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Research

Pest Management

Association of Insect-Derived Ear Injury With Yield and Aflatoxin of Maize Hybrids Varying in Bt Transgenes

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Abstract

Environmental factors have been associated with the production of aflatoxin in maize, *Zea mays* L., and it is inconclusive whether transgenic, *Bacillus thuringiensis* (Bt), maize has an impact on aflatoxin accumulation. Maize hybrids differing in transgenes were planted in two locations from 2014 to 2017. Yield, aflatoxin, and ear injury caused by corn earworm, *Helicoverpa zea* (Boddie), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), were measured across three groups of hybrids differing in transgenes including near-isogenic hybrids, and water-stressed conditions. The hybrid groups consisted of non-Bt hybrids with no Bt transgenes, a second group with one or more Cry-Bt transgenes, and the third group with vegetative insecticidal Bt protein and Cry-Bt transgenes (Cry/Vip-Bt). Across the six data sets derived from 11 experiments, the Cry-Bt and Cry/Vip-Bt hybrids had less ear injury and aflatoxin on average than non-Bt hybrids. The effects of ear injury on yield and aflatoxin were more prominent and consistent in Corpus Christi, TX, where hybrids experienced more water-limited conditions than in College Station, TX. The trend of increased aflatoxin among hybrids with increased ear injury was further resolved when looking at Cry-Bt and Cry/Vip-Bt isogenic hybrids in Corpus Christi. The results supported that the maize hybrids with the inclusion of Cry-Bt and Cry/Vip-Bt transgenes warrant further investigation in an integrated approach to insect and aflatoxin management in sub-tropical rain-fed maize production regions. Research outcomes may be improved by focusing on areas prone to water-stress and by using hybrids with similar genetic backgrounds.

Key words: corn earworm, fall armyworm, vegetative insecticidal protein, *Bacillus thuringiensis*, *Aspergillus flavus*

The United States produced 383.5 billion kilograms of maize, *Zea mays* (L.), in 2016 (U.S. Department of Agriculture [USDA] 2017). Much of U.S. maize production is concentrated in the mid-west states, but the amount of maize produced in the southern United States accounts for a substantial minority despite more challenging growing conditions (13%) (USDA 2017). Maize grown in the southern United States is more likely to experience ear injury and aflatoxin accumulation than maize grown in the mid-west due to high periodic populations of fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and corn earworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), and environmental factors conducive to *Aspergillus flavus* (Link) (Deuteromycetes: Moniliales) infection (Abbas et al. 2007).

Aspergillus flavus is a fungus that produces the secondary metabolite aflatoxin B_1, commonly referred to as aflatoxin (Abbas et al. 2009). *Aspergillus* species are the most common producers of aflatoxins, and *A. flavus* is the most common producer of aflatoxin in maize. Due to the toxic nature of aflatoxin to humans, livestock, and wildlife, aflatoxin levels are regulated by the U.S. Food and Drug Administration, with *A. flavus* management costs estimated at $163 million annually (U.S. Food and Drug Administration [U.S. FDA] 1994, Wu 2014).

Ear-feeding insects, such as larvae of fall armyworm and corn earworm, feed on silks and developing maize kernels, thereby potentially affecting quality and yield (Vickery 1929, Smith and Riley 1992). Ear injury caused by these insects can also increase maize susceptibility to *A. flavus* infection by creating mechanical wounds on the kernels and silks which provide entry of *A. flavus* conidia (Abbas et al. 2013). Insects can also serve as active sources of inoculum by proliferating *A. flavus* spores in the hindgut as seen with the corn earworm, or passively by carrying the fungal spores on their bodies, appendages, and mouthparts (Dowd et al. 2005, Abbas et al. 2007).

Transgenic *Bacillus thuringiensis* (Cry)-Bt field maize was released in 1998 to the southeastern United States to primarily target the European corn borer, *Ostrina nubilalis* (Hübner) (Lepidoptera: Crambidae) but was also found to control corn earworm and fall armyworm to varying degrees (Bowen et al. 2014). Maize hybrids with crystalline (Cry)-Bt express delta-endotoxins in a parasporal crystal systemically throughout the plant (Chakroun et al. 2016), although they are less concentrated in the reproductive structures of the maize plant such as silks and kernels (Estruch et al. 1996). The endotoxins are retained within the crystalline structure until ingested and denatured by the digestive enzymes of the larvae which release the active endotoxins (Chakroun et al. 2016). Since the release of Cry-Bt maize, its use has steadily increased. In 2017, 82% of maize grown in the United States contained at least one Cry-Bt transgene (U.S. Department of Agriculture – Economic Research Service [USDA-ERS] 2018).

In 2011, a newer homology of Bt endotoxin became commercially available (Hodgson and Gassmann 2010). The vegetative insecticidal protein (Vip)-Bt transgene is incorporated into the maize genome with selected Cry-Bt transgenes that express increased toxicity to a broader range of lepidopteran pests than maize with only Cry-Bt transgenes (Palma et al. 2012). Unlike Cry-Bt endotoxins, Vip-Bt endotoxins are secreted by the bacterial cell and do not require sporulation (Ruiz de Escudero et al. 2014). Vip-Bt endotoxins are expressed in relatively higher levels in reproductive structures (Estruch et al. 1996). Ear-feeding lepidopteran larvae such as the corn earworm tend to enter the ear through the silk channel (Deer 1952) and may be exposed to higher concentrations of Vip-Bt endotoxins than hybrids incorporating only the Cry-Bt transgenes. In concept, the higher mortality of early instar larvae caused by combining Cry/Vip-Bt toxins may reduce ear injury sufficiently to also negatively affect *A. flavus* infection.

The general association of ear injury and aflatoxin experiences a gap in integrated research involving yield, ear injury, and aflatoxin of maize hybrids containing these Bt transgenes. Comparisons of Cry-Bt and Cry/Vip-Bt hybrids of similar background genetics are uncommon to date. It is well documented that there is variation in yield, lepidopteran insect damage, *A. flavus* infection, and aflatoxin accumulation due to maize genetic background (Betrán et al. 2005, Wahl et al. 2016). Reducing variation attributed to genetic background can be achieved by comparing near-isogenic hybrids that differ in Bt transgenes (Barros et al. 2010). Hybrids not adapted to a region in which they are planted may result in greater variation in yield and susceptibility to insect ear-feeding and *A. flavus* infection (Betrán et al. 2005, Williams et al. 2005). This issue has become more challenging in the southern United States as most maize breeding is done in the midwestern United States which tends to select for traits that exacerbate aflatoxin (Farfan et al. 2013, Murray et al. 2019). Last, the relationship between Bt maize, ear injury, yield, and aflatoxin are confounded across experiments conducted in different environments (Dowd et al. 2005, Abbas et al. 2007). Typically, environmental conditions that favor aflatoxin production include water-stress, high temperatures, high humidity, and sizeable lepidopteran insect populations (Payne and Widstrom 1992, Buntin et al. 2004, Abbas et al. 2007). Environmental conditions common to sub-tropical regions of the southern United States, which includes our study areas, provide these environmental stress conditions more consistently compared to more temperate growing regions.

The objectives of this study were to describe the association of fall armyworm and corn earworm ear injury with yield and aflatoxin levels as mediated by Cry/Vip-Bt and Cry-Bt endotoxins in adapted maize hybrids grown in areas varying in environmental stress. Our main intentions were to explore the relationships among ear injury, yield, and aflatoxin of ears in maize hybrids with and without Bt transgenes and clarify whether Bt transgenes affect these associations.

