UPDATE ON BT RESISTANCE IN *HELICOVERPA ZEA* AND THE VALUE OF TREATING BT COTTON WITH INSECTICIDES

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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 2021, we continued monitoring for Bt resistance and investigating the benefit of treating Bt cotton with insecticides for bollworm. We detected high incidences of resistance to Cry1Ac and Cry2Ab2 Bt proteins in Texas and the Mid-South but did not detect any resistance to Vip3Aa. Bt susceptibility among bollworm collections from Bt corn planted in small plots alongside non-Bt corn, suggest that the Cry proteins in corn strongly select for resistance to corresponding Cry proteins, but that these Cry resistant selected populations tend to be more susceptible to Vip3Aa than the larvae collected from non-Bt. Cotton bollworm infestations in Texas and the Mid-South were low in 2021, relative to recent years, and Bollgard 2 provided adequate protection from bollworm at

6 out of 7 locations, with Tillar, AR representing the only location were BG2 failed. Treatment with Vantacor benefitted the non-Bt at 3 out of the 7 locations, and for BG2 only at Tillar, AR. The cotton technologies expressing Vip3Aa (WideStrike 3, TwinLink Plus and Bollgard 3) provided good bollworm control and did not benefit from insecticide treatment. The 6% damaged fruit threshold appeared to serve as a reliable indicator of when treatment for bollworms in justified.

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 protein 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. Because of resistance to the Cry proteins, Vip3Aa is considered the only Bt protein that provides consistent bollworm control in corn and cotton.

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 three Bt proteins: Cry1Ac, Cry2Ab2, and Vip3a. Cry1Ac protein was provided by Bayer CropScience as lyophilized MVPII powders with 20.0% 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 13 field populations of *H. zea* were collected from across the Mid-South and Texas in 2021. The F_1 generation was used for all collections except CBW-LA-Alexandria-TRE from which the F_2 generation was used. The susceptible strain (CBW-BZ-SS) Benzon Research Inc., Carlisle, PA was utilized to compute resistance ratios.

Insect Bioassays

For each population, a diet-overlay bioassay was used to evaluate the larval susceptibility of *H. zea* to Cry1Ac, Cry2Ab2, and Vip3A toxins. Assays were conducted at the Entomological Research Laboratory at Texas A&M University. 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 µg/cm^2, and concentrations for Vip3Aa39 were from 0, 0.01, 0.0316, 0.1, 0.316, 1.0, to 3.16 µg/cm^2. Repeater pipets were used 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 each 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 ºC, 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. For purposes of defining resistance, we are considering resistance ratios e 10-fold as resistant.

Bt Cotton Technology Field Performance

Tests were conducted at seven locations across the Mid-South and one location in Texas to determine the efficacy of Bt cotton technologies and if treated these technologies for bollworms with insecticides results in higher yields. The Bt cotton technologies evaluated included: WideStrike 3 (WS3) as PHY 390 W3FE (Cry1Ac+Cry1F+Vip3A19) TwinLink Plus (TL+) as ST 5450 GLTP (Cry1Ab+Cry2Ae+Vip3A19), Bollgard II (BG2) as DP 1646 B2XF (Cry1Ac+Cry2A+Vip3A19), and Bollgard 3 (BG3) as DP 1845 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 College Station, TX, Alexandria, LA, Winnsboro, LA, Stoneville, MS, Glendora, MS, Tillar, AR and Jackson, TN. Vantacor (chlorantraniliprole) was applied at 1.7 fl-oz/ac at first bloom at all locations and then as needed but no sooner than 14 days following application 1.

Field Data Analysis

Insect densities, plant terminal, square, bloom and boll injury were determined prior to foliar treatment and weekly thereafter. At each evaluation, 25-50 terminals, squares, bloom, and bolls were sampled per plot. Square and boll damage was combined to calculate percent damaged fruit. Seasonally, percent fruit damage was calculated by averaging percent fruit damage over the entire sampling period at each location. At all locations, the middle two rows of each plot were 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. Difference in yield due to insecticide application was determine for each Bt technology by subtracting the yield of the insecticide-treated plot from the yield of the non-sprayed plot. Only percent seasonal fruit damage and change in yield due to insecticide treatment are reported.

