# VALUE OF BT TECHNOLOGY FOR BOLLWORM MANAGEMENT

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## Abstract

Since the introduction of Bt cotton in the United States in 1996, management of the bollworm, *Helicoverpa zea*, has become much less problematic. However, there are still incidents where unacceptable fruit injury is experienced and insecticidal oversprays are utilized to prevent yield loss. There has been much speculation surrounding the reasons for control failures among Bt cotton technologies including Bt resistance or tolerance, inadequate expression of Bt toxins expression due to plant phenology or environmental stressors. The objective of this project was to evaluate the efficacy of second and third generation Bt cotton at six locations within the Mid-South and one location in Texas for efficacy towards bollworms and to determine if spraying Bt cotton for bollworms results in a reduction in damage and increased yields relative to non-Bt cotton. Our data demonstrates that all currently available Bt cotton technologies may suffer unacceptable bollworm injury. Widestrike appears to be most sensitive to experiencing control failures and benefits from insecticide applications targeting bollworms, if these applications are made in a timely manner. Bollgard II and Twinlink may also suffer control failures, but are less common than Widestrike. Cotton varieties containing the Vip3A toxin provided the greatest level of bollworm control.

## Introduction

The introduction in 1996 of transgenic cotton containing genes expressing *Bacillus thuringi*ensis Berliner (Bt) proteins ushered in a new era in cotton insect pest management. The first Bt cotton introduced in the U.S. was Bollgard I which expressed the Cry1Ac endo-toxin. This toxin was highly effective towards tobacco budworm, *Chloridea virescens* (F.), but moderately toxic towards bollworm, *Helicoverpa zea* Bodie. Insecticide applications targeting tobacco budworm were completely eliminated, while those targeting bollworm were greatly reduced. To increase efficacy and for resistance management, dual and multi-Bt gene cotton varieties have since been introduced including Bollgard II, Bollgard 3, Twinlink, Twinlink Plus, Widestrike, Widestrike 3. Although these introductions have increased the efficacy of transgenic cotton targeting lepidoteran pests, including bollworm, there are still incidents where unacceptable fruit injury is experienced and insecticidal oversprays are required to preserve yield. In recent years these incidents have become common and extensive in some areas including portions of Texas, the Mid-South and North Carolina. Bollworm resistance to Cry1Ac and Cry2Ab has been implicated as a primary causal factor contributing to Bt cotton efficacy failures.

The objective of this project was to evaluate the efficacy of second and third generation Bt cotton for efficacy towards bollworms and to determine if overspraying Bt cotton results in a reduction in damage and increased yields relative to non-Bt cotton.

## Materials and Methods

Tests were conducted at six locations across the Mid-South and one location in Texas to determine the impact of foliar insecticide applications targeting bollworms on injury and yields in second and third generation Bt cottons. The Bt cotton technologies evaluated included: ST 494GLT, TwinLink™ (TL; Cry1Ab, Cry2Ae), ST 4946GLB2, Bollgard II® (BG2; Cry1Ac, Cry2Ab), PHY 333WRF, WideStrike® (WS; Cry1Ac, Cry1F), and PHY 330WRF3, WideStrike 3® (WS3; Cry1Ac, Cry1F, Vip3A). At one location ST 5575GLTP, TwinLink Plus™ (TL+; Cry1Ab, Cry2Ae, Vip3A) was included. A non-Bt variety (NBT) was included as a check, DP 1441RF.

All tests were 5 x 2 factorials with factor A being the cotton technology and factor B being entries sprayed for bollworms with Prevathon at 20 fl-oz/ac, or non-sprayed. Plots were 4 rows wide x 40-60 ft in length. Each factorial combination was replicated 4 times. Test locations included Pine Bluff and Rowher, AR, Stoneville and Starkville, MS, Jackson, TN, Winnsboro, LA and College Station, TX. Foliar applications were made in accordance with the occurrence of larvae in the non-Bt cotton plots at each individual location. Prevathon applications occurred as follows: Pinebluff, AR (July 24), Rohwer, AR (July 31), Stoneville, MS (July 31 and Aug 17), Starkville, MS (July 14), College Station, TX (July 12), Jackson, TN (Aug 3 and 29)