Materials and Methods

Locations and Agronomics

Multiple adapted maize hybrid families were examined for yield, ear injury caused by the fall armyworm and corn earworm, and *A. flavus*-derived aflatoxin in maize ears at two locations (Corpus Christi and College Station, TX) over 4 yr (2014–2017) in replicated small plot experiments. Individual plot sizes varied between 4.6 and 6.1 m in length, two to six rows, and 76–96 cm between row centers. Hybrids were selected based on the best adaptation to southern and central Texas. Hybrids were assigned to a family of one to four members that had similar or near-isogenic backgrounds that varied in pyramided configurations of Bt transgenes including non-Bt versions (Table 1). All seed were treated with seed protectants by the seed suppliers for pre-emergence pest and disease control. Plots were machine-seeded with a research cone-planter targeting a density of 74,000 plants/ha (Corpus Christi) and between 60,000 and 85,000 plants/ha (College Station). Plots were planted following cotton and maize from the past season. Planting in Corpus Christi occurred in early April, and between late March and late April in College Station, which was considered late for these regions. The late plantings increased the likelihood of high temperatures and humidity during the flowering period of the maize life cycle and fall armyworm and corn earworm ear-feeding.

Glyphosate was sprayed post-emergence to control weeds when only glyphosate-tolerant hybrids were in the experiment (experiments 5–7). Preemergent, mechanical, and individual plant weed control was used in all other experiments because glyphosate-tolerant hybrids used for in these experiments were planted alongside selected developmental hybrids without herbicide resistance. Hybrids were grown under dryland conditions, except when irrigation was applied as a separate treatment when drought conditions provided opportunity to contrast two levels of water-stress by using supplemental irrigation. To assure a positive aflatoxin test environment, plots were inoculated with toxigenic *A. flavus* NRRL 3357 by placing approximately 50 g of inoculated maize kernels between rows (Isakeit et al. 2010), except in experiment 7, where no inoculation was performed and *A. flavus* was limited to that present in the environment.

Environmental Conditions

The Corpus Christi experiments (Table 2) were conducted in a humid sub-tropical portion of the Texas Gulf Coast region from 2014 to 2017. Corpus Christi experienced mild winters, with average high and low ambient temperatures in January at 18.3°C and 7.2°C, respectively. This type of climate is conducive to *A. flavus* persistence in the soil (Wicklow et al. 1993), and winter survival of fall armyworm and corn earworm (Vickery 1929, Harding 1976). Summers were hot and humid, with June through August average high temperatures ranging between 32.2 and 36.1°C. The average relative humidity during the maize-flowering cycle during our experiments (23 May–9 June) ranged from 76 to 84% (National Oceanic and Atmospheric Administration [NOAA] 2017). Water-stress conditions were common most years in Corpus Christi, with only 2015 receiving rainfall at levels seen in companion experiments conducted in College Station. Supplemental irrigation was applied using drip-tube placed in every other row to raise water inputs during maize fertilization and ear-filling growth stages. A smaller amount of irrigation

Table 1. Hybrid family composition, data sets used in analysis, and Bt transgene identification guide for all hybrids planted in Corpus Christi and College Station, TX, 2014–2017

Hybrid family	Family members [a]	Data sets [b]	Bt transgenes targeting Lepidoptera
1	1	A, B, C	N/A
2	1	A, B, C	N/A
3	1	A, B, C	*Cry1A.105*, *Cry2Ab2*
4	1	A, B, C	*Cry1A.105*, *Cry2Ab2*
5	1	A, B, C	N/A
6	1	A, B, C	N/A
7	1	A, B, C	*Cry1A.105*, *Cry2Ab2*
	2	A, B, C	*Cry1A.105*, *Cry2Ab2*
8	1	A, B, C	*Cry1A.105*, *Cry2Ab2*, *Cry1F*
	2	A, B, C	*Cry1A.105*, *Cry2Ab2*
9	1	A, B, C, D, E, F	N/A
	2	A, B, C, D, E, F	*Cry1A.105*, *Cry2Ab2*
10	1	A, B, C, D, E, F	*Cry1A.105*, *Cry2Ab2*, *Cry1F*
	2	A, B, C, D, E, F	N/A
11	1	A, B, C, D, E, F	N/A
	2	A, B, C, D, E, F	*Cry1A.105*, *Cry2Ab2*
12	1	A, B, C, D, E, F	*Cry1Ab*, *Vip3Aa20*
	2	A, B, C, D, E, F	N/A
	3	A, B, C, D, E, F	*Cry1Ab*
13	1	A, B, C, D, E, F	N/A
	2	A, B, C, D, E, F	*Cry1Ab*, *Cry1F*
	3	A, B, C, D, E, F	*Cry1Ab*, *Cry1F*, *Vip3Aa20*
14	1	A, B, C, D, E, F	N/A
	2	A, B, C, D, E, F	*Cry1A.105*, *Cry2Ab2*, *Cry1F*
	3	A, B, C, D, E, F	*Cry1A.105*, *Cry2Ab2*
	4	A, B, C, D, E, F	*Cry1A.105*, *Cry2Ab2*

[a]Hybrids included in a specific hybrid family with similar or near-isogenic background and varying in Bt transgenes.
[b]Data sets in which the hybrid were included for analyses (see text and Table 2 for structure of data sets that considered variation across locations, hybrid similarity, and water-stress).

Table 2. Locations and experimental conditions of field experiments to compare the association of insect-derived ear injury with yield and aflatoxin of maize hybrids varying in Bt transgenes, 2014–2017

Exp	Loc	Hybrid families [a]	Near-isogenic hybrids [b]	Year	Planting date	Total rainfall [c]	Non-stress water app. [d]	Stressed water app. [e]
1	CC	7	3	2014	1 Apr. 2014	0.12	0.033	0.011
2	CC	5	2	2015	4 Apr. 2015	0.27	–	–
3	CC	6	3	2016	6 Apr. 2016	0.17	–	–
4	CC	3	3	2017	1 Apr. 2017	0.15	0.032	0.009
5	CC	5	3	2016	6 Apr. 2016	0.17	–	–
6	CC	4	3	2017	1 Apr. 2017	0.15	0.032	0.010
7	CC	1	1	2014	1 Apr. 2014	0.12	–	0.010
8	CS	7	3	2014	21 Mar. 2014	0.20	–	–
9	CS	5	2	2015	2 Apr. 2015	0.22	–	–
10	CS	6	3	2016	9 Apr. 2016	0.31	–	–
11	CS	3	3	2017	26 Apr. 2017	0.36	–	–

Exp = number label of separate experiments; Loc = experimental location; CC = Corpus Christi, TX; CS, College Station, TX.
[a]Number of hybrid family, each composed of hybrids with similar background genetics; hybrid family members are all from the same source.
[b]Subset of families composed of hybrids with the same parent genetics that differ by Bt transgenes.
[c]Total rainfall accrued over planting season in hectare meters.
[d]Irrigation applied to non-stressed plots in hectare meters.
[e]Irrigation applied to stressed plots in hectare meters.

was applied to the dryland-designated plots to avoid plant wilt and fertility problems (Table 2). The soil composition at the testing sites in Corpus Christi was primarily a mixture of Orelia sandy-clay loam (Brewer et al. 2014).

The College Station experiments (Table 2) were in a temperate portion of central Texas. College Station experienced mild winters with average January high and low temperature of 16.1 and 4.5°C, respectively, 2014–2017. Summers in College Station were hot but less humid than Corpus Christi. June to August average daily high temperature ranged between 32.2 and 36.7°C. The average relative humidity during the maize-flowering cycle (28 May–17 June) in College Station ranged from 70 to 81% (NOAA 2017). Rainfall from 2014 to 2017 was sufficient for maize growth from planting through grain-fill (Table 1). The soil composition was primarily Ships Clay (Kiniry and Bockholt 1998).