All field data were analyzed using ANOVA and means were separated using an F-protected LSD (P=0.05).

Results and Discussion

LC_{50} Values and Resistance Ratios of Field-Collected Populations of *H. Zea* to Bt Proteins

Detailed data of LC_{50} values for four Bt proteins are listed in **Tables 1-3** for Cry1Ac, Cry2Ab2, and Vip3Aa39, respectively. The LC_{50} value of the susceptible CBW-BZ-SS to Cry1Ac was 0.09 µg/cm^2 with a 95% CL of 0.07-0.12 µg/cm^2 (**Table 1**). Twelve of thirteen field populations (92%) exhibited significantly lower susceptibility to Cry1Ac protein. The only susceptible field-collected population, CBW-Malone-NBT, which was collected from non-Bt corn. Among the other field-collected populations, LC_{50} values ranged, 6.27 – 250,860 µg/cm^2, resulting in the resistance ratios (RR) from 69.7 - ~2.8 million-fold.

The LC_{50} value of the CBW-BZ-SS on Cry2Ab2 was 0.11 µg/cm^2 with a 95% CL of 0.08-0.16 µg/cm^2 (**Table 2**). Compared to CBW-BZ-SS, 12 of 13 field populations (92%) exhibited significantly high LC_{50} values, ranging 1.23-9.10 µg/cm^2 resulting in resistance ratios of 11.2 – 82.7-fold. Only the collection from Alexandria, LA (CBW-LA-Alexandria-TRE) which was collected from flooded Trecepta corn (Cry1A.105 + Cry2Ab2 + Vip3Aa) tested susceptible to Cry2Ab2. However, due to the low number larvae collected this population was evaluated at the F_2 generation which may have affected the results.

The LC_{50} value of the CBW-BZ-SS to Vip3Aa39 was 0.33 µg/cm^2 with a 95% CL of 0.16-0.78 µg/cm^2 (**Table 3**). It should be noted that the CBW-BZ-SS is considered to be more tolerant to Vip3Aa than most susceptible field collected populations. Compared to CBW-BZ-SS, all 13 field populations were susceptible to the Vip3Aa39 protein, with the LC_{50} values ranging from <0.03 – 0.13 µg/cm^2. These LC_{50} values resulted in resistance ratios ranging from <0.09 – 0.39-fold. Thus, all of the field collected populations were more susceptible to Vip3Aa39 than the susceptible strain. The population originating from flooded Trecepta corn (evaluated at the F2 generation) was found to be highly susceptible to Vip3Aa39 suggesting the Trecepta corn was probably not expressing Vip3Aa well.

These data suggest that susceptibility to Cry1Ac and Cry2Ab2 have been largely compromised in the Mid-South and in Texas. Susceptibility to Vip3Aa appeared to remain be high in 2021.

At three collection locations we were able to compare resistance ratios from collections from non-Bt corn and Bt corn hybrids in paired planting. At Taylor, TX, *H. zea* collected from Intersect corn (Cry1Ab + Cry1F) exhibited a Cry1Ac RR of 1,543-fold relative to the non-Bt with a resistant ratio of 142.6-fold; a 974.51% decrease in susceptibility (**Figure 1**). Larvae collected from DoublePro corn (Cry1A.105 + Cry2Ab2) at Taylor, TX, Malone, TX, and Winnsboro, LA exhibited RRs to Cry1Ac of 364.1-fold, 539.6-fold, and 154.6-fold, respectively. Relative to collections from non-Bt corn at each location, these resulted in a 153.48% and 6,248.24% decrease in susceptibility at Taylor and Malone, respectively, and a 41.03% increase in susceptibility at Winnsboro. The Malone non-Bt population was the only collection found to be susceptible to Cry1Ac and thus selection pressure had the greatest influence. These data suggest Cry proteins in corn are selecting for resistance.