Insect densities, plant terminal, square, bloom and boll injury were determined prior to foliar treatment and weekly thereafter using one of two sampling techniques depending on the location. At the Stoneville and Starkville, MS locations a modified whole plant sampling procedure of 20-25 plants per plot. The top 4-5 nodes plus one white or pink bloom and one small to medium-sized boll were be sampled on each plant. If larvae or fresh injury was observed, the entire plant was sampled. Total counts of larvae, total numbers of damaged squares, and total numbers of damaged bolls (including flowers) were recorded in each plot. At the other locations 25 of each, terminals, squares, bloom and bolls were sampled per plot. The number of bollworm larvae and damage plant tissue were recorded. At all locations, the middle two rows of each plot was harvested using a mechanized cotton picker. Seed cotton yields were converted to lint yields in lbs per acre based on an estimated 40% lint turnout. All field data were analyzed using ANOVA and means were separated using a F protected LSD ($P < 0.05$).

## Results and Discussion

Across all locations fruit damage was greatest in the untreated NBT plots seasonally averaging 17.95 and 8.75% damaged squares and bolls, respectively. Injury in the NBT plots was followed by injury in the untreated WS plots which averaged 8.34 and 5.56% damaged squares and bolls, respectively. Across locations, the remaining untreated Bt technology entries exhibited similar fruit damage, where square and boll damage respectively averaged 2.37 and 1.60% for BG2, 3.30 and 2.32 for TL, and 2.08 and 0.30% for WS3.

At individual locations, although the NBT plots had the highest average square damage, at Rowher, AR and College Station, TX, the untreated WS was not statistically different (Table 1). Boll injury was similar except the untreated NBT did not differ from the untreated WS at the Rowher, AR and Jackson, TN locations. At most locations, the untreated WS had higher square damage than the other untreated Bt entries. The exceptions were at Stoneville, MS and Winnsboro, LA where untreated WS did not differ from TL. At Pine Bluff, AR, BG2, TL, WS3 and TL+ did not differ in square damage, but WS3 and TL+ had fewer damaged bolls than TL. There were no significant differences in fruit injury to BG2, TL or WS3 at Rohwer, AR, Starkville, MS, Winnsboro, LA or Colleges Station, TX. At Stoneville, MS, WS3 had fewer damaged bolls than TL, but there were no differences in square damage. At Jackson, TN, WS3 had fewer damaged squares than TL, and WS3 and BG2 had fewer damaged bolls than TL.

Comparing fruit damage of the untreated Bt technologies to the untreated NBT as an indicator of efficacy, WS was the least effective Bt technology (Table 2). Reduction in square damage across locations ranged from -7.41% to 69.05%, and averaged 43.46%. Average reduction square damage among the remaining Bt technologies was similar; values ranged from 66.67% to 100%. On average, BG2 had 82.69% fewer damaged squares, and TL and WS3 had 81.33 and 86.19% less respectively. Boll damage was similar with reductions of 43.69, 84.30 and 79.60% in WS,

BG2 and TL respectively. WS3 offered the greatest boll protection with a reduction in 96.47% relative to the untreated NBT. At the single location where evaluated, TL+ reduced square and boll damage by about 96%.

The value of applying Prevathon for preventing fruit loss due to bollworm feeding was inconsistent (Table 1). Whereas the NBT benefitted from Prevathon applications in fewer damaged squares across all locations, the benefit of treating WS, BG2, TL and WS3 was realized at 3, 1, 1 and 0 locations, respectively. Results were similar regarding boll damage, where damage was less in the Prevathon treated entries for WS at 5 locations, 2 locations for TL, and at no locations for BG2 or WS3. At Pine Bluff, AR where TL+ was evaluated fruit damage between the treated and untreated plots did not differ. These results were despite Bt resistance being detected for Cry1Ac at all locations, and for Cry2Ab everywhere but Rohwer, AR (Pine Bluff, AR not evaluated) (Table 5) (Yang et al. 2018).

Based on the percentage reduction in damaged fruit (Table 2), it is evident that Cry2A is still providing significant control of bollworm except under high population events. The apparent lack of consistent benefit from treating with Prevathon is most likely due to application timing. Because of the small plots in the randomized experimental design, insecticide applications had to be applied using ground driven equipment. The 2017 production season was characterized as extremely wet in the Mid-South which interfered with application timing at some locations. At the College Station, TX location Prevathon was applied 4 days later than scheduled, due to weather. The lack of timely control was most evident for WS where the untreated averaged 16.88% damaged squares and the Prevathon treated averaged 11.20% damaged squares.

Benefit in yield from applying Prevathon was also variable (Table 3). Three locations exhibited no statistical yield differences among any treatment x entry combinations. Prevathon application(s) resulted in higher yields for NBT at 3 locations, for WS at 4 locations and for only 1 location for BG2, TL and WS3. Undoubtedly environmental conditions resulting in boll shed and poor Prevathon application timing contributed to the lack of significant differences. On average across all locations, the NBT plots exhibited a 30.92% increase in yield from being treated with Prevathon, WS 15.13%, BG2 -0.33%, TL 11.04% and WS3 4.71% (Table 4).

These studies illustrate that bollworm injury and yield loss is common throughout much of the southern cotton belt and is most likely due to resistance to the Bt cry proteins and high bollworm pressure. Although the Bt technologies still have great value for managing tobacco budworm, *Chloridea virescen,* and other lepidopteran pests, activity towards bollworm has greatly deteriorated. The Widestrike technology appears to be fully compromised for bollworm control since Cry1F is ineffective and there appears to be widespread resistance to Cry1Ac. Resistance to the Cry2A toxins appears to be high in many areas as well, but this toxin appears to still offer adequate control except under high bollworm pressure. The Vip cotton varieties appear to offer the greatest level of bollworm control, but with resistance to Cry1A and Cry2A toxin there is concern that too much selection pressure is being exerted on this toxin.

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## References

Yang, F., D. Kerns, J. Gore, A. Catchot, D. Cook, F. Musser, G. Lorenz, N. Seiter, S. Stewart and S. Brown. 2018. Continuous monitoring of susceptibility of *Helicoverpa zea* in the Southern U.S. to different Bt proteins. In Proc. Beltwide Cotton Conf., National Cotton Council, Memphis, TN, In Press.

Table 1. Seasonal mean square and boll damage among Bt cotton technology traits untreated or treated with Prevathon at 20 fl-oz/ac, 2017.[1]

| Bt tech[2] | Insecticide treatment | Pinebluff, AR % Damaged | | Rohwer, AR % Damaged | | Stoneville, MS Damaged/20 plants | | Starkville, MS Damaged/25 plants | | Winnsboro, LA % Damaged | | College Station, TX % Damaged | | Jackson, TN % Damaged | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Squares | Bolls | Squares | Bolls | Squares | Bolls | Squares | Bolls | Squares | Bolls | Squares | Bolls | Squares | Bolls |
| NBT | Untreated | 52.50 a | 19.75 a | 6.75 a | 8.25 a | 4.50 a | 5.63 a | 4.50 a | 3.38 a | 9.90 a | 1.25 a | 19.08 a | 6.33 a | 28.40 a | 16.63 a |
| | Prevathon | 8.25 c | 3.08 cd | 1.00 b | 2.50 b | 1.69 bc | 1.31 bcd | 0.00 c | 0.81 c | 5.21 b | 0.00 b | 15.53 ab | 2.50 b | 1.20 de | 0.58 c |
| WS | Untreated | 16.25 b | 8.08 b | 7.25 a | 11.00 a | 2.00 b | 1.69 b | 2.25 b | 2.38 b | 3.96 bc | 0.16 b | 16.88 ab | 1.33 bc | 9.80 b | 14.25a |
| | Prevathon | 5.00 cde | 2.25 cd | 1.00 b | 3.25 b | 1.19 bcd | 0.50 de | 0.08 c | 0.19 c | 2.50 cd | 0.00 b | 11.20 bc | 1.00 bc | 0.60 de | 1.00 c |
| BG2 | Untreated | 3.25 def | 3.50 cd | 2.25 b | 2.25 b | 0.81 d | 0.75 cde | 0.17 c | 0.44 c | 2.60 bcd | 0.00 b | 4.13 d | 1.33 bc | 3.40 cd | 2.92 c |
| | Prevathon | 1.25 f | 1.33 cd | 0.25 b | 0.25 b | 0.88 cd | 0.63 bc | 0.17 c | 0.19 c | 0.63 d | 0.00 b | 3.42 d | 0.33 bc | 0.00 e | 0.38 c |
| TL | Untreated | 6.00 cd | 4.58 c | 2.00 b | 2.00 b | 1.44 bcd | 1.38 bc | 0.00 c | 0.38 c | 0.94 d | 0.00 b | 5.82 cd | 1.33 bc | 5.00 c | 6.58 b |
| | Prevathon | 2.50 def | 0.92 d | 0.50 b | 2.00 b | 0.88 cd | 0.88 b-e | 0.17 c | 0.00 c | 1.77 cd | 0.00 b | 3.38 d | 0.67 bc | 0.60 de | 0.17 c |
| WS3 | Untreated | 4.25 def | 0.83 d | 1.00 b | 0.50 b | 1.00 cd | 0.44 e | 0.17 c | 0.19 c | 1.25 d | 0.00 b | 6.30 cd | 0.00 c | 0.60 de | 0.17 c |
| | Prevathon | 0.75 f | 0.50 d | 0.25 b | 0.50 b | 0.81 d | 0.13 e | 0.00 c | 0.19 c | 1.56 cd | 0.00 b | 5.95 cd | 0.00 c | 0.20 e | 0.00 c |
| TL+ | Untreated | 2.25 ef | 0.83 d | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | Prevathon | 0.75 f | 0.75 d | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |

Means in a column followed by the same letter are not significantly different based on a F protected LSD ($P < 0.05$).
[1]Prevathon at 20 fl-oz/ac applications: Pinebluff, AR (July 24), Rohwer, AR (July 31), Stoneville, MS (July 31 and Aug 17), Starkville, MS (July 14), College Station, TX (July 12), Jackson, TN (Aug 3 and 29).
[2]NBT (non-Bt, DP 1441RF, WS (WideStrike, PHY 333WRF, BG2 (Bollgard II, ST 4946GLB2), TL (TwinLink, ST 4949GLT), TL+ (TwinLink Plus, ST 5575GLTP).

Table 2. Percentage reduction in square and boll damage of non-treated Bt cotton technologies relative to a non-treated non-Bt cotton, 2017.

| Bt tech[1] | Pinebluff, AR % Reduction | | Rohwer, AR % Reduction | | Stoneville, MS % Reduction | | Starkville, MS % Reduction | | Winnsboro, LA % Reduction | | College Station, TX % Reduction | | Jackson, TN % Reduction | | Across locations % Reduction | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Squares | Bolls | Squares | Bolls | Squares | Bolls | Squares | Bolls | Squares | Bolls | Squares | Bolls | Squares | Bolls | Squares | Bolls |
| WS | 69.05 | 59.09 | -7.41 | -33.33 | 55.56 | 69.98 | 50.00 | 29.59 | 60.00 | 87.20 | 11.53 | 78.99 | 65.49 | 14.31 | 43.46 | 43.69 |
| BG2 | 93.81 | 82.28 | 66.67 | 72.72 | 82.00 | 86.68 | 96.22 | 86.98 | 73.73 | 100.00 | 78.35 | 78.99 | 88.03 | 82.44 | 82.69 | 84.30 |
| TL | 88.57 | 76.81 | 70.37 | 75.76 | 68.00 | 75.49 | 100.00 | 88.75 | 90.51 | 100.00 | 69.50 | 79.99 | 82.39 | 60.43 | 81.33 | 79.60 |
| WS3 | 91.90 | 95.80 | 85.19 | 93.93 | 77.78 | 92.19 | 96.22 | 94.38 | 87.37 | 100.00 | 66.98 | 100.00 | 97.89 | 98.98 | 86.19 | 96.47 |
| TL+ | 95.70 | 95.80 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |

[1]WS (WideStrike, PHY 333WRF, BG2 (Bollgard II, ST 4946GLB2), TL (TwinLink, ST 4949GLT), TL+ (TwinLink Plus, ST 5575GLTP).

Table 3. Yields (lbs-lint/ac) among Bt cotton technology traits untreated or treated with Prevathon at 20 fl-oz/ac, 2017.[1]

| Bt tech[2] | Insecticide treatment | Pinebluff, AR | Rohwer, AR | Stoneville, MS | Starkville, MS | Winnsboro, LA | College Station, TX | Jackson, TN |
|---|---|---|---|---|---|---|---|---|
| NBT | Untreated | 1155.5 e | 937.1 a | 1176.2 c | 575.5 a | 807.1 a | 1354 b | 683.8 e |
| | Prevathon | 1499.4 ab | 1055.8 a | 1306.6 abc | 843.8 a | 921.5 a | 1709 a | 1202.9 abc |
| WS | Untreated | 1296.5 d | 868.4 a | 1278.4 bc | 704.5 a | 966.2 a | 1122 cd | 1108.5 cd |
| | Prevathon | 1478.8 ab | 1097.0 a | 1469.1 a | 816.3 a | 935.3 a | 1373 b | 1281.1 ab |
| BG2 | Untreated | 1566.5 a | 1005.9 a | 1448.8 ab | 660.6 a | 919.1 a | 1153 bcd | 1004.7 d |
| | Prevathon | 1488.7 ab | 889.0 a | 1445.4 ab | 722.6 a | 854.4 a | 1109 cd | 1165.1 bc |
| TL | Untreated | 1303.4 d | 1211.3 a | 1152.7 c | 597.0 a | 865.3 a | 1022 d | 795.9 e |
| | Prevathon | 1323.6 cd | 1090.1 a | 1211.0 c | 722.6 a | 886.8 a | 1244 bcd | 1077.8 cd |
| WS3 | Untreated | 1464.7 ab | 1097.0 a | 1171.9 c | 693.3 a | 958.8 a | 1197 bcd | 1154.1 bc |
| | Prevathon | 1370.5 bcd | 1117.7 a | 1301.9 abc | 709.7 a | 999.1 a | 1274 bc | 1309.1 a |
| TL+ | Untreated | 1447.8 abc | -- | -- | -- | -- | -- | -- |
| | Prevathon | 1404.8 bcd | -- | -- | -- | -- | -- | -- |

Means in a column followed by the same letter are not significantly different based on a F protected LSD ($P < 0.05$).
[1]Prevathon at 20 fl-oz/ac applications: Pinebluff, AR (July 24), Rohwer, AR (July 31), Stoneville, MS (July 31 and Aug 17), Starkville, MS (July 14), College Station, TX (July 12), Jackson, TN (Aug 3 and 29)
[2]NBT (non-Bt, DP 1441RF, WS (WideStrike, PHY 333WRF, BG2 (Bollgard II, ST 4946GLB2), TL (TwinLink, ST 4949GLT), TL+ (TwinLink Plus, ST 5575GLTP).

Table 4. Percentage change in yield in Bt cotton technology from treatment with Prevathon, 2017.[1]

| Bt tech[2] | Pinebluff, AR | Rohwer, AR | Stoneville, MS | Starkville, MS | Winnsboro, LA | College Station, TX | Jackson, TN | Across locations |
|---|---|---|---|---|---|---|---|---|
| NBT | 29.76* | 12.67 | 11.09 | 46.62 | 14.17 | 26.22* | 75.91* | 30.92* |
| WS | 14.06* | 26.32 | 14.92* | 15.87 | -3.20 | 22.37* | 15.57* | 15.13* |
| BG2 | -4.97 | -11.62 | -0.23 | 9.39 | -7.04 | -3.82 | 15.96* | -0.33 |
| TL | 1.55 | -10.01 | 5.06 | 21.04 | 2.48 | 21.72 | 35.42* | 11.04 |
| WS3 | -6.43 | 1.89 | 11.09 | 2.37 | 4.20 | 6.43 | 13.43* | 4.71 |
| TL+ | -2.97 | -- | -- | -- | -- | -- | -- | -- |

Values within a Bt tech with a * indicate a significant ($P < 0.05$) change in yield between non-treated and Prevathon-treated plots within a Bt technology.
[1]Prevathon at 20 fl-oz/ac applications: Pinebluff, AR (July 24), Rohwer, AR (July 31), Stoneville, MS (July 31 and Aug 17), Starkville, MS (July 14), College Station, TX (July 12), Jackson, TN (Aug 3 and 29)
[2]NBT (non-Bt, DP 1441RF, WS (WideStrike, PHY 333WRF, BG2 (Bollgard II, ST 4946GLB2), TL (TwinLink, ST 4949GLT), TL+ (TwinLink Plus, ST 5575GLTP).

Table 5. Resistance ratios of field collected bollworms (relative to a susceptible strain) originating near each test location, 2017.

| Bt toxin | Pinebluff, AR | Rohwer, AR | Stoneville, MS | Starkville, MS | Winnsboro, LA | College Station, TX | Jackson, TN |
|---|---|---|---|---|---|---|---|
| Cry1Ac | >109.8 | 30.5 | >109.8 | >109.8 | 68.8 | >109.8 | >109.8 |
| Cry2Ab2 | -- | 6.1 | >50.0 | 46.1 | >50.0 | >50.0 | 32.3 |