Experimental Design and Hybrids

Across all experiments, plots were arranged in a randomized complete block design of the hybrid treatment or a split-plot design when including a water-stress treatment. For experiments 1, 4, and 6, the main plot was the water-stress treatment (two levels: irrigated and dryland), and the hybrids were randomized within the main plot. In all other experiments, the hybrid treatment was the only experimental factor. The treatments were replicated four times for each experiment. There were 26 hybrids used across all experiments partitioned over 14 hybrid families obtained from different sources. Near-isogenic hybrids in six hybrid families included members with Bt transgene configurations paired with a non-Bt member. The other eight families included one or two Bt and non-Bt hybrids with background genetics that expressed similar agronomic and yield characteristics (Table 1). The Cry-Bt only hybrids expressed several Bt proteins in various hybrids from several sources: *Cry1A.105* + *Cry 2Ab2* + *Cry1F* (Monsanto, St. Louis, MO), and *Cry1Ab* + *mCry3A* (Syngenta, Basel, Switzerland). The Cry/Vip-Bt hybrids expressed *Vip3Aa20* + *Cry1Ab* (Monsanto), and *Vip3Aa20* + *Cry1Ab* + *Cry1Fa2* (Syngenta)

Experimental Measurements

To measure ear injury attributed to corn earworm and fall armyworm, a subset of 10–20 ears was evaluated per plot once large larvae (fourth instar or greater) represented more than 90% of the larvae found in ears sampled in border rows. A sample of the larvae ($n > 100$) in each experiment was examined for species identification. Depth of ear injury regardless of species was measured as the length of visual evidence of feeding from all larvae within a single ear, including tip injury when present (Curley 2000). If the depth of ear injury was not continuous, the lengths of ear injury were summed together and reported as one total depth of ear injury. Area of ear injury was recorded as the sum of total area of ear injury (cm^2) throughout a single ear. Between 4.72 and 6.1 m of the row were hand-harvested (experiments 1, 2, and 7) or combine-harvested (experiments 3–6 and 8–11). Hand-harvested ears were threshed using a low-profile plot thresher (Almaco, Nevada, IA). Moisture was measured with a grain analysis computer (DICKEY-john, Auburn, IL).

To quantify the presence of aflatoxin, between 0.4 and 5 kg samples of maize were finely ground and homogenized with a Romer mill (Romer Labs, Union, MO) for experiments 1–4 and 8–10. Aflatoxin concentration (ppb) from a thoroughly mixed subsample was quantified using the VICAM Afla-test fluorometer USDA-FGIS procedure (VICAM, Watertown, MA). In experiments 5, 6, and 7, maize extracts taken from a thoroughly mixed subsample and combined with 70% methanol were cleaned with 0.45 ml nylon syringe filters. Twenty microliters of the cleaned sample were separated on a Waters Nova-pak C18 column in a Dionex Ultimate high-pressure liquid chromatography (HPLC) system with post-column photochemical derivatization (PHRED, Aura Industries, New York, NY) and fluorescence detection (Abbas et al. 2015). Samples were compared with commercially available aflatoxin standards (aflatoxin B1, B2, G1 and G2, Sigma Chemical Company, St. Louis, MO) to estimate concentration, with a limit of quantitation of 0.2 ng/g (Weaver et al. 2017).

Data Analysis

Six data sets used for analyses were derived from the 11 experiments and the three groups that varied in Bt transgenes, which represented an array of environmental conditions and potential measurement variation. The six data sets were 1) all hybrid families in all experiments, 2) hybrid families in College Station, 3) hybrid families in Corpus Christi, 4) hybrid families limited to near-isogenic hybrids in College Station, 5) hybrid families limited to near-isogenic hybrids in Corpus Christi, and 6) hybrid families limited to near-isogenic hybrids in Corpus Christi under water-stressed conditions.

For each of the six data sets, descriptive statistics (means, SEMs, and coefficients of variation [CV = SD divided by the mean * 100]) were calculated for the two ear injury measurements, aflatoxin, and yield for the three Bt groups of all hybrid families. The two ear injury measurements were compared with correlation analysis across all Bt groups and all experiments (Freund and Littell 2000). The correlation analysis, descriptive statistics, and preliminary analysis of covariate regression described below were used to determine if one or both ear injury measurements were needed to interpret ear injury on yield and aflatoxin as influenced by the three Bt groups.

Yield was regressed against the amount of ear injury and included the Bt group factor as a covariate (Freund and Littell 2000). The analysis was repeated for each of the six data sets. The covariate model tested the hypothesis that yield was affected by ear injury (a significant slope) and the three Bt groups affected the relationship (when there was a significant interaction of ear injury and Bt groups). The Bt group factor was treated as a fixed effect. A common yield–ear injury regression was estimated for all Bt groups when no interaction was detected. If a significant ear injury and Bt group interaction was detected ($P \leq 0.05$), the slopes of the yield–ear injury regressions for the Bt groups were compared using indicator variables to test the null hypothesis that the relationship between ear injury and yield was equal across the non-Bt hybrid group, Cry-Bt hybrid group, and Cry/Vip-Bt hybrid group (Neter et al. 1985). Three paired regression comparisons were conducted, each comparing a regression of one Bt group to the aggregate of the other two Bt groups. The statistical criteria of distinguishing slopes using the paired comparisons was set at $\alpha = 0.10$ to aid in identifying differences in the yield–ear injury regressions, as protected by a significant interaction of ear injury and Bt groups ($P \leq 0.05$) (Neter et al. 1985). The individual slope of a Bt group was reported if it differed from the aggregate of the other two across the three paired comparisons. If two or three of the comparisons were significant, the individual slope for each Bt group were reported. In instances where the three paired comparison were not significant at $\alpha = 0.10$, a common regression slope for the three Bt groups was reported, and the interaction detected was more likely representative of non-linear differences. Scatter plots of the data were presented along with regressions for each of the six data sets.

The second series of covariate regressions for the six data sets were performed for aflatoxin concentration (ppb values transformed by the $\log_{10} + 1$) regressed against the amount of ear injury using the same three Bt groups as a covariate. The same procedures and presentations were followed as done for comparing yield to ear injury.

Results

Descriptive Statistics

Ear injury was predominantly caused by the corn earworm which outnumbered the fall armyworm by 4-fold, and fall armyworm injury to ears, whorls, and leaves was minimal. Across 11 experiments conducted at two locations, the non-Bt hybrid group incurred more ear injury measured by depth and area of ear injury than hybrids expressing either Cry-Bt or Cry/Vip-Bt transgenes (Table 3). Ear injury in Cry-Bt and Cry/Vip-Bt hybrids was similar in both locations

Table 3. Mean depth of ear injury, area of ear injury, aflatoxin content, and yield of hybrids grouped by Bt transgene at experimental sites in College Station and Corpus Christi, TX, 2014–2017