For our paired corn hybrid plantings, the RR for the population collected from Intersect corn at Taylor had an RR for Cry2Ab2 of 0.09-fold, resulting in a 75% decrease in susceptibility relative to the collection from non-Bt corn. (**Figure 2**). Larvae collected from DoublePro corn from Taylor, Malone, and Winnsboro exhibited RRs to Cry2Ab2 of 61.6-fold, 67.3-fold, and 79.4-fold, respectively. Relative to collections from non-Bt corn at each location, these resulted in a 450%, 275.98%, and 119.94% decrease in susceptibility at Taylor, Malone, and Winnsboro, respectively. These data further suggest that Cry proteins in corn are selecting for resistance.

For our paired corn hybrid plantings, the resistance ratio for the population collected from Intersect corn at Taylor had an RR for Vip3Aa39 of 0.09-fold, resulting in a 75% increase in susceptibility to Vip3Aa39 relative to the collection form the non-Bt (**Figure 3**). Larvae collected from DoublePro corn from Taylor, Malone, and Winnsboro exhibited RRs to Vip3Aa39 of 0.09-fold, 0.09-fold, and 0.21-fold, respectively. Relative to collections from non-Bt corn at each location, these resulted in a 75%, 40%, and 44.15% increase in susceptibility at Taylor, Malone, and Winnsboro, respectively. Although, the increase in susceptibility is not great, these data suggest that where Cry resistance is evident, susceptibility to Vip3Aa increases. Thus, inferring possible negative cross-resistance.

Bt Cotton Technology Field Performance

Overall, in 2021, bollworm infestation pressure was very low relative to previous years. This may be due to high rainfall and/or colder than normal temperatures during January and February. At Alexandria and Winnsboro, LA, seasonal fruit damage was 0.75% and 2.67%, respectively, and did not differ from any of the Bt traits (**Table 4**), and none of the Bt technology entries benefited in yield from being treated with insecticide (**Table 5**). Neither of these locations ever attained 20% egg lay or a 6% damaged fruit threshold for any varietal entry.

Infestation was also low at College Station, TX and Stoneville, MS which averaged 4.79% and 6.79% seasonal fruit damage in the non-Bt, respectively. At College Station, seasonal percent fruit damage in the non-Bt was statistically greater than the Bt traits, and BG2 (0.63% fruit damage) was statistically greater than the entries expressing Vip3Aa (**Table 4**). Treating with insecticides benefitted only the non-Bt entry. The College Station location never attained 20% egg lay across varieties and exceeded the 6% damaged fruit threshold only within the non-Bt on 12 Aug with a value of 8.17% damaged fruit. Consequently, only the non-Bt benefitted in yield from being treated with Vantacor (**Table 5**). At Stoneville, percent seasonal damaged fruit in the non-Bt was greater than the Bt entries, and none of the Bt entries differed from each other (**Table 4**), but no differences were detected in yield benefit from treating with insecticide (**Table 5**). At Stoneville, the non-Bt exceeded the 6% damage threshold on all three sample dates, but none of the Bt entries ever exceeded threshold.

Infestations were higher at Glendora, MS, Tillar, AR and Jackson, TN, and averaged 26.38%, 26.18%, and 29.75% seasonal damaged fruit in the non-Bt, respectively (**Table 4**). At Glendora and Jackson, seasonal fruit damage was significantly higher in the non-Bt than the Bt traits, but none of the Bt traits differed from each other. Whereas, at Tillar, seasonal percent fruit damage for BG2 (21.73%) did not differ from the non-Bt. Tillar was the only location where BG2 failed to offer significant bollworm protection. At Jackson, the only the non-Bt benefitted in yield from insecticide treatment, while at Glendora, TLP had a significantly higher yield when treated (**Table 5**). At Jackson, the non-Bt exceeded the 6% damage threshold on both sample dates, while none of the Bt entries suffered any bollworm damage. At Glendora, the non-Bt exceed the 6% damage threshold on both sample dates, but no benefit in yield was detected from spraying with insecticide. At Tillar, the non-Bt and BG2 exceeded the 6% damage threshold on all five samples dates, and WS3 exceeded threshold on the first sample date. However, benefit in yield from spraying was detected for only the non-Bt and BG2 entries (**Table 5**).

