The exact trend was observed for the percentage of bolls sustaining heliothine damage as that of square damage and infested bolls. Significant differences were observed among treatment combinations (P<0.0001), with the non-sprayed, non-Bt genotype suffering 72% boll damage. The non-sprayed PHY440W and PHY470WR genotypes reduced boll damage to 8.3 and 7.5%, respectively, but sustained significantly higher levels of boll damage than any other treatment combination (0-1.3%). Although the WideStrike genotypes provided high levels of control against heliothines, this technology was not as potent against bollworm as was Bollgard II. Assuming similarity in Cry1Ac expression among technologies, differences in efficacy against bollworm should be related to the difference in efficacy between Cry2Ab and Cry1F. Karim et al. (2000) reported that although bollworm was less susceptible to Cry2A than to Cry1A proteins, activity of Cry2A was greater than that of Cry1F against bollworm. Thus, cottons containing Cry1Ac and Cry2Ab would likely be more efficacious against bollworm than those containing Cry1Ac and Cry1F.

The percentage of bolls sustaining heliothine damage in the non-sprayed, non-Bt genotype (72%) was closely related to the percent yield loss between the insecticide-treated and non-treated, non-Bt subplots (61%) (Table 3). However, seedcotton yields for the non-sprayed, Bt genotypes were compromised by damage from hemipteran pests; yield losses sustained by the Bt genotypes ranged from 17-20%. Obviously, this level of yield reduction was not caused by the 1.3-8.3% peak boll damage observed for these same genotypes. Thus, the strength of these technologies cannot be related to the yields recorded in this test. Nevertheless, these results demonstrate that plant bugs and stink bugs have the capabilities to substantially devalue these transgenic technologies.

Only a moderate heliothine population infested the field test in Edgecombe Co., but a significant proportion of this population was tobacco budworm (~20%), as observed at the Martin Co. site. The percentage of squares infested with a live heliothine larva differed among treatment combinations (P=0.0013), where the non-sprayed, non-Bt contained the highest infestation level at 6.5% (Table 4). All treatment combinations reduced the percentage of squares with a live larva below that of the non-sprayed non-Bt, ranging from 0-1% of squares infested; no differences were observed between these treatment combinations. A significant difference also existed among treatment combinations with regard to the percentage of squares sustaining heliothine damage (P<0.0001). Square damage reached 18.5% in the non-sprayed, non-Bt genotype, which was significantly higher than any other treatment combination. No differences were observed between the non-sprayed WideStrike and Bollgard genotypes, as well as the insecticide-sprayed non-Bt. Only the non-treated Bollgard II and insecticide-treated Bollgard reduced square damage below that of non-sprayed WideStrike. With no difference detected between WideStrike, Bollgard, and Bollgard II with regard to larval infestation of squares and only a minimal difference found for square damage, it appears that WideStrike genotypes, like current Bollgard varieties, provide effective control of low-moderate bollworm populations.

With regard to the percentage of bolls infested with a live larva, significant differences were observed among treatment combinations (P<0.0001). Twelve percent of the bolls in the non-sprayed non-Bt were infested with a heliothine larva (Table 5). All other treatment combinations significantly reduced the infestation to 1% or lower; no differences existed among these treatment combinations. The percentage of bolls sustaining damage from heliothine feeding peaked at 54% in the non-sprayed non-Bt, which was significantly higher than all other treatment combinations (P<0.0001). As with square damage, no differences existed among the non-sprayed Bollgard and WideStrike genotypes, as well as the insecticide-sprayed non-Bt. Insecticide-treated Bollgard and non-sprayed Bollgard II sustained less boll damage than the insecticide-treated non-Bt; however, no difference in boll damage levels was observed between the WideStrike and Bollgard II varieties. As demonstrated by larval infestation of squares and associated damage, WideStrike and Bollgard varieties are likely to provide levels of bollworm control equal to that of Bollgard II under conditions of low to moderate populations.
The Edgecombe Co. test results were similar to those in the Martin Co. test as boll damage estimates in the non-sprayed non-Bt (54%) mirrored the yield loss estimated between the insecticide-sprayed and non-sprayed non-Bt (52%) (Table 6). Yield loss in the Bollgard genotype was somewhat elevated above the boll damage estimate (10% vs. 6%); however, as in the Martin Co. test, damage by hemipteran pests may have reduced yields in the non-sprayed, Bt genotypes.

These studies demonstrated that the pyramided toxins in the WideStrike genotypes provided a high level of heliothine control; however, efficacy of the dual-gene Bollgard II against bollworm was greater than that of

WideStrike under conditions of high bollworm populations. Results from both tests also showed that WideStrike varieties will likely require supplemental insecticide oversprays for adequate bollworm control, possibly even under conditions of moderate bollworm populations. Furthermore, yield losses to hemipteran pests indicated that control of plant bugs and stink bugs was critical during the period in which heliothines infested the tests. Thus, the difference observed in bollworm efficacy between WideStrike and Bollgard II cottons will likely be minimized in areas where bug pests typically reach economic thresholds during the period in which heliothines are present in these cottons. Instead of using organophosphorus insecticides for bug control, a pyrethroid typically provides excellent control of bollworms and adequate control of plant bugs and stink bugs; utilization of this chemistry when heliothines are present will devalue the potential for increased bollworm control of Bollgard II over WideStrike. Because bug pests appear to be rising in pest status across the cotton belt, it is likely that producers will choose varieties based upon improved agronomic characteristics instead of insect control technology.

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Literature Cited

Burd, A. D., J. R. Bradley, Jr., J. W. Van Duyn, and F. Gould. 2000. Resistance of bollworm, *Helicoverpa zea*, to cry1A(c) toxin. Proc. Beltwide Cotton Conf. 923-926.

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

Huckaba, R. M., R. B. Lassiter, X. Huang, C. A. Blanco, V. B. Langston, L. B. Braxton, F. J. Haile, J. M. Richardson, and J. Pellow. 2003. Field efficacy of Dow AgroSciences MXB-13 transgenic cotton for control of cotton bollworm and tobacco budworm. Proc. Beltwide Cotton Conf. 1293-1298.

Jackson, R. E., J. R. Bradley, Jr., and J. W. Van Duyn. 2003. Field performance of transgenic cottons expressing one or two *Bacillus thuringiensis* endotoxins against bollworm, *Helicoverpa zea* (Boddie). J. Cotton Science. 7: 57-64.

Karim, S., S. Riazuddin, F. Gould, and D. H. Dean. 2000. Determination of receptor binding properties of *Bacillus thuringiensis* delta-endotoxins to cotton bollworm (*Helicoverpa zea*) and pink bollworm (*Pectinophora gossypiella*) midgut brush border membrane vesicles. Pestic. Biochem. And Physiol. 67:198-216.

Lambert, A. L., J. R. Bradley, Jr., and J. W. Van Duyn. 1996. Effects of natural enemy conservation and planting date on the susceptibility of *Bt* cotton to *Helicoverpa zea* in North Carolina. Proc. Beltwide Cotton Conf. 931-935.

Lambert, A. L., J. R. Bradley, Jr., and J. W. Van Duyn. 1997. Interactions of *Helicoverpa zea* and *Bt* cotton in North Carolina. Proc. Beltwide Cotton Conf. 870-873.

Langston, V. B., R. B. Lassiter, L. B. Braxton, E. A. Flora, F. J. Haile, B. Haygood, R. M. Huckaba, J. W. Pellow, J. M. Richardson, and J. S. Richburg. 2004. Efficacy of WideStrike Cotton against heliothine insects. Proc. Beltwide Cotton Conf. 1348-1352.

Mahaffey, J. S., J. S. Bacheler, J. R. Bradley, Jr., and J. W. Van Duyn. 1994. Performance of Monsanto's transgenic *B.t.* cotton against high populations of lepidopterous pests in North Carolina. Proc. Beltwide Cotton Conf. 1061-1063.

Mahaffey, J. S., J. R. Bradley, Jr., and J. W. Van Duyn. 1995. *B. t.* cotton: field performance in North Carolina under conditions of unusually high bollworm populations. Proc. Beltwide Cotton Conf. 795-798.

SAS Institute. 1990. SAS/STAT User's Guide, Vol. 2. SAS Institute, Cary, NC, 795 pp.

Table 1. Percent peak live bollworm larvae and damage on squares in insecticide-sprayed and non-sprayed WideStrike, Bollgard II, and non-Bt cotton genotypes in Martin Co., NC. 2004.

Cotton Genotype	Insecticide	% Live Larvae[a]	% Square Damage[a]
PHY410R	No	30.0 a	65.8 a
PHY440W	No	2.5 b	11.3 b
PHY470WR	No	1.3 bc	7.0 b
PHY410R	Yes	0.8 bc	2.5 c
DP424BGII/RR	No	0.0 c	0.8 c
PHY440W	Yes	0.0 c	0.0 c
PHY470WR	Yes	0.0 c	0.0 c
DP424BGII/RR	Yes	0.0 c	0.0 c

[a]Means within the same column and followed by the same letter are not significantly different, Fisher's Protected LSD ($P \leq 0.05$).

Table 2. Percent peak live bollworm larvae and damage on bolls in insecticide-sprayed and non-sprayed WideStrike, Bollgard II, and non-Bt cotton genotypes in Martin Co., NC. 2004.

Cotton Genotype	Insecticide	% Live Larvae[a]	% Boll Damage[a]
PHY410R	No	30.0 a	72.0 a
PHY440W	No	3.8 b	8.3 b
PHY470WR	No	5.0 b	7.5 b
DP424BGII/RR	No	0.0 c	1.3 c
PHY410R	Yes	0.0 c	1.3 c
PHY440W	Yes	0.0 c	0.0 c
PHY470WR	Yes	0.0 c	0.0 c
DP424BGII/RR	Yes	0.0 c	0.0 c

[a]Means within the same column and followed by the same letter are not significantly different, Fisher's Protected LSD ($P \leq 0.05$).

Table 3. Seedcotton yields from insecticide-sprayed and non-sprayed WideStrike, Bollgard II, and Non-Bt cotton genotypes in Martin Co., NC. 2004.

Cotton Genotype	Insecticide	Lbs. Seedcotton / Acre[a]
DP424BGII/RR	Yes	4460 a
PHY440W	Yes	4175 ab
PHY410R	Yes	4075 ab
PHY470WR	Yes	4071 ab
DP424BGII/RR	No	3589 bc
PHY440W	No	3473 c
PHY470WR	No	3281 c
PHY410R	No	1592 d

[a]Means within the same column and followed by the same letter are not significantly different, Fisher's Protected LSD ($P \leq 0.05$).

Table 4. Percent peak live bollworm larvae and damage on squares in insecticide-sprayed and non-sprayed WideStrike, Bollgard, Bollgard II, and non-Bt cotton genotypes in Edgecombe Co., NC. 2004.

Cotton Genotype	Insecticide	% Live Larvae[a]	% Square Damage[a]
PHY410R	No	6.5 a	18.5 a
PHY470WR	No	1.0 b	2.0 b
PHY410R	Yes	1.5 b	1.5 bc
SG215BR	No	0.5 b	0.5 bc
DP424BGII/RR	No	0.0 b	0.0 c
SG215BR	Yes	0.0 b	0.0 c

[a]Means within the same column and followed by the same letter are not significantly different, Fisher's Protected LSD ($P \leq 0.05$).

Table 5. Percent peak live bollworm larvae and damage on bolls in insecticide-sprayed and non-sprayed WideStrike, Bollgard, Bollgard II, and non-Bt cotton genotypes in Edgecombe Co., NC. 2004.

Cotton Genotype	**Insecticide**	**% Live Larvae**[a]	**% Boll Damage**[a]
PHY410R	No	12.0 a	54.0 a
PHY410R	Yes	1.0 b	7.0 b
SG215BR	No	1.0 b	6.0 bc
PHY470WR	No	1.0 b	5.5 bc
SG215BR	Yes	0.5 b	1.5 c
DP424BGII/RR	No	0.0 b	1.0 c

[a]Means within the same column and followed by the same letter are not significantly different, Fisher's Protected LSD ($P \leq 0.05$).

Table 6. Seedcotton yields from insecticide-sprayed and/or non-sprayed WideStrike, Bollgard II, and Non-Bt cotton genotypes in Edgecombe Co., NC. 2004.

Cotton Genotype	**Insecticide**	**Lbs. Seedcotton / Acre**[a]
PHY410R	Yes	2920 a
PHY470WR	No	2671 a
SG215BR	Yes	2245 b
DP424BGII/RR	No	2132 b
SG215BR	No	2012 b
PHY410R	No	1405 c

[a]Means within the same column and followed by the same letter are not significantly different, Fisher's Protected LSD ($P \leq 0.05$).