Data set [a]	Exp [b]	Bt group [c]	Depth ± SEM [d]	Depth CV [d]	Area ± SEM [e]	Area CV [e]	Aflatoxin ± SEM [f]	Aflatoxin CV [f]	Yield ± SEM [g]	Yield CV [g]
College Station and Corpus Christi (all hybrids)										
		Non-Bt	5.18 ± 0.18	45.64	9.54 ± 0.32	45.01	325.27 ± 39.36	162.78	4,800.71 ± 112.32	31.48
A	1–11	Cry-Bt	3.42 ± 0.10	45.39	5.70 ± 0.21	56.54	173.32 ± 13.16	118.13	7,024.94 ± 161.17	35.76
		Cry/Vip-Bt	0.11 ± 0.02	196.21	0.12 ± 0.03	207.83	66.54 ± 12.47	166.61	7,405.48 ± 311.54	37.39
College Station (all hybrids)										
		Non-Bt	4.44 ± 0.33	55.04	8.01 ± 0.65	60.18	71.69 ± 14.34	148.30	10,029.47 ± 241.14	17.83
B	8–11	Cry-Bt	3.86 ± 0.26	54.36	6.67 ± 0.59	70.64	155.78 ± 25.01	128.43	10,290.53 ± 198.86	15.46
		Cry/Vip-Bt	0.22 ± 0.07	148.45	0.28 ± 0.08	143.90	62.44 ± 17.68	141.61	10,780.30 ± 306.15	14.20
Corpus Christi (all hybrids)										
		Non-Bt	5.50 ± 0.20	41.10	10.21 ± 0.35	37.93	435.97 ± 53.32	137.28	4,434.72 ± 134.80	34.12
C	1–7	Cry-Bt	3.27 ± 0.10	39.09	5.36 ± 0.18	45.04	179.61 ± 15.47	115.27	5,774.07 ± 108.26	25.08
		Cry/Vip-Bt	0.06 ± 0.02	194.27	0.06 ± 0.02	211.05	68.44 ± 16.40	176.08	5,843.07 ± 209.22	26.31
College Station (near-isogenic hybrids only)										
		Non-Bt	4.87 ± 0.39	52.56	8.80 ± 0.77	57.32	83.37 ± 17.17	135.08	9,970.48 ± 286.20	18.82
D	8–11	Cry-Bt	4.05 ± 0.32	47.89	8.29 ± 0.92	66.28	125.81 ± 32.75	156.20	9,876.01 ± 266.43	16.19
		Cry/Vip-Bt	0.22 ± 0.07	148.45	0.28 ± 0.08	143.90	62.44 ± 17.68	141.61	10,780.30 ± 306.15	14.20
Corpus Christi (near-isogenic hybrids only)										
		Non-Bt	5.62 ± 0.23	41.96	10.33 ± 0.40	39.60	478.60 ± 62.47	133.75	4,360.40 ± 153.06	35.97
E	1–7	Cry-Bt	3.41 ± 0.12	36.81	5.77 ± 0.23	42.58	172.51 ± 18.66	115.19	5,734.81 ± 127.71	23.88
		Cry/Vip-Bt	0.06 ± 0.02	194.27	0.06 ± 0.02	211.05	68.45 ± 16.40	176.08	5,843.07 ± 209.22	26.31
Corpus Christi (near-isogenic hybrids only) (water-stressed plots only)										
		Non-Bt	6.02 ± 0.43	45.83	10.86 ± 0.70	42.01	557.50 ± 115.38	135.71	3,807.59 ± 246.61	42.47
F	1, 4, and 7	Cry-Bt	3.65 ± 0.19	35.56	6.20 ± 0.37	41.23	207.86 ± 33.60	112.00	5,124.33 ± 99.80	13.49
		Cry/Vip-Bt	0.02 ± 0.02	375.35	0.01 ± 0.01	354.71	111.91 ± 41.16	161.32	5,436.52 ± 317.46	25.45

[a]Data set—grouping of experimental sites by location, Bt group, and water-stress for analysis (A = College Station and Corpus Christi, B = College Station, C = Corpus Christi, D = College Station near-isogenic hybrids only, E = Corpus Christi near-isogenic hybrids only, F = Corpus Christi near-isogenic hybrids only under water-stressed conditions).

[b]Experiment numbers included in a specific data set. See Table 1 for experimental conditions.

[c]Bt group—hybrid group based on transgenic traits of the hybrid.

[d]Depth (cm) ± SEM of ear injury caused by fall armyworm and corn earworm, followed by the coefficient of variation of the measure.

[e]Area (cm^2) ± SEM of ear injury caused by fall armyworm and corn earworm, followed by the coefficient of variation of the measure.

[f]Aflatoxin ± SEM in parts per billion (ppb), followed by the coefficient of variation of the measure.

[g]Yield ± SEM in kilograms per hectare (kg/ha), followed by the coefficient of variation of the measure.

(Table 3). Aflatoxin was on average higher in Corpus Christi than in College Station, particularly for the non-Bt hybrid group (Table 3). Data subsets of near-isogenic hybrids only or near-isogenic hybrids only in water-stress conditions (data sets D, E, and F) did not appear to further reduce the variability of these measurements by inspecting the CVs (Table 3).

The two ear injury measurements were significantly correlated, and the association was positive, aggregating data across all experiments and hybrids (r = 0.62, n = 502, $P \leq 0.01$). CVs were also similar for the two measurements (Table 3). Review of the covariate analyses using yield regressed on both ear injury measurements indicated the depth of penetration produced marginally more significant regressions and regression line comparisons. Therefore, the depth of penetration was the ear injury measurement reported for the covariate analyses of the six data sets.

Association of Ear Injury to Yield

The interactions of ear injury and Bt group factor were significant ($P \leq 0.05$) for each of the six data sets. Proceeding with the paired comparisons of the regression lines across the three Bt groups at α = 0.10 revealed selective differences that varied modestly when inspecting results from the six data sets. All slope estimates calculated were negative when $R^2 > 0.05$ (except for the Cry/Vip-Bt group which had very low ear injury), indicating that in general yield declined as ear injury increased. With very low ear injury, the relevance of the Cry/Vip-Bt hybrid group in a regression analysis framework was relatively minor compared to the other two Bt groups. It more reasonably reflected the variation in yield potential of the hybrids, extended the ear injury ranges to zero or near-zero.

For the combined Corpus Christi and College Station data sets, paired comparisons of non-Bt, Cry-Bt, and Cry/Vip-Bt yield–ear injury regression slopes differed significantly using indicator variables; therefore, the three regression line coefficients were estimated separately (Table 4, Fig. 1A). Very low ear injury was detected for the Cry/Vip-Bt hybrid group, resulting in a clear distinction of the yield–ear injury regression from the regressions of the other two hybrid groups (P = 0.0038) and poor fit of the data to the Cry/Vip-Bt regression line (R^2 = 0.067). The fit of the other two regression lines was also weak (R^2 = 0.092) ($P < 0.01$), likely due to major differences in mean yields between College Station and Corpus Christi (Table 4, Fig. 1A).

Decomposing the results by location, the College Station yield–ear injury regression line for the non-Bt group differed significantly from the common regression for the two Bt groups (Table 4, Fig. 1B). The non-Bt group experienced a sharper decline in yield as ear injury increased when compared to the other Bt groups (R^2 = 0.23) (Table 4, Fig. 1B). In Corpus Christi, the non-Bt

Table 4. Yield by depth of ear injury regression comparisons using Bt group as a covariate, and relevant regression equations, conducted for six data sets derived from 11 experiments representing an array of experimental conditions, 2014–2017

Bt group	Regression comparison [a]	Regression line [a]	R^2
A: College Station and Corpus Christi all hybrids [b]			
Non-Bt	t = −1.81; df = 1, 498; P = 0.072	y = −192.79x + 5,814.1	0.092
Cry-Bt	t = 4.18; df = 1, 498; P < 0.0001	y = 63.23x + 6,808.6	<0.01
Cry/Vip-Bt	t = 2.91; df = 1, 498; P = 0.0038	y = 3,292.73x + 7,037.7	0.067
B: College Station all hybrids [b]			
Non-Bt	t = −1.67; df = 1, 140; P = 0.097	y = −356.0x + 11,610	0.23
Cry-Bt	t = 0.60; df = 1, 140; P = 0.547	–	–
Vip-Bt	t = −0.12; df = 1, 140; P = 0.906	–	–
Common (Cry-Bt and Cry/Vip-Bt)		y = −171.97x + 10,916	0.069
C: Corpus Christi all hybrids [b]			
Non-Bt	t = −1.48; df = 1, 354; P = 0.139	–	–
Cry-Bt	t = 0.34; df = 1, 354; P = 0.737	–	–
Cry/Vip-Bt	t = 0.19; df = 1, 354; P = 0.849	–	–
Common (all)		y = −255.8x + 6,234.9	0.14
D: College Station near-isogenic hybrids only [b]			
Non-Bt	t = −1.32; df = 1, 100; P = 0.188	–	–
Cry-Bt	t = 0.9; df = 1, 100; P = 0.370	–	–
Cry/Vip-Bt	t = 0.13; df = 1, 100; P = 0.899	–	–
Common (all)	–	y = −240.2x + 10,965	0.14
E: Corpus Christi near-isogenic hybrids only [b]			
Non-Bt	t = −1.68; df = 1, 269; P = 0.093	y = −231.7x + 5,684.3	0.12
Cry-Bt	t = 0.27; df = 1, 269; P = 0.790	–	–
Cry/Vip-Bt	t = 0.20; df = 1, 269; P = 0.844	–	–
Common (Cry-Bt and Cry/Vip-Bt)		y = −257.8x + 6,166.0	0.014
F: Corpus Christi near-isogenic hybrids only water-stressed only [b]			
Non-Bt	t = −0.55; df = 1, 105; P = 0.586	–	–
Cry-Bt	t = 0.56; df = 1, 105; P = 0.579	–	–
Cry/Vip-Bt	t = 0.38; df = 1, 105; P = 0.701	–	–
Common (all)		y = −225.8x + 5,575.4	0.21

Yield measured in kilograms per hectare (kg/ha); depth of ear injury measured in centimeter.

[a]If a significant ear injury and Bt group interaction was detected, regression coefficients (slopes of the yield–ear injury regressions for each hybrid group) were compared using indicator variables to test the null hypothesis that the relationship between ear injury and yield was equal across the non-Bt, Cry-Bt, and Cry/Vip-Bt hybrids (regression comparison). Separate regressions by hybrid group and common lines across hybrid groups (either all groups or pairs of groups as listed) provided based on results of hypothesis tests (regression lines).

[b]Data sets correspond to experimental conditions. See Tables 2 and 3 for full details.

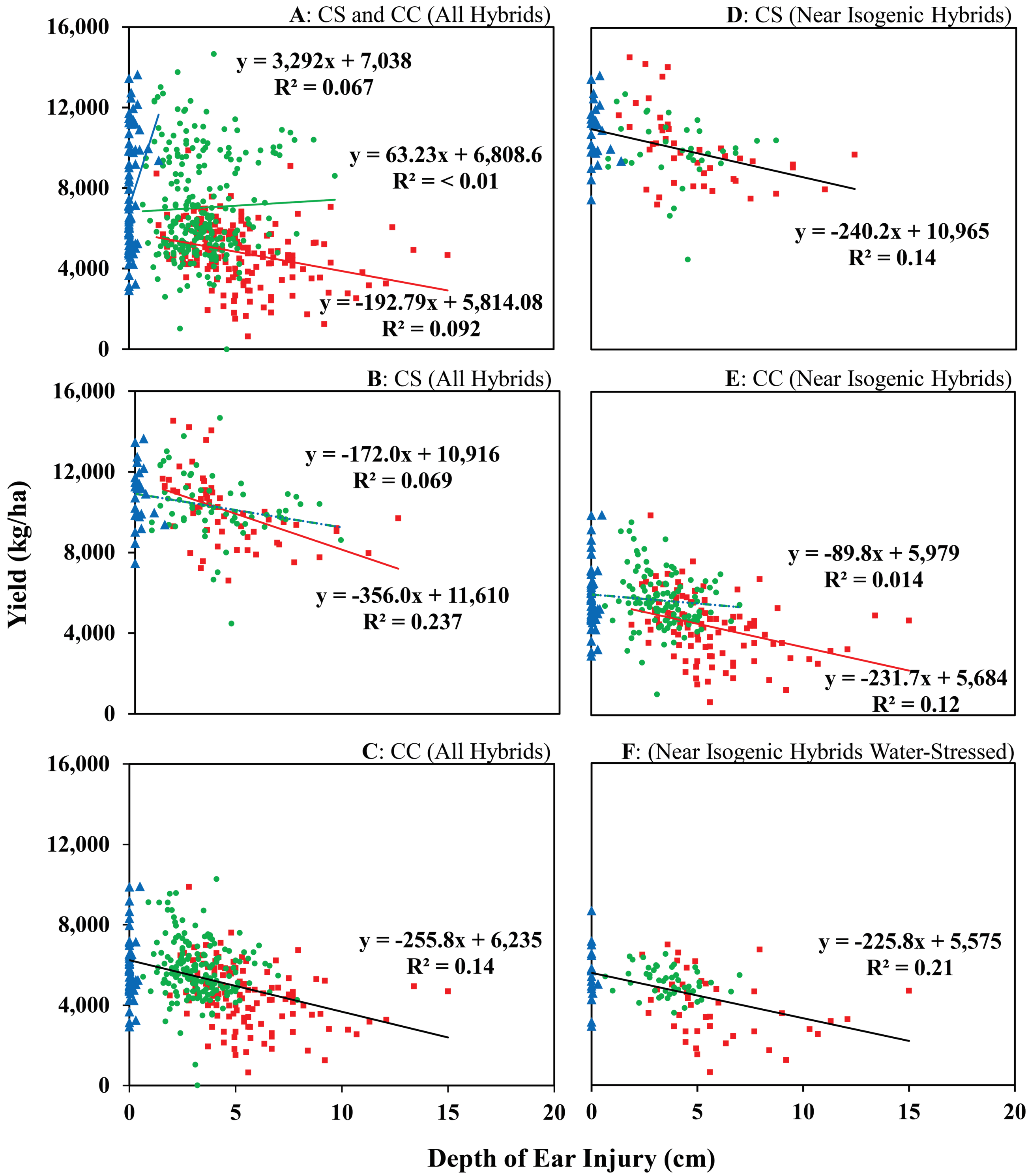

Fig. 1. Yield (kg/ha) by the depth of ear injury (cm) regressions using Bt groups as a covariate. Regressions presented separately for (A) all hybrids tested in Corpus Christi and College Station, (B) all hybrids tested in College Station, (C) all hybrids tested in Corpus Christi, (D) near-isogenic hybrids only in College Station, (E) near-isogenic hybrids only in Corpus Christi, and (F) near-isogenic hybrids only in Corpus Christi under water-stressed conditions only. Solid light-shaded (blue online) triangles and lines represent data and regressions from Cry/Vip-Bt hybrids. Solid medium-shaded (green online) and lines represent data and regressions from Cry-Bt hybrids, and solid dark-shaded (red online) squares and lines represent data and regressions from non-Bt hybrids. Based on regression line comparison results (Table 4), common regression for all hybrid groups are represented by a solid black line, and a common regression for Cry-Bt and Cry/Vip-Bt groups are represented by a green-blue dash-dotted line.

group yielded on average 1,000 kg/ha less than the two Bt groups (Table 3). However, the slopes of thee three hybrid groups did not differ significantly in the paired comparisons using indicator variables ($\alpha = 0.10$); therefore, one common regression line for all Bt groups was estimated. The downward slope of this regression line appeared to reflect the yield depression experienced with the

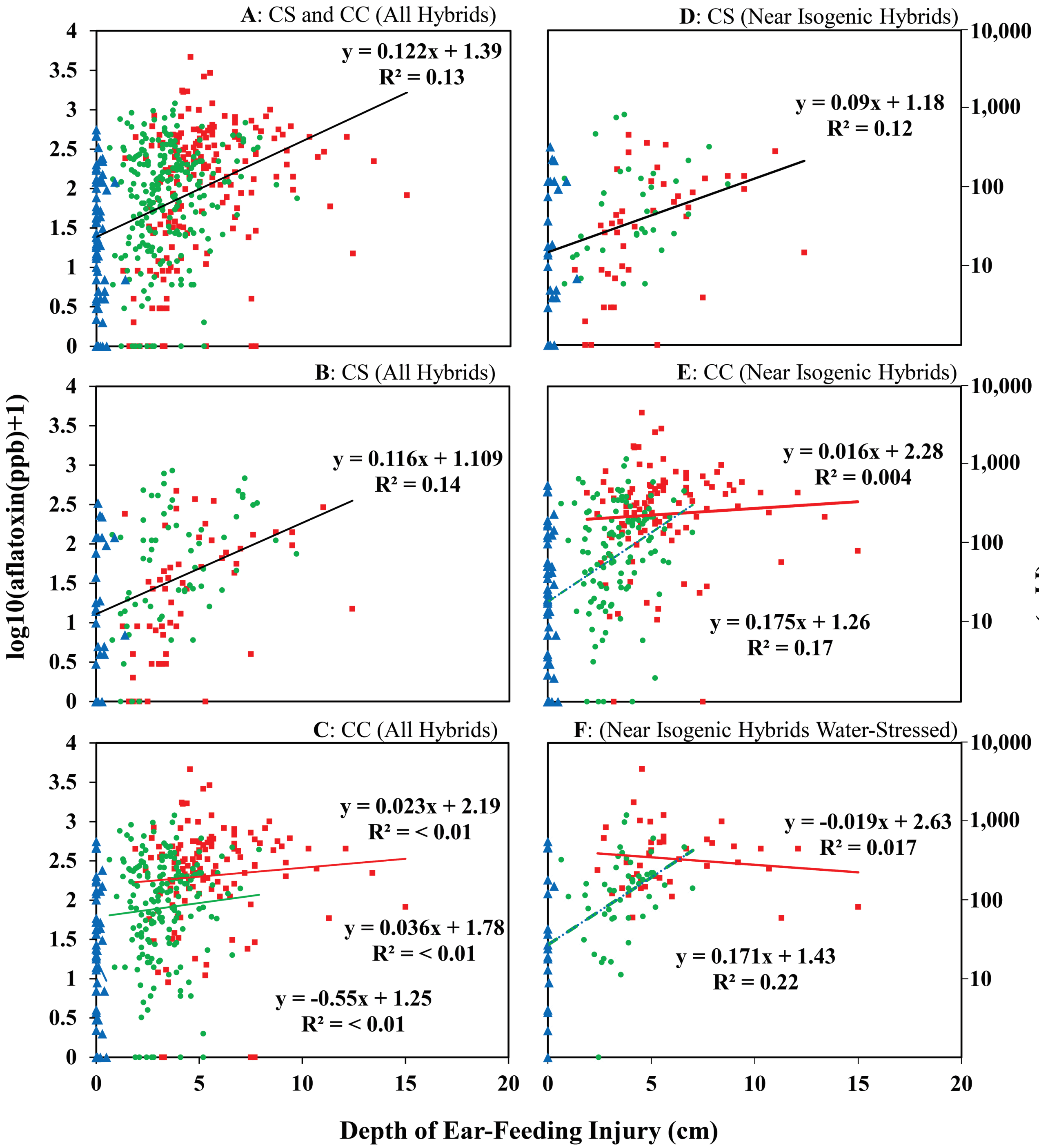

Fig. 2. Aflatoxin (ppb, data $\log_{10}$ +1 transformed) by the depth of ear injury using linear regression. Regressions presented separately for (A) all hybrids tested in Corpus Christi and College Station, (B) all hybrids tested in College Station, (C) all hybrids tested in Corpus Christi, (D) near-isogenic hybrids only in College Station, (E) near-isogenic hybrids only in Corpus Christi, and (F) near-isogenic hybrids only in Corpus Christi under water-stressed conditions only. Solid light-shaded (blue online) triangles and lines represent data and regressions from Cry/Vip-Bt hybrids. Solid medium-shaded (green online) and lines represent data and regressions from Cry-Bt hybrids, and solid dark-shaded (red online) squares and lines represent data and regressions from non-Bt hybrids. Based on regression line comparison results (Table 5), a common regression for all hybrid groups are represented by a solid black line, and a common regression for Cry-Bt and Cry/Vip-Bt groups are represented by a green-blue dash-dotted line.

Cry-Bt and non-Bt hybrids as the amount of ear injury increased (R^2 = 0.14) (Table 4, Fig. 1C).

Further breaking down our analysis, we examined yield–ear injury regressions by Bt group across near-isogenic subsets of hybrid families to minimize confounding effects caused by hybrid genetics. In College Station, the three yield–ear injury regression lines did not differ significantly in the paired comparisons; therefore, a common regression line was estimated (R^2 = 0.14) (Table 4, Fig. 1D). Analyzing the near-isogenic subset of hybrid families, we found significant differences between the yield–ear injury regressions of non-Bt and the two Bt groups in Corpus Christi (α = 0.10). The non-Bt group had a lower intercept possibly causing a more gradual

Table 5. Aflatoxin by depth of ear injury regression comparisons using Bt group as a covariate, and relevant regression equations, conducted for six data sets derived from 11 experiments

Bt group	Regression comparison [a]	Regression line [a]	R^2
A: College Station and Corpus Christi all hybrids [b]			
Non-Bt	$t = -1.21$; df = 1, 498; $P = 0.2256$	–	–
Cry-Bt	$t = -1.56$; df = 1, 498; $P = 0.1206$	–	–
Cry/Vip-Bt	$t = 0.14$; df = 1, 498; $P = 0.8868$	–	–
Common (all)	–	$y = 0.122x + 1.39$	0.13
B: College Station all hybrids [b]			
Non-Bt	$t = 0.04$; df = 1, 140; $P = 0.9698$	–	–
Cry-Bt	$t = 1.07$; df = 1, 140; $P = 0.2860$	–	–
Cry/Vip-Bt	$t = 0.14$; df = 1, 140; $P = 0.8883$	–	–
Common (all)	–	$y = 0.116x + 1.11$	0.14
C: Corpus Christi all hybrids [b]			
Non-Bt	$t = -3.03$; df = 1, 354; $P = 0.0026$	$y = 0.023x + 2.19$	< 0.01
Cry-Bt	$t = -2.16$; df = 1, 354; $P = 0.0313$	$y = 0.036x + 1.78$	< 0.01
Cry/Vip-Bt	$t = 0.75$; df = 1, 354; $P = 0.4540$	$y = -0.55x + 1.25$	< 0.01
D: College Station near-isogenic hybrids only [b]			
Non-Bt	$t = 0.29$; df = 1, 100; $P = 0.7754$	–	–
Cry-Bt	$t = 0.02$; df = 1, 100; $P = 0.9863$	–	–
Cry/Vip-Bt	$t = 0.22$; df = 1, 100; $P = 0.8292$	–	–
Common (all)	–	$y = 0.09x + 1.18$	0.12
E: Corpus Christi near-isogenic hybrids only [b]			
Non-Bt	$t = -3.96$; df = 1, 269; $P < 0.0001$	$y = 0.017x + 2.28$	< 0.01
Cry-Bt	$t = 0.51$; df = 1, 269; $P = 0.6124$	–	–
Cry/Vip-Bt	$t = -0.79$; df = 1, 269; $P = 0.4311$	–	–
Common (Cry-Bt and Cry/Vip-Bt)		$y = 0.14x + 1.43$	0.17
F: Corpus Christi near-isogenic hybrids only water-stressed only [b]			
Non-Bt	$t = -4.06$; df = 1, 105; $P < 0.0001$	$y = -0.019x + 2.64$	0.017
Cry-Bt	$t = 0.53$; df = 1, 105; $P = 0.5987$	–	–
Vip-Bt	$t = 1.17$; df = 1, 105; $P = 0.2435$	–	–
Common (Cry-Bt and Cry/Vip-Bt)	–	$y = 0.109x + 1.70$	0.22

Aflatoxin measured in parts per billion (ppb), data $\log_{10}$ transformed; depth of ear injury measured in cm.

[a]If a significant ear injury and Bt group interaction was detected, regression coefficients (slopes of the aflatoxin–ear injury regressions for each hybrid group) were compared using indicator variables to test the null hypothesis that the relationship between ear injury and aflatoxin was equal across the non-Bt, Cry-Bt, and Cry/Vip-Bt hybrids (regression comparison). Separate regressions by hybrid group and common lines across hybrid groups (either all groups or pairs of groups as listed) provided based on results of hypothesis tests (regression lines).

[b]Data sets correspond to experimental sites and conditions. See Tables 2 and 3 for full details.

reduction in yield decline than the two Bt groups, which shared a common regression estimate (Table 4, Fig. 2E). The differences were minor and may reflect the sensitivity of the analysis with the number of hybrids and plots at the Corpus Christi site (Table 4, Fig. 2E).

To further explore the effects of water-limiting conditions on yield–ear injury association, another regression was performed for the same near-isogenic groups in Corpus Christi using hybrids that were exposed to water-stressed conditions. The three yield–ear injury regression lines did not differ significantly in the paired comparisons (Table 4); therefore, one common regression line with good fit was estimated for all three Bt groups ($R^2 = 0.21$) (Table 4, Fig. 2F). The rate of yield decline may have been reduced because of slightly lower yield potential from plots that were not provided supplemental irrigation during flowering (Table 2).

Association of Ear Injury and Aflatoxin Accumulation

The interactions of ear injury and the Bt group were significant ($P \le 0.05$) for each of the six data sets. The comparisons of the regression lines across the three Bt groups revealed selective differences across the six data sets. Across the data sets all slope estimates calculated were positive when $R^2 > 0.05$, indicating a general increase in aflatoxin as ear injury increased.

For the combined data sets of College Station and Corpus Christi, the non-Bt, Cry-Bt, and Cry/Vip-Bt aflatoxin–ear injury regression lines did not differ significantly for each of the three paired comparisons. A common regression line with a positive slope was estimated for all three groups, indicating as ear injury increased aflatoxin tended to increase ($R^2 = 0.13$) (Table 5, Fig. 2A).

Further decomposing the data by location, in College Station, the aflatoxin–ear injury regression lines did not differ significantly among the three Bt groups using indicator variables. The common regression line had a modest fit ($R^2 = 0.14$) showing a tendency of aflatoxin to increase as ear injury increased. College Station rainfall always exceeded 0.20 ha-m across 4 yr of observations (Table 2). In contrast, Corpus Christi experienced relatively low and variable rainfall and high temperatures which were associated with reduced variability in aflatoxin and relatively higher aflatoxin than in College Station (Table 2). Significant differences in regression lines in the three paired comparisons were detected in Corpus Christi. Therefore, the three regression line coefficients were estimated separately. However, all regression lines had a poor fit ($R^2 < 0.01$), likely caused by the variable aflatoxin accumulation of individual hybrids (Table 5, Fig. 2C).

We examined the aflatoxin–ear injury relationship across near-isogenic subsets of hybrid families to minimize confounding effects in hybrid genetics. Focusing on College Station data, aflatoxin–ear injury regression lines among the three Bt groups did not differ significantly from one another ($\alpha = 0.10$) (Table 5); therefore, a common regression line was estimated (Table 5, Fig. 2D). Again, the inability to detect differences among Bt groups may be due to the high variability of aflatoxin occurrence in the College Station environment. In contrast, analysis in Corpus Christi using the near-isogenic subset of hybrid families, significant differences between the aflatoxin levels of non-Bt and the two Bt groups were detected ($\alpha = 0.10$) (Table 5). Aflatoxin contamination of non-Bt hybrids was variable in the high-stress environment of Corpus Christi, and the data fit was poor ($R^2 < 0.01$) (Table 5, Fig. 2E). The common regression line for the two Bt groups had an improved fit and followed the trend of increased aflatoxin among hybrids with increased ear injury ($R^2 = 0.17$) (Table 5, Fig. 2E).

In order to further explore the effects of water-limiting conditions on the aflatoxin–ear injury association, another regression analysis was performed for the same near-isogenic groups in Corpus Christi that were exposed to water-stressed conditions. The common regression line estimates for the two Bt groups showed a better fit and a slightly less positive relationship between ear injury and aflatoxin accumulation in water-stress plots (slope = 0.171, $R^2 = 0.22$, Fig. 2F) as compared to the same hybrids in non-water-stressed conditions (slope = 0.175, $R^2 = 0.17$, Fig. 2E). Under water-stressed conditions, the mean aflatoxin levels of non-Bt hybrids was slightly higher (Fig. 2F) when compared to non-stressed plots (Fig. 2E), with highly variable aflatoxin concentration which reflected the flat slopes and poor fit to the regression line ($R^2 = 0.017$) (Fig. 2F). Water-stress affected ear injury, aflatoxin accumulation, and yield. Comparing hybrids in Corpus Christi planted in non-water-stressed plots (dataset E) to water-stressed plots (dataset F), there was a 7% increase in ear injury, a 18% increase in aflatoxin accumulation, and a 10% decrease in yield averaging across all Bt groups (Table 2).

Discussion

The literature describing the association between Bt transgenes and aflatoxin reduction showed mixed findings. Multiple studies in the southern United States reported reductions in aflatoxin concentrations among hybrids with Cry-Bt transgenes compared to their non-Bt counterparts (Benedict et al. 1998, Windham et al. 1999, Williams et al. 2004, Wiatrak et al. 2005, Accinelli et al. 2008, Abbas et al. 2013), while others reported mixed findings of aflatoxin associated with Cry-Bt hybrids (Williams et al. 2002, Bruns and Abbas 2006, Williams et al. 2010, Bowen et al. 2014). The high variation in aflatoxin accumulation associated with ear injury among these studies may have reflected the environmental conditions where and when the experiments were conducted. It may also reflect that only non-Bt and Cry-Bt transgenes were used for comparison which did not offer as wide of range of ear injury as if Cry/Vip-Bt transgenes were included. Across the six data sets derived from the 11 experiments of our study, the Cry/Vip-Bt hybrids had negligible ear injury and relatively low aflatoxin accumulation on average than the non-Bt hybrids (Table 3, Fig. 2).

Ear injury by insects can contribute to yield losses (Cox et al. 2009) and aflatoxin accumulation in maize (Xinzhi et al. 2011). However, results are highly variable due to environmental conditions and to a lesser extent hybrid genetics (Guo et al. 2005, Abbas et al. 2007, Reay-Jones and Reisig 2014). Establishing wider ranges of variation in ear injury using Bt transgenes, and wider ranges of water-stress by using supplemental irrigation allowed us to measure how variation among environmental conditions common to sub-tropical regions of Texas affect yield, ear injury, and aflatoxin accumulation. In College Station where yield was high and aflatoxin occurrence was low, Bt groups had no detectable effects on yield loss and aflatoxin accumulation (Tables 4 and 5, Figs. 1 and 2). The effects of ear injury on yield and aflatoxin were more prominent and consistent in Corpus Christi where hybrids experienced more water-limiting conditions and heat stress (Tables 4 and 5, Figs 1 and 2). The trend of increased aflatoxin among hybrids with increased ear injury was further resolved when looking at Cry-Bt and Cry/Vip-Bt isogenic hybrids in Corpus Christi. Our study provided an example of how wide range of environmental stress and diverse maize genetics can affect yield and aflatoxin accumulation in maize, and allows for better detection of the interactions that occur between hybrid genetics and screening environment.

The results here clarified trends that merit more detailed research. Across all six data sets, the Cry and Cry/Vip-Bt hybrid groups had the lowest average amounts of ear injury and aflatoxin accumulation, and the highest yields (Table 3). In College Station, the association of ear injury to aflatoxin accumulation was subtle, likely due to increased variation in aflatoxin occurrence caused by low environmental stress (Xinzhi et al. 2011) (Tables 3 and 5, Fig. 2B and D). These findings support that aflatoxin concentrations are highly variable but on average higher in stressful environments (Abbas et al. 2007, Dolezal et al. 2014), such as seen in Corpus Christi (Table 5, Fig. 2). The results suggest maize hybrids with the inclusion of Cry-Bt and Cry/Vip-Bt transgenes should be further investigated as part of an integrated approach to insect and aflatoxin management in sub-tropical rain-fed maize production regions. Research outcomes may be improved screening hybrids in areas prone to heat and water-limiting stress and by making comparisons among maize hybrids with related genetic background.

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References Cited

Abbas, H. K., T. Shier, and R. D. Cart. 2007. Effect of temperature, rainfall and planting date on aflatoxin and fumonisin contamination in commercial Bt and non-Bt corn hybrids in Arkansas. Phytoprotection 88: 41–50.

Abbas, H. K., J. R. Wilkinson, R. M. Zablotowicz, C. Accinelli, C. A. Abel, H. A. Bruns, and M. A. Weaver. 2009. Ecology of *Aspergillus flavus*, regulation of aflatoxin production, and management strategies to reduce aflatoxin contamination of corn. Toxin Rev. 28: 142–153.

Abbas, H. K., R. M. Zablotowicz, M. A. Weaver, W. T. Shier, H. A. Bruns, N. Bellaloui, C. Accinelli, and C. A. Abel. 2013. Implications of Bt traits on mycotoxin contamination in maize: overview and recent experimental results in the southern United States. Amer. Chem. Soc. 61: 11759–11770.

Abbas, H. K., R. M. Zablotowicz, W. T. Shier, B. J. Johnson, N. A. Phillips, M. A. Weaver, C. A. Abel, H. A. Bruns. 2015. Aflatoxin and fumonisin in corn (*Zea mays*) infected by common smut *Ustilago maydis*. Plant Dis. 99: 1236 - 1240.

Accinelli, C., H. K. Abbas, R. M. Zablotowicz, and J. R. Wilkinson. 2008. *Aspergillus flavus*, aflatoxin occurrence and expression of aflatoxin biosynthesis genes in soil. Can. J. Microbiol. 54: 371–379.

Barros, E., S. Lezar, M. J. Anttonen, J. P. Van Dijk, R. M. Rohlig, E. J. Kok, and K. H. Engel. 2010. Comparison of two GM maize varieties with a near-isogenic non-GM variety using transcriptomics, proteomics, and metabolomics. J. Plant Biotech. 8: 436–451.

Benedict, J., D. Fromme, J. Cosper, C. Correa, G. N. Odvody, and R. D. Parker. 1998. Efficacy of Bt corn events MON810, Bt11, and E176 in controlling corn earworm, fall armyworm, sugarcane borer and aflatoxin. Texas A&M University System, College Station, TX. (http://lubbock.tamu.edu/ipm/AgWeb/r_and_d/1998/Roy%20Parker/Bt%20Corn/BtCorn.html) (accessed 10 July 2018).

Betrán, J., T. Isakeit, G. N. Odvody, and K. Mayfield. 2005. Breeding corn to reduce preharvest aflatoxin contamination, pp. 353–379. *In* H. K. Abbas (ed.) Aflatoxin and food safety. Taylor and Francis, Boca Raton, FL.

Bowen, K. L., K. L. Flanders, A. K. Hagan, and B. Ortiz. 2014. Insect damage, aflatoxin content, and yield of Bt corn in Alabama. J. Econ. Entomol. 107: 1818–1827.

Brewer, M. J., G. N. Odvody, D. J. Anderson, and J.C. Remmers. 2014. Comparison of Bt transgene, hybrid background, water stress, and insect stress effects on corn leaf and ear injury and subsequent yield. Environ. Entomol. 43: 828–839.

Bruns H. A. and Abbas H. K. 2006. Planting date effects on Bt and non-Bt corn in the mid-South USA. Agron. J. 98: 100–106.

Buntin, D. G., K. L. Flanders, and R. E. Lynch. 2004. Assessment of experimental Bt events against fall armyworm and corn earworm in field corn. J. Econ. Entomol. 97: 259–264.

Chakroun, M., N. Banyuls, Y. Bel, B. Escriche, and J. Ferré. 2016. Bacterial vegetative insecticidal proteins (Vip) from entomopathogenic bacteria. Microbiol. Mol. Biol. Rev. 80: 329–350.

Cox, W. J., J. Hanchar, and E. Shields. 2009. Stacked corn hybrids show inconsistent yield and economic responses in New York. Agron. J. 101: 1,530–1,537.

Curley, M. D. 2000. Yield loss of field corn from insects, pp. 61–116. *In* R. K. D. Perterson and L. G. Higley (eds.), Biotic stress and yield loss. CRC, Boca Raton, FL.

Deer, J. A. 1952. Corn earworm control. L-143. Agricultural Ext. Serv, College Station, TX.

Dolezal, A. L., X. Shu, G. R. OBrian, D. M. Nielsen, C. P. Woloshuk, R. S. Boston, and G. A. Payne. 2014. *Aspergillus flavus* infection induces transcriptional and physical changes in developing maize kernels. Front. Microbiol. 384: 1–10.

Dowd, P. F., E. T. Johnson, and W. P. Williams. 2005. Strategies for insect management targeted toward mycotoxin management, pp. 517–541. *In* H. K. Abbas (ed.), Aflatoxin and food safety. Taylor and Francis, Boca Raton, FL.

Estruch, J. J., G. W. Warren, M. A. Mullins, G. J. Nye, J. A. Craig, and M. G. Koziel. 1996. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. Proc. Natl Acad. Sci. 93: 5389–5394.

Farfan, I.D.B., S. C. Murray, D. Pietsch, and S. Labar. 2013. A multi-environment trial analysis of commercial maize shows a slight grain yield improvement in Texas. Field Crops Res. 149:167–176.

Freund, R. J., and R. C. Littell. 2000. SAS system for regression, 3rd ed. SAS Institