BT RESISTANCE AND BT TECHNOLOGY PERFORMANCE FOR TEXAS AND THE MID-SOUTH IN 2019

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Abstract

When Bt cotton was introduced in the United States in 1996, management of the cotton bollworm, *Helicoverpa zea*, had become much less problematic. However, in recent years unexpected bollworm damage has become common place in dual-gene Bt cotton and insecticide applications targeting bollworm have been necessary to avoid unacceptable injury and yield loss. Over the past three years we have demonstrated that bollworms in the southern U.S. have developed resistance to Cry1Ac, Cry1F, and Cry2Ab, and that treating dual-gene cotton for bollworms can reduce crop injury and preserve yield. In 2019, we continued monitoring for Bt resistance and investigating the benefit of treating Bt cotton with insecticides for bollworm. Similar to 2017 and 2018, we detected high incidences of resistance to all of the Cry toxins in Texas and the Mid-South, and we found one population collected in Mississippi from Leptra corn (Cry1Ab+Cry1F+Vip3Aa) suspected of having low resistance to Vip3A. Cotton bollworm infestations in Texas and the Mid-South were low in 2019, relative to recent years, and although Bollgard 2 benefited in lower fruit injury when treated with Prevathon, the benefit in yield protection was variable. Overall, the cotton technologies expressing Vip3Aa (WideStrike 3, TwinLink Plus and Bollgard 3) did not benefit from Prevathon treatment.

Introduction

The cotton bollworm, *Helicoverpa zea*, is one of the most damaging pests of cotton grown in the southern U.S. In 1996, transgenic cotton containing genes expressing Bacillus thuringiensis (Bt) proteins were introduced into U.S. cotton and 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*, but moderately toxic towards bollworm, *H. zea*. 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. Currently, Bt proteins used in transgenic cotton plants in the U.S. are categorized into three groups: Cry1, which contains Cry1Ab, Cry1Ac, and Cry1F; Cry2, which includes Cry2Ab and Cry2Ae; and Vip3a. The currently available Bt cotton technologies include: Bollgard 2 (Cry1Ac + Cry2Ab), WideStrike (Cry1Ac + Cry1F), TwinLink (Cry1Ab + Cry2Ae), WideStrike 3 (Cry1Ac + Cry1F + Vip3a), TwinLink Plus (Cry1Ab + Cry2Ae + Vip3a) and Bollgard 3 (Cry1Ac + Cry2Ab + Vip3a).

Although new Bt toxin introductions have increased the efficacy of transgenic cotton targeting lepidopteran 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 objectives of this project were to: 1) To estimate the susceptibility of the field populations of *H. zea* to Bt proteins, 2) To evaluate the efficacy of second and third generation Bt cotton for efficacy towards *H zea*, and 3) To determine if spraying Bt cotton for *H. zea* results in a reduction in fruit damage and increased yields relative to non-Bt cotton.

Materials and Methods

Bacillus thuringiensis proteins

Susceptibility of *H. zea* was determined against four Bt proteins: Cry1Ac, Cry1F, Cry2Ab2, and Vip3a. Cry1Ac protein was provided by Bayer CropScience as lyophilized MVPII powders with 20.0% AI. Cry1F protein was provided by Corteva AgroScience as lyophilized powders with 53.0% AI. Cry2Ab2 protein was provided by Bayer CropScience in the form of lyophilized (freeze-dried) Bt-corn leaf powder expressing ~4 mg of Cry2Ab2 protein/g. Juan Luis Jurat-Fuentes, University of Tennessee provided the Vip3Aa39 protein with a purity of 0.7 μg/ml.

Insect sources

A total of 20 field populations of *H. zea* were collected from across the Mid-South and Texas in 2019. Primarily F_1, and a few F_2 generations of these field-collected populations were used for the bioassays described below. In addition, two susceptible colonies were also used in the current study. One was obtained from a commercial source Benzon Research Inc., Carlisle, PA (CBW-BZ-SS); the other (CBW-TX-SS) was collected by Dr. David Kerns in Winnsboro, LA. The CBW-TX-SS strain was evaluated only at TAMU.

Insect bioassays

For each population, a diet-overlay bioassay was used to evaluate the larval susceptibility of *H. zea* to Cry1Ac, Cry2Ab2, Cry1F and Vip3A toxins. Assays were conducted at two laboratories, the Entomological Research Laboratory at Texas A&M University (Drs. Kerns and Yang) and the Southern Insect Management Unit USDA-ARS Laboratory in Stoneville, MS (Dr. Little). Each bioassay included 7-8 concentrations plus one untreated control. Diet-overlay concentrations for Cry1Ac and Cry2Ab2 ranged from 0, 0.01, 0.0316, 0.1, 0.316, 1.0, 3.16, to 10.0 $\mu g/cm^2$; diet-overlay concentrations for Vip3a1 were from 0, 0.01, 0.0316, 0.1, 0.316, 1.0, to 3.16 $\mu g/cm^2$; and diet-overlay concentrations for Cry1F ranged from 0, 0.02, 0.04, 0.2, 0.4, 2.0, 4.0, to 8.0 $\mu g/cm^2$. We used repeater pipets to dispense 0.8 ml per well of liquid diet (Southland Product, Inc. Lake Village, AR) into 128-well bioassay trays (C-D International, Pitman, NJ). Once the diet cooled and solidified, Bt protein solution suspended in 0.1% Triton-X100 was overlaid onto the diet surface of each well and allowed to air dry. A constant volume of 40 µl Bt protein solution was overlaid into each well for Cry1Ac, Cry1F, and Vip3a1 proteins, while a volume of 200 µl Bt protein solution per well was used for Cry2Ab2 protein. One neonate (< 24 h) of *H. zea* was released on the diet surface in each well. After larval inoculation, wells were covered with vented lids (C-D International, Pitman, NJ). Each combination of insect population by Bt protein concentration was replicated four times with 16-32 larvae in each replication. The bioassay trays were placed in an environmental chamber maintained at 26 ± 1 °C, 50% RH, and a 14:10 (L:D) h photoperiod. Larval mortality, and larval instar were recorded on the 7th day after inoculation.

Bioassay data analysis

Larval mortality was calculated based on the number of dead larvae plus survivors that were still in the first instar (mortality = dead+L1) divided by the total number of insects assayed, and was used to determine LC_{50} values and the corresponding 95% confidence limit (CL). Larval mortality at each concentration was first corrected based on the control mortality using the method of Abbott (1925), followed by Probit analysis to determine the LC_{50} that caused 50% mortality and the corresponding 95% confidence limit (CL). Where the LC_{50} 95% CLs for each field collected population did not overlap with the LC_{50} 95% CL of the CBW-BZ-SS susceptible colony, resistance ratio was calculated using the LC_{50} of a field population divided by the LC_{50} of the CBW-BZ-SS susceptible strain. In some cases, the LC_{50} value of an insect population was considered to be greater than the highest Bt protein concentration used in the bioassay if its larval mortality was <50% at the highest concentration assayed. If the LC_{50} value of an insect population was smaller than that of the CBW-BZ-SS, a negative sign was assigned to the resistance ratio. For purposes of defining resistance, we are considering resistance ratios ≥10-fold as resistant.

Bt cotton technology field performance

Tests were conducted at seven locations across the Mid-South and one locations in Texas to determine the impact of foliar insecticide applications targeting bollworms on fruit injury and yields in second and third generation Bt cottons. The Bt cotton technologies evaluated included: WideStrike3 (WS3) as PHY 330W3FE (Cry1Ac+Cry1F+Vip3A19) TwinLink Plus (TL+) as ST 5471 GLTP (Cry1Ab+Cry2Ae+Vip3A19), Bollgard II (BG2) as ST 1518 B2XF (Cry1Ac+Cry2A+Vip3A19), and Bollgard 3 (BG3) as DP 1835 B3XF (Cry1Ac+Cry2A+Vip3A19). A non-Bt variety (NBT) DP 1822 XF was included as a check.

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 untreated. Plots were 4 rows wide x 40-60 ft in length. Each factorial combination was replicated 4 times. Test locations included two plantings at Tillar, AR, Stoneville and Glendora, MS (the Glendora site was lost due to herbicide drift), Jackson, TN, Alexandria, 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: Tillar, AR1 (July 24), Tillar, AR2 (July 31), Stoneville, MS (July 11 and July 29), College Station, TX (July 3), and Jackson, TN (July 29 and Aug 15).

Field data analysis

Insect densities, plant terminal, square, bloom and boll injury were determined prior to foliar treatment and weekly thereafter. At each evaluation, 25 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 PROC GLIMMIX (PROC GLIMMIX SAS Institute Inc. 2011). Data was analyzed across test sites using the random effects of Site, Rep (site) and Bt Tech*Rep (Site). Where significant interactions (P = 0.05) were detected between Bt tech and spray, the SLICEDIFF option of the LSMEANS statement was utilized to determine if a given Bt tech differed, sprayed vs. non-sprayed for difference in yield response.

Results and Discussion

LC_{50} values of field-collected populations of H. zea to Bt proteins

Detailed data of LC_{50} values for four Bt proteins are listed in **Tables 1-4** for Cry1Ac, Cry2Ab2, Cry1F and Vip3Aa39, respectively. The LC_{50} values of the susceptible CBW-BZ-SS was 0.11 μg/cm^2 with a 95% CL of 0.07-0.16 μg/cm^2 in the TAMU lab and 0.18 μg/cm^2 with a 95% CL of 0.15-0.22 μg/cm^2 for the USDA lab (**Table 1**). Compared to CBW-BZ-SS, 29 of 30 field populations (95%) exhibited significantly lower susceptibility to Cry1Ac protein. The only susceptible field-collected population, CBW-TX-SK-Leptra, originated from Leptra corn and was not evaluated for resistance to Cry1Ac until the F2 generation which may have affected the results. Among the other field-collected populations, LC_{50} values ranged, 2.37 - >100.0 μg/cm^2, resulting in the resistance ratios from 21.5 - >909.1-fold.

The LC_{50} value of the CBW-BZ-SS on Cry2Ab2 diet was 0.63 μg/cm^2 with a 95% CL of 0.37-1.09 μg/cm^2 for the TAMU lab and 0.98 μg/cm^2 with 95% CL of 0.75-1.33 μg/cm^2 for the USDA lab (**Table 2**). The CBW-TX-SS-TAMU strain LC_{50} was 0.70 μg/cm^2 with 95% CL of 0.51-0.97 μg/cm2. Compared to CBW-BZ-SS, 22 of 30 field populations (73.3%) exhibited significantly high LC_{50} values, ranging 7.33- >20.0 μg/cm^2 resulting in resistance ratios of 11.6 - >31.7-fold. Susceptible populations were detected in collections from Hillsboro, TX (CBW-TX-HO-VT2P), Snook, TX (CBW-TX-SK-BG3 and CBW-TX-SK-Leptra), Winnsboro, LA (CBW-LA-WB-VT2P), Stoneville, MS (CBW-MS-SE-Leptra), Warren, MS (CBW-MS-WA-CC), Lafayette, AR (CBW-AR-VT2P) and Pickens, AR (CBW-AR-PK-NBt corn). Among the 8 Cry2Ab2 susceptible collections, 3 originated from non-Bt sources (non-Bt corn and crimson clover), 3 from crops expressing Vip3A (Bollgard 3 and Leptra) and 3 from VT DoublePro corn. Those hosts expressing Cry2Ab included Bollgard 3 and VT DoublePro. The reason collections were possible from hosts expressing Cry2Ab, yet tested susceptible, is unclear.

The LC_{50} value of the CBW-BZ-SS on Cry1F diet was 0.73 μg/cm^2 with a 95% CL of 0.55-0.97 μg/cm^2 (**Table 3**). All 7 of the populations evaluated on Cry1F diet exhibited resistance, with all LC_{50}s >8.00 μg/cm^2, resulting in resistance ratios >10.9-fold. Note that Cry1F at the highest concentration of 8.0 μg/cm^2 failed to result in 100%

mortality of the susceptible CBW-BZ-SS (93.75 ± 4.42%), indicating that Cry1F is generally not a highly effective toxin to *H. zea.*

The LC_{50} value of the CBW-BZ-SS on Vip3Aa39 diet was 0.69 μg/cm^2 with a 95% CL of 0.56-0.87 μg/cm^2 at the TAMU lab and 0.39 μg/cm^2 with a 95% CL of 0.31-0.49 μg/cm^2 at the USDA lab (**Table 4**). It should be noted that the LC_{50} values for CBW-BZ-SS between the two laboratories were significantly different based on non-overlapping 95% CLs. This indicates that there may be slight differences between the CBW-BZ-SS strains utilized at the two laboratories or slight differences in bioassay technique. For this reason, calculations of resistance ratios reported are within each lab. Additionally, the susceptible strain CBW-TX-SS-TAMU (0.17 μg/cm^2 with a 95% CL of 0.14-0.21 μg/cm^2) was significantly more susceptible than the CBW-BZ-SS-TAMU strain. Compared to CBW-BZ-SS, all 26 field populations were susceptible to the Vip3Aa39 protein, with the LC_{50} values ranging from 0.04 to 2.21 μg/cm^2. These LC_{50} values resulted in resistance ratios ranging from -9.75 to 3.2-fold. Only on population resulted in a positive resistance ratio, CBW-MS-SE-Leptra. The CBW-BZ-SS strain is thought to have some tolerance to Vip3A which would result in at large number of field-collected strains testing more susceptible. Thus, the CBW-BZ-SS strain may not be the most suitable reference strain for Vip3A toxins. Relative to the CBW-TX-SS susceptible strain, 7 field-collected population were significantly more tolerant to Vip3Aa39. All of the populations collected from Vip3A expressing crops had significantly elevated resistance ratios relative to CBW-TX-SS, but only one population. CBW-MS-SE-Leptra was the only population that had a resistance ratio (13.0-fold) that exceeded the 10-fold resistance threshold.

These data suggest that susceptibility to Cry1Ac, Cry2Ab2 and Cry1F have been largely compromised in the Mid-South and in Texas. Susceptibility to Vip3A appears to be high, but based on comparisons to the CBW-TX-SS susceptible strain, resistance occurred in 3.84% of the populations evaluated.

Bt cotton technology field performance

Overall in 2019, bollworm infestation pressure was lower than recent years. This may be due to high rainfall during February-June, and later than normal corn plantings over a wide range of dates, resulting in a trap crop scenario. Across all locations, seasonally averaged square and boll damage was consistently greatest in the untreated NBT, ranging 0.50-56.3% and 0.00-22.00% for damaged squares and bolls, respectively **(Table 5)**. The College Station, TX site suffered the greatest square damage to NBT, seasonally averaging 56.3%, however, most of this damage occurred pre-bloom and very early-bloom. Square damage was lowest at the Winnsboro, LA site which reported 8.34% square damage. Seasonal boll damage was greatest at the Tillar, AR1 site which averaged 22.00%, and lowest at the Alexandria, LA site with no boll damage detected. Prevathon applications reduced square and boll damage in the NBT at all locations with the exception of Alexandria, LA.

At Alexandria, fruit damage was almost non-existent and there were no differences among technologies, sprayed or untreated. Among the other locations, square and boll damage was consistently greater in the NBT relative to the Bt technologies. At the Tillar, AR1 location among the Bt technologies BG2 suffered greater square damage than WS3 and TL+, but did not differ from BG3 **(Tables 5 & 6)**. Boll damage was similar but BG2 only suffered greater damage than TL+. The Prevathon application at this location significantly reduced damage, within a technology, only in the NBT for squares, and only within the NBT for bolls. At the Tillar, AR2 location BG2 had higher boll damage than all of the other Bt technologies. Prevathon resulted in fewer damaged squares for only the NBT, and fewer damaged bolls for NBT and BG2. Among the Bt technologies at the Stoneville, MS location, square and boll damage was greatest in BG2, although square damage did not significantly differ from WS3, and only BG2 significantly benefited from treatment with Prevathon. At the Jackson, TN and College Station, TX locations there were no differences in square or boll damage among the Bt technologies, and only the NBT benefitted from treatment with Prevathon.

Yields across locations was highly variable, especially between varieties due to agronomic differences and poor weather conditions **(Table 7)**. When making comparison of Prevathon-treated to untreated within a variety/technology, NBT benefited in yield from treatment only at the Stoneville, MS, Alexandria, LA and Jackson, TN locations. The Tillar, AR1 & 2 locations suffered inclement weather that may negatively impacted yields and may have masked spray benefit, and the College Station, TX location may have been able to compensate for the early fruit damage **(Tables 7 & 8)**. At the Stoneville, MS location BG2 also benefited in higher yield from Prevathon applications, and at the Jackson, TN location, TL+ benefitted whereas yield was significantly lower in the where BG3 was treated. Across all locations, only the NBT demonstrated increased yield, 13.11%, from treating with Prevathon.

Table 1. Cry1Ac, LC_{50} and 95% confidence limits (CL) based on larval mortality of *Helicoverpa zea* to three Bt proteins in 2019.

Insect strain	N[a]	LC_{50} (95% CL) (μg/cm²)[b]	Slope ± SE	X²	df	Resistance ratio[c]
CBW-BZ-SS-TAMU	640	0.11 (0.07, 0.16)	1.10 ± 0.11	60.8	34	1.0
CBW-TX-SS-TAMU	640	0.09 (0.07, 0.12)	1.53 ± 0.13	39.0	34	0.8
CBW-TX-HO-VT2P	512	6.01 (2.68, 14.71)	0.78 ± 0.14	107.6	26	54.6*
CBW-TX-JN-VT2P	512	>100.0	/	/	/	> 909.1*
CBW-TX-LK-NBt	512	>100.0	/	/	/	> 909.1*
CBW-TX-NA-BG2	512	71.79 (38.36, 177.00)	0.65 ± 0.08	31.0	26	652.6*
CBW-TX-SK-BG3	512	10.92 (4.45, 40.46)	0.41 ± 0.08	42.9	26	99.3*
CBW-TX-SK-Leptra	512	0.57 (0.46, 0.70)	2.05 ± 0.19	14.8	26	5.2
CBW-TX-WN-NBt	512	>100.0	/	/	/	> 909.1*
CBW-AR-LE-VT2P	512	>100.0	/	/	/	> 909.1*
CBW-AR-TR-VT2P	512	>100.0	/	/	/	> 909.1*
CBW-LA-AA-BG2	512	>100.0	/	/	/	> 909.1*
CBW-LA-AA-VT2P	512	8.97 (5.90, 14.29)	0.93 ± 0.10	39.8	26	81.5*
CBW-LA-WB-VT2P	512	15.64 (9.11, 30.75)	0.76 ± 0.10	47.9	26	142.2*
CBW-TN-JN-VT2P	512	>100.0	/	/	/	> 909.1*
CBW-MS-SE-Leptra	512	15.64 (9.11, 30.75)	0.76 ± 0.10	47.9	26	142.2*
CBW-MS-SE-VT2P	512	2.37 (1.72, 3.26)	1.53 ± 0.14	33.2	26	21.5*
CBW-MS-SK-VT2P	512	>100.0	/	/	/	> 909.1*
CBW-BZ-SS-USDA	640	0.18 (0.15, 0.22)	1.97 ± 0.16	146.37	33	1.0
CBW-PK-AR-NBt corn	512	27.71 (15.42, 63.60)	0.75 ± 0.09	72.28	26	153.9*
CBW-HB-LA-CC	512	12.30 (7.39, 24.14)	0.71 ± 0.08	81.73	26	68.3*
CBW-CA-MS-CC	512	> 31.60	/	/	/	> 175.6*
CBW-CO-MS-CC	512	> 31.60	/	/	/	> 175.6*
CBW-FA-MS-Bt corn	512	14.26 (9.19, 25.22)	0.88 ± 0.09	88.25	26	79.2*
CBW-GD-MS-CC	512	> 31.60	/	/	/	> 175.6*
CBW-MK-MS-CC	512	> 31.60	/	/	/	> 175.6*
CBW-MB-MS-NBt corn	512	> 31.60	/	/	/	> 175.6*
CBW-NZ-MS-CC	512	> 31.60	/	/	/	> 175.6*
CBW-OB-MS-CC	384	> 31.60	/	/	/	> 175.6*
CBW-RF-MS-Bt corn	512	> 31.60	/	/	/	> 175.6*
CBW-SV-MS-NBt corn	512	> 31.60	/	/	/	> 175.6*
CBW-TL-MS-TL-F2	512	6.23 (3.84, 11.43)	0.67 ± 0.07	85.83	26	34.6*
CBW-WA-MS-CC	512	> 31.60	/	/	/	> 175.6*

[a] Total number of neonates assayed.

[b] The LC_{50} value of an insect strain was considered to be greater than the highest Bt protein concentration used in the bioassay if its larval mortality was <50% at the highest concentration. Larval mortality was calculated based on the number of dead larvae plus survivors that were still in the first instar (mortality = dead+L1) divided by the total number of insects assayed.

[c] Resistance ratio for Bt protein were calculated by dividing the LC_{50} value of an insect population by that of the susceptible strain (CBW-BZ-SS). If the LC_{50} an insect population was smaller than that of the CBW-BZ-SS, a negative sign was assigned to the resistance ratio.

* indicates significant resistance ratios that were ≥ 10-fold.

Table 2. Cry2Ab2, LC_{50} and 95% confidence limits (CL) based on larval mortality of *Helicoverpa zea* to three Bt proteins in 2019.

Insect strain	N[a]	LC_{50} (95% CL) (μg/cm²)[b]	Slope ± SE	X^2	df	Resistance ratio[c]
CBW-BZ-SS-TAMU	576	0.63 (0.37, 1.09)	1.50 ± 0.21	62.5	30	1.0
CBW-TX-SS-TAMU	512	0.70 (0.51, 0.97)	1.53 ± 0.15	40.2	26	1.1
CBW-TX-HO-VT2P	512	3.14 (2.44, 4.08)	1.41 ± 0.12	29.6	26	5.0
CBW-TX-JN-BG2	512	>20.0	/	/	/	> 31.7*
CBW-TX-LK-NBt	512	7.33 (5.29, 10.82)	1.10 ± 0.11	28.1	26	11.6*
CBW-TX-NA-BG2	512	19.18 (8.72, 81.63)	0.93 ± 0.18	44.6	26	30.4*
CBW-TX-SK-BG3	512	2.18 (1.39, 3.40)	2.28 ± 0.39	42.4	22	3.5
CBW-TX-SK-Leptra	512	2.29 (1.31, 4.25)	1.27 ± 0.19	59.5	26	3.6
CBW-TX-WN-NBt	512	19.54 (12.08, 42.65)	1.28 ± 0.21	16.8	26	31.0*
CBW-AR-LE-VT2P	512	4.42 (3.18, 6.35)	1.39 ± 0.15	24.2	26	7.0
CBW-AR-TR-VT2P	512	8.06 (5.60, 12.56)	0.99 ± 0.10	33.4	26	12.8*
CBW-LA-AA-BG2	512	>20.0	/	/	/	> 31.7*
CBW-LA-AA-VT2P	512	15.12 (8.80, 34.39)	1.03 ± 0.15	32.5	26	24.0*
CBW-LA-WB-VT2P	512	1.62 (1.24, 2.12)	1.27 ± 0.10	27.1	26	2.6
CBW-TN-JN-VT2P	512	>20.0	/	/	/	> 31.7*
CBW-MS-SE-Leptra	448	2.05 (1.22, 3.51)	1.56 ± 0.24	69.6	22	3.3
CBW-MS-SE-VT2P	512	>20.0	/	/	/	> 31.7*
CBW-MS-SK-VT2P	512	>20.0	/	/	/	> 31.7*
CBW-BZ-SS-USDA	640	0.98 (0.75, 1.33)	1.11 ± 0.08	193.37	33	1.0
CBW-PK-AR-NBt corn	512	3.16 (1.30, 12.89)	0.37 ± 0.06	33.57	26	3.23
CBW-HB-LA-CC	512	> 10.0	/	/	/	> 10.2*
CBW-CA-MS-CC	512	> 10.0	/	/	/	> 10.2*
CBW-CO-MS-CC	512	> 10.0	/	/	/	> 10.2*
CBW-FA-MS-Bt corn	512	> 10.0	/	/	/	> 10.2*
CBW-GD-MS-CC	512	> 10.0	/	/	/	> 10.2*
CBW-MK-MS-CC	512	> 10.0	/	/	/	> 10.2*
CBW-MB-MS-NBt corn	512	> 10.0	/	/	/	> 10.2*
CBW-NZ-MS-CC	512	> 10.0	/	/	/	> 10.2*
CBW-OB-MS-CC	384	> 10.0	/	/	/	> 10.2*
CBW-RF-MS-Bt corn	512	> 10.0	/	/	/	> 10.2*
CBW-SV-MS-NBt corn	512	> 10.0	/	/	/	> 10.2*
CBW-TL-MS-TL-F2	512	1.86 (1.04, 4.02)	0.53 ± 0.07	63.06	26	1.90
CBW-WA-MS-CC	512	> 10.0	/	/	/	> 10.2*

[a] Total number of neonates assayed.

[b] The LC_{50} value of an insect strain was considered to be greater than the highest Bt protein concentration used in the bioassay if its larval mortality was <50% at the highest concentration. Larval mortality was calculated based on the number of dead larvae plus survivors that were still in the first instar (mortality = dead+L1) divided by the total number of insects assayed.

[c] Resistance ratio for Bt protein were calculated by dividing the LC_{50} value of an insect population by that of the susceptible strain (CBW-BZ-SS). If the LC_{50} an insect population was smaller than that of the CBW-BZ-SS, a negative sign was assigned to the resistance ratio.

* indicates significant resistance ratios that were ≥ 10-fold.

Table 3. Cry1F, LC_{50} and 95% confidence limits (CL) based on larval mortality of *Helicoverpa zea* to three Bt proteins in 2019.

Insect strain	N[a]	LC_{50} (95% CL) (μg/cm^2)[b]	Slope ± SE	X^2	df	Resistance ratio[c]
CBW-BZ-SS-TAMU	512	0.73 (0.55, 0.97)	1.68 ± 0.15	28.9	26	1.0
CBW-TX-JN-BG2	512	>8.00	/	/	/	> 10.9*
CBW-TX-LK-NBt	512	>8.00	/	/	/	> 10.9*
CBW-TX-NA-BG2	512	>8.00	/	/	/	> 10.9*
CBW-TX-SK-BG3	512	>8.00	/	/	/	> 10.9*
CBW-TX-WN-NBt	512	>8.00	/	/	/	> 10.9*
CBW-LA-AA-BG2	512	>8.00	/	/	/	> 10.9*
CBW-TN-JN-VT2P	512	>8.00	/	/	/	> 10.9*

[a] Total number of neonates assayed.

[b] The LC_{50} value of an insect strain was considered to be greater than the highest Bt protein concentration used in the bioassay if its larval mortality was <50% at the highest concentration. Larval mortality was calculated based on the number of dead larvae plus survivors that were still in the first instar (mortality = dead+L1) divided by the total number of insects assayed.

[c] Resistance ratio for Bt protein were calculated by dividing the LC_{50} value of an insect population by that of the susceptible strain (CBW-BZ-SS). If the LC_{50} an insect population was smaller than that of the CBW-BZ-SS, a negative sign was assigned to the resistance ratio.

* indicates significant resistance ratios that were ≥ 10-fold.

Table 4. Vip3Aa39, LC_{50} and 95% confidence limits (CL) based on larval mortality of *Helicoverpa zea* to three Bt proteins in 2019.

Insect strain	N[a]	LC_{50} (95% CL) (μg/cm²)[b]	Slope ± SE	X^2	df	Resistance ratio[c] (BZ-SS)	Resistance ratio[c] (TX-SS)
CBW-BZ-SS-TAMU	448	0.69 (0.56, 0.87)	3.66 ± 0.53	8.5	22	1.0	0.24
CBW-TX-SS-TAMU	448	0.17 (0.14, 0.21)	2.84 ± 0.29	10.5	22	-2.3	1.0
CBW-TX-HO-VT2P	448	0.30 (0.25, 0.35)	3.64 ± 0.41	10.8	22	-2.3	1.8
CBW-TX-JN-BG2	448	0.20 (0.03, 1.17)	3.02 ± 1.32	19.5	22	-3.5	1.2
CBW-TX-LK-NBt	448	0.28 (0.24, 0.34)	2.89 ± 0.29	7.3	22	-1.4	1.7
CBW-TX-NA-BG2	448	0.08 (0.07, 0.10)	3.06 ± 0.34	14.1	22	-8.6	0.5
CBW-TX-SK-BG3	448	0.50 (0.40, 0.63)	1.85 ± 0.16	16.3	22	-1.4	2.9
CBW-TX-SK-Leptra	448	0.66 (0.49, 0.89)	1.79 ± 0.19	34.9	22	-1.1	3.9
CBW-TX-WN-NBt	448	0.24 (0.19, 0.29)	2.09 ± 0.19	19.5	22	-2.9	0.2
CBW-AR-LE-VT2P	448	0.39 (0.33, 0.47)	2.77 ± 0.27	18.6	22	-1.8	2.3
CBW-AR-TR-VT2P	448	0.15 (0.13, 0.19)	2.53 ± 0.25	6.9	22	-0.2	0.9
CBW-LA-AA-BG2	448	0.24 (0.20, 0.29)	2.50 ± 0.24	16.8	22	-2.9	1.4
CBW-LA-AA-VT2P	448	0.23 (0.18, 0.28)	2.16 ± 0.19	24.3	22	-3.0	1.4
CBW-LA-WB-VT2P	448	0.14 (0.12, 0.17)	2.48 ± 0.25	11.8	22	-4.9	-1.2
CBW-TN-JN-VT2P	448	0.32 (0.24, 0.44)	2.50 ± 0.33	38.8	22	-2.2	1.9
CBW-MS-SE-Leptra	448	2.21 (1.27, 4.44)	1.59 ± 0.29	57.2	22	3.2	13.0
CBW-MS-SE-VT2P	448	0.08 (0.07, 0.10)	2.94 ± 0.33	10.2	22	-8.6	-2.1
CBW-MS-SK-VT2P	448	0.16 (0.13, 0.19)	2.80 ± 0.28	16.7	22	-4.3	0.2
CBW-BZ-SS-USDA	640	0.39 (0.31, 0.49)	1.49 ± 0.10	217	33	1.0	--
CBW-PK-AR-NBt corn	512	0.21 (0.17, 0.26)	2.15 ± 0.19	132.4	26	-1.9	--
CBW-FA-MS-Bt corn	512	0.22 (0.17, 0.27)	1.69 ± 0.13	160.0	26	-1.77	--
CBW-GD-MS-CC	512	0.09 (0.07, 0.11)	2.41 ± 0.23	113.0	26	-4.33	--
CBW-MK-MS-CC	512	0.16 (0.12, 0.20)	1.35 ± 0.10	166.6	26	-2.44	--
CBW-MB-MS-NBt corn	512	0.14 (0.12, 0.17)	2.40 ± 0.22	116.4	26	-2.79	--
CBW-OB-MS-CC	384	0.12 (0.09, 0.15)	2.42 ± 0.26	89.4	19	-3.25	--
CBW-RF-MS-Bt corn	512	0.16 (0.13, 0.20)	2.11 ± 0.18	134.4	26	-2.44	--
CBW-SV-MS-NBt corn	512	0.32 (0.26, 0.39)	2.36 ± 0.21	123.8	26	-1.22	--
CBW-TL-MS-TL-F2	512	0.04 (0.04, 0.06)	1.87 ± 0.18	110.9	26	-9.75	--
CBW-WA-MS-CC	512	0.15 (0.12, 0.19)	1.62 ± 0.13	156.7	26	-2.60	--

[a] Total number of neonates assayed.
[b] The LC_{50} value of an insect strain was considered to be greater than the highest Bt protein concentration used in the bioassay if its larval mortality was <50% at the highest concentration. Larval mortality was calculated based on the number of dead larvae plus survivors that were still in the first instar (mortality = dead+L1) divided by the total number of insects assayed.
[c] Resistance ratio for Bt protein were calculated by dividing the LC_{50} value of an insect population by that of the susceptible strain (CBW-BZ-SS). If the LC_{50} an insect population was smaller than that of the CBW-BZ-SS, a negative sign was assigned to the resistance ratio.
* indicates significant resistance ratios that were ≥ 10-fold.

Table 5. Seasonal mean square and boll damage among Bt cotton technology traits untreated or treated with Prevathon at 20 fl-oz/ac, 2019.[1]

Bt tech[2]	Insecticide treatment	Tillar, AR1 % Damaged		Tillar, AR2 % Damaged		Stoneville, MS % Damaged		Alexandria, LA % Damaged		Jackson, TN % Damaged		College Station, TX % Damaged	
		Squares	Bolls	Squares	Bolls	Squares	Bolls	Squares	Bolls	Squares	Bolls	Squares	Bolls
NBT	untreated	17.0 a	22.0 a	8.4 a	18.3 a	14.8 a	13.3 a	0.5 a	0.0 a	11.0 a	12.5 a	56.3 a	8.7 a
	Prevathon	4.7 bc	4.8 bc	3.5 b	3.8 c	3.3 bcd	2.0 bc	0.0 a	0.0 a	0.0 b	0.0 b	11.0 b	1.7 b
WS3	untreated	2.5 c	3.0 bc	1.5 bc	2.0 cd	3.8 bc	1.5 c	0.3 a	0.0 a	1.0 b	0.0 b	1.7 b	0.3 b
	Prevathon	3.5 bc	1.3 c	0.5 c	0.3 d	1.8 cde	0.8 c	0.0 a	0.0 a	0.0 b	0.0 b	1.0 b	0.0 b
TL+	untreated	1.5 c	1.3 c	1.0 bc	0.7 d	2.0 cde	0.8 c	0.0 a	0.0 a	0.0 b	0.0 b	2.0 b	0.0 b
	Prevathon	4.0 bc	3.0 bc	1.0 bc	1.7 cd	1.5 de	0.2 c	0.0 a	0.0 a	0.0 b	0.0 b	1.0 b	0.0 b
BG2	untreated	5.5 b	6.5 b	1.0 bc	8.1 b	5.3 b	5.3 b	0.3 a	0.0 a	1.0 b	1.5 b	4.7 b	1.0 b
	Prevathon	1.5 c	3.0 bc	1.5 bc	0.3 d	1.0 e	0.7 c	0.0 a	0.0 a	0.0 b	0.0 b	0.7 b	0.0 b
BG3	untreated	3.0 bc	3.7 bc	2.0 bc	1.0 d	2.0 cde	0.3 c	0.3 a	0.0 a	0.0 b	0.0 b	3.3 b	0.0 b
	Prevathon	1.5 c	1.3 c	0.5 c	0.7 d	0.5 e	0.7 c	0.0 a	0.0 a	0.0 b	0.0 b	0.3 b	0.0 b

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: Tillar, AR1 (July 24), Tillar, AR2 (July 31), Stoneville, MS (July 11 and July 29), Alexandria, LA (July 10), Jackson, TN (July 29 and Aug 15), College Station, TX (July 3).
[2]NBT (non-Bt, DP 1822XF, WS3 (WideStrike3, PHY 330W3FE, TL+ (TwinLink Plus, ST 5471 GLTP), BG2 (Bollgard II, ST 1518 B2XF), BG3 (Bollgard 3, DP 1835 B3XF).

Table 6. Percentage reduction in square and boll damage of non-treated Bt cotton technologies relative to a non-treated non-Bt cotton, 2019.

Bt tech[1]	Tillar, AR2 % Reduction		Tillar, AR2 % Reduction		Stoneville, MS % Reduction		Alexandria, LA % Reduction		Jackson, TN % Reduction		College Station, TX % Reduction		Across locations % Reduction	
	Squares	Bolls	Squares	Bolls	Squares	Bolls	Squares	Bolls	Squares	Bolls	Squares	Bolls	Squares	Bolls
WS3	85.29 ab	86.36 ab	82.14 a	89.09 a	74.17 ab	88.75 a	50.00 a	--	90.91 a	100.00 a	97.04 a	96.19 a	79.93 ab	92.08 a
TL+	91.18 a	93.94 a	88.10 a	96.36 a	86.51 a	93.77 a	100.00 a	--	100.00 a	100.00 a	96.45 a	100.00 a	93.71 a	96.81 a
BG2	67.65 c	70.52 b	88.10 a	55.98 b	64.06 b	60.02 b	50.00 a	--	90.91 a	88.00 a	91.71 a	88.47 a	75.41 b	72.60 b
BG3	82.35 bc	83.33 ab	79.19 a	94.55 a	86.51 a	97.53 a	50.00 a	--	100.00 a	100.00 a	94.09 a	100.00 a	82.02 ab	95.08 a

[1]WS3 (WideStrike3, PHY 330W3FE, TL+ (TwinLink Plus, ST 5471 GLTP), BG2 (Bollgard II, ST 1518 B2XF), BG3 (Bollgard 3, DP 1835 B3XF).

Table 7. Yields (lbs-lint/ac) among Bt cotton technology traits untreated or treated with Prevathon at 20 fl-oz/ac, 2019.[1]

Bt tech[2]	Insecticide treatment	Tillar, AR1	Tillar, AR2	Stoneville, MS	Alexandria, LA	Jackson, TN	College Station, TX
NBT	untreated	773.8 a	728.5 de	1200.7 f	920.6 c	1173.8 d	1379.1 d
	Prevathon	1064.4 a	614.4 e	1548.8 e	1178.1 b	1482.5 a	1447.7 cd
WS3	untreated	944.0 a	994.9 ab	1604.9 de	1221.8 a	1254.4 cd	1498.4 cd
	Prevathon	1064.4 a	756.8 b-e	1742.2 bcd	1280.9 a	1425.5 ab	1418.3 cd
TL+	untreated	957.8 a	981.0 abc	1872.2 abc	1244.1 ab	1187.6 d	1772.9 ab
	Prevathon	1117.7 a	732.9 cde	1900.1 ab	1286.76 a	1317.3 bc	1797.4 a
BG2	untreated	1035.1 a	1084.34 a	1731.3 cd	1160.3 b	1447.1 a	1542.5 cd
	Prevathon	988.7 a	854.6 a-e	1941.8 a	1217.7 ab	1150.2 ab	1531.0 cd
BG3	untreated	1095.3 a	1039.8 a	1701.1 d	1220.6 ab	1423.5 ab	1581.7 bc
	Prevathon	1214.4 a	876.8 a-d	1736.2 cd	1291.2 a	1278.0 cd	1527.8 cd

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: Tillar, AR1 (July 24), Tillar, AR2 (July 31), Stoneville, MS (July 11 and July 29), Alexandria, LA (July 10), Jackson, TN (July 29 and Aug 15), College Station, TX (July 3).

[2]NBT (non-Bt, DP 1822XF, WS3 (WideStrike3, PHY 330W3FE, TL+ (TwinLink Plus, ST 5471 GLTP), BG2 (Bollgard II, ST 1518 B2XF), BG3 (Bollgard 3, DP 1835 B3XF).

Table 8. Percentage change in yield in Bt cotton technology from treatment with Prevathon, 2019.[1]

Bt tech[2]	Tillar, AR1	Tillar, AR2	Stoneville, MS	Alexandria, LA	Jackson, TN	College Station, TX	Across locations
NBT	27.30	-18.57	22.48*	21.86*	20.82*	4.74	13.11*
WS3	11.31	-31.47	7.88	4.62	12.00*	-5.65	-0.22
TL+	14.31	-33.86	1.47	3.32	9.85*	1.36	-0.59
BG2	-4.70	-26.88	10.84*	4.71	-25.81	-0.75	-7.10
BG3	9.71	-6.45	2.02	5.47	-11.38*	-3.53	-0.69

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: Tillar, AR1 (July 24), Tillar, AR2 (July 31), Stoneville, MS (July 11 and July 29), Alexandria, LA (July 10), Jackson, TN (July 29 and Aug 15), College Station, TX (July 3).

[2]NBT (non-Bt, DP 1822XF, WS3 (WideStrike3, PHY 330W3FE, TL+ (TwinLink Plus, ST 5471 GLTP), BG2 (Bollgard II, ST 1518 B2XF), BG3 (Bollgard 3, DP 1835 B3XF).

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