Across locations, there was very little variability in fruit damage for Bt entries expressing Vip3Aa, and the median reduction in fruit damage relative to the non-Bt differed less than 5% (Figure 4). BG2 was more variable, primarily driven by the Tillar location (17% reduction), and somewhat by College Station (86.85% reduction), and Glendora (82.45% reduction). The percent reduction in fruit damage relative to the non-Bt was 100%, 90.46%, 92.05%, and 100% for Alexandria, Winnsboro, Stoneville, and Jackson, respectively. The median percent reduction in fruit damage for BG2, relative to the non-Bt, was 90.64% and the mean was 81.28%. These values suggest that BG2 failure is geographically variable, and that under low bollworm pressure, BG2 will provide adequate protection.

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Table 1. LC_{50} and 95% confidence limits (CL) based on larval mortality of *Helicoverpa zea* to Cry1Ac protein in 2021.

Insect strain	N[a]	LC_{50} (95% CL) ($\mu g/cm^2$)[b]	Slope ± SE	X2	df	Resistance ratio[c]
CBW-BZ-SS	512	0.09 (0.07, 0.12)	1.19 ± 0.10	38.2	26	1.0
CBW-LA-Alexandria-CS	512	6.27 (3.63, 11.63)	0.51 ± 0.07	23.9	26	69.7 *
CBW-LA-Alexandria-NBT	512	23.96 (15.0, 43.20)	0.71 ± 0.08	37.0	26	266.2 *
CBW-LA-Winnsboro-NBT	512	23.58 (13.01, 52.55)	0.70 ± 0.09	41.6	26	262.0 *
CBW-LA-Winnsboro-VT2P	512	13.91 (6.94, 34.64)	0.27 ± 0.08	66.1	26	154.6 *
CBW-TX-Thrall-Intrasect	512	250860 (3589, 1.46251E13)	0.48 ± 0.13	33.0	26	2787333.0 *
CBW-TX-Thrall-VT2P	512	8.22 (5.38, 13.20)	0.70 ± 0.07	29.3	26	91.3 *
CBW-TX-Malone-NBT	512	0.77 (0.15, 2.16)	0.51 ± 0.12	84.1	26	8.5
CBW-TX-Malone-VT2P	512	48.56 (26.49, 116.34)	0.80 ± 0.11	40.1	26	539.6 *
CBW-TX-Taylor-Intrasect	512	138.89 (25.23, 30575)	0.36 ± 0.10	64.5	26	1543.2 *
CBW-TX-Taylor-NBT	512	12.92 (6.44, 33.24)	0.41 ± 0.06	15.8	26	143.6 *
CBW-TX-Taylor-VT2P	512	32.77 (16.94, 83.62)	0.51 ± 0.07	30.3	26	364.1 *
CBW-TX-Wharton-VT2P	512	78.12 (37.97, 241.75)	0.71 ± 0.10	37.1	26	868.0 *

[a] Total number of neonates assayed.
[b]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).
* indicates significant resistance ratios that were e 10-fold.

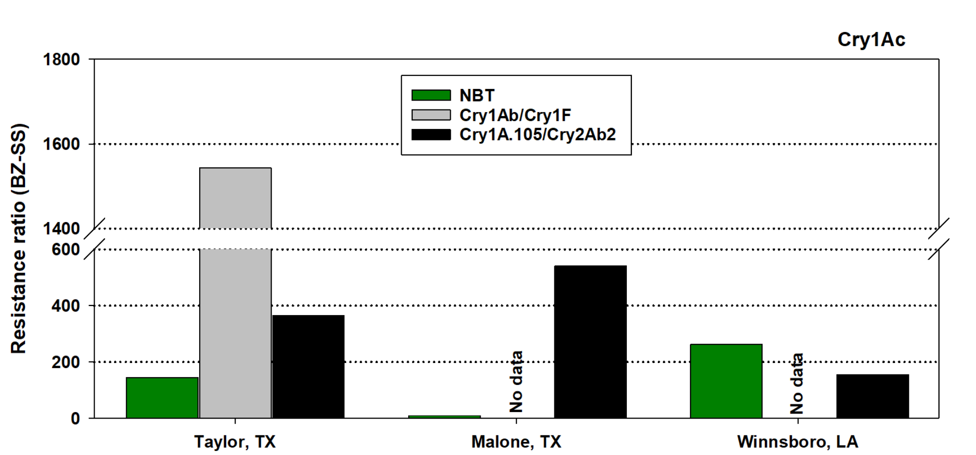

Figure 1. Resistance ratios for Cry1Ac for bollworms collected from paired plantings of non-Bt, Intersect (Cry1Ab + Cry1F) and DoublePro (Cry1A.105 + Cry2Ab2) corn hybrids at three locations.

Table 2. LC_{50} and 95% confidence limits (CL) based on larval mortality of *Helicoverpa zea* to Cry2Ab2 protein in 2021.

Insect strain	N[a]	LC_{50} (95% CL) ($\mu g/cm^2$)[b]	Slope ± SE	X2	df	Resistance ratio[c]
CBW-BZ-SS	512	0.11 (0.08, 0.16)	1.33 ± 0.13	36.7	26	1.0
CBW-LA-Alexandria-CS	512	4.88 (3.59, 6.90)	1.11± 0.10	13.0	26	44.4 *
CBW-LA-Alexandria-NBT	512	9.10 (6.82, 11.59)	1.41 ± 0.77	84.2	26	82.7 *
CBW-LA-Winnsboro-NBT	512	3.97 (2.42, 6.98)	1.44 ± 0.22	46.6	26	36.1 *
CBW-LA-Winnsboro-VT2P	512	8.73 (4.18, 29.23)	1.31 ± 0.32	62.0	26	79.4 *
CBW-TX-Thrall-Intrasect	512	7.13 (4.14, 14.83)	0.98 ± 0.14	47.6	26	64.8 *
CBW-TX-Thrall-VT2P	512	3.11 (2.01, 5.11)	1.09 ± 0.13	41.5	26	28.3 *
CBW-TX-Malone-NBT	512	1.97 (1.11, 3.75)	0.80 ± 0.11	59.8	26	17.9 *
CBW-TX-Malone-VT2P	512	7.40 (5.16, 11.42)	1.42 ± 0.18	32.5	26	67.3 *
CBW-TX-Taylor-Intrasect	512	1.61 (1.21, 2.16)	1.13 ± 0.09	35.4	26	14.6 *
CBW-TX-Taylor-NBT	512	1.23 (0.91, 1.66)	1.08 ± 0.09	34.7	26	11.2 *
CBW-TX-Taylor-VT2P	512	6.78 (3.57, 17.01)	0.86 ± 0.14	60.1	26	61.6 *
CBW-TX-Wharton-VT2P	512	8.67 (4.85, 19.45)	0.75 ± 0.10	38.8	26	78.8*
CBW-LA-Alexandria-TRE	320	0.584 (0.052, 1.353)	0.65 ± 0.20	25.5	14	5.31

[a] Total number of neonates assayed.
[b]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).
* indicates significant resistance ratios that were e 10-fold.

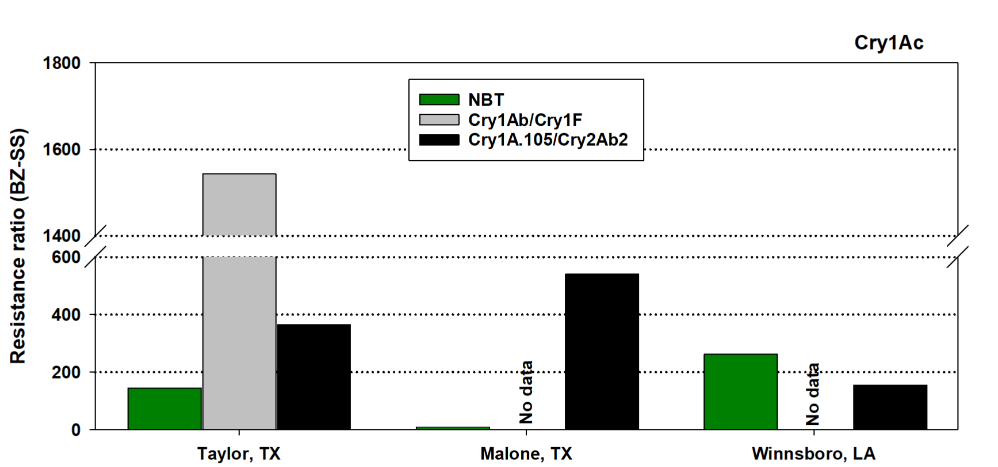

Figure 2. Resistance ratios for Cry2Ab2 for bollworms collected from paired plantings of non-Bt, Intersect (Cry1Ab + Cry1F) and DoublePro (Cry1A.105 + Cry2Ab2) corn hybrids at three locations.

Table 3. LC_{50} and 95% confidence limits (CL) based on larval mortality of *Helicoverpa zea* to Vip3Aa39 protein in 2021.

Insect strain	N[a]	LC_{50} (95% CL) ($\mu g/cm^2$)[b]	Slope ± SE	X2	df	Resistance ratio[c]
CBW-BZ-SS	512	0.33 (0.16, 0.78)	1.80 ± 0.42	77.2	26	1.0
CBW-LA-Alexandria-CS	448	0.05 (0.04, 0.07)	2.27 ± 0.29	12.9	22	0.15
CBW-LA-Alexandria-NBT	448	0.03 (0.02, 0.04)	2.29 ± 0.35	10.2	22	0.09
CBW-LA-Winnsboro-NBT	448	0.13 (0.10, 0.15)	2.60 ± 0.27	14.3	22	0.39
CBW-LA-Winnsboro-VT2P	448	0.07 (0.04, 0.10)	1.76 ± 0.33	73.7	22	0.21
CBW-TX-Thrall-Intrasect	448	< 0.03	/	/	/	< 0.09
CBW-TX-Thrall-VT2P	448	0.10 (0.09, 0.12)	3.37 ± 0.37	21.0	22	0.30
CBW-TX-Malone-NBT	448	0.05 (0.04, 0.08)	2.67 ± 0.33	14.2	22	0.15
CBW-TX-Malone-VT2P	448	< 0.03	/	/	/	< 0.09
CBW-TX-Taylor-Intrasect	448	0.03 (0.01, 0.04)	1.42 ± 0.33	51.5	22	0.09
CBW-TX-Taylor-NBT	448	0.12 (0.11, 0.18)	1.95 ± 0.19	26.0	22	0.36
CBW-TX-Taylor-VT2P	448	< 0.03	/	/	/	< 0.09
CBW-TX-Wharton-VT2P	448	0.03 (0.02, 0.05)	1.87 ± 0.28	10.5	22	0.09
CBW-LA-Alexandria-TRE	448	< 0.03	/	/	/	< 0.09

[a] Total number of neonates assayed.
[b]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).
* indicates significant resistance ratios that were e 10-fold.

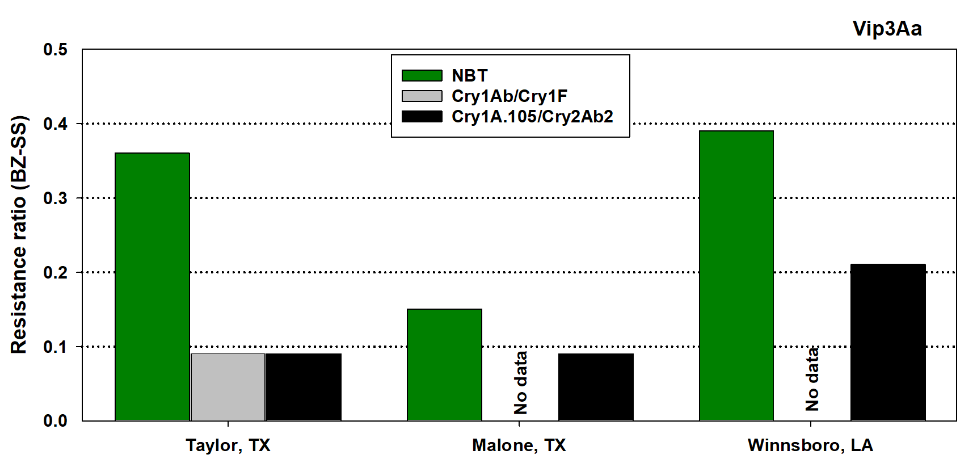

Figure 3. Resistance ratios for Vip3Aa39 for bollworms collected from paired plantings of non-Bt, Intersect (Cry1Ab + Cry1F) and DoublePro (Cry1A.105 + Cry2Ab2) corn hybrids at three locations.

Table 4. Seasonal mean percentage fruit (square & boll) damage among five Bt technologies for seven locations in Texas and the Mid-South, 2021.

	Seasonal mean % fruit damage				
Location	Non-Bt	WideStrike 3	TwinLink Plus	Bollgard 2	Bollgard 3
College Station, TX	4.79 a	0.17 c	0.21 c	0.63 b	0.04 c
Alexandria, LA	0.75 a	0.00 a	0.00 a	0.00 a	0.00 a
Winnsboro, LA	2.67 a	0.25 b	0.25 b	0.25 b	0.00 b
Stoneville, MS	6.79 a	0.08 b	0.04 b	0.54 b	0.08 b
Glendora, MS	26.38 a	0.63 b	0.50 b	4.63 b	1.50 b
Tillar, AR	26.18 a	1.73 b	1.30 b	21.73 b	1.28 b
Jackson, TN	29.75 a	0.00 b	0.00 b	0.00 b	0.00 b

Means within a row followed by the same letter are not significantly difference based on an F-protected LSD (P=0.05).

Table 5. Benefit in yield from spraying five Bt technologies with Vantacor at 1.7 fl-oz/ac for seven locations in Texas and the Mid-South, 2021.

	Difference in yield (lbs.-lint/ac) between sprayed vs. non-sprayed				
Location	Non-Bt	WideStrike 3	TwinLink Plus	Bollgard 2	Bollgard 3
College Station, TX	339.60*	-101.23	-4.90	48.98	195.92
Alexandria, LA	43.59	20.42	46.33	8.33	24.16
Winnsboro, LA	271.03	63.68	73.47	215.52	21.23
Stoneville, MS	-120.34	111.68	-15.70	287.13	210.93
Glendora, MS	115.84	-164.01	321.14*	20.65	154.83
Tillar, AR	907.90*	170.23	-199.46	1060.93*	170.23
Jackson, TN	253.63*	-41.29	-96.34	19.67	39.33

Values within location and Bt trait followed by an * indicates that there was a significant (P=0.05) effect from threating that trait with Vantacor.

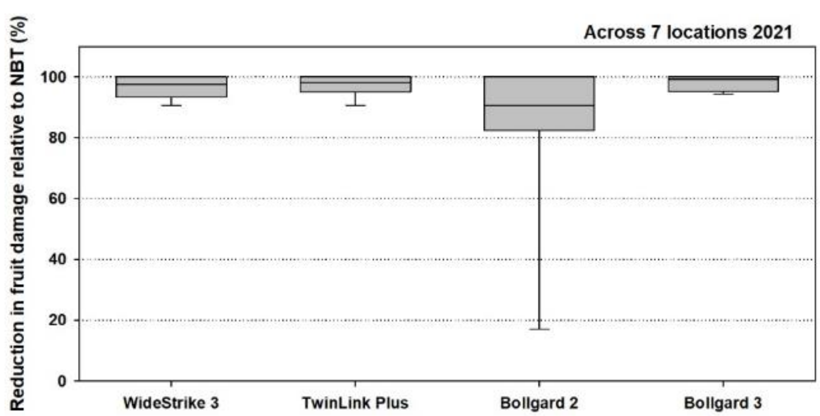

Figure 4. Box and whisker plots depicting the percent reduction in fruit damage among Bt cotton technologies relative non-Bt cotton across seven locations in 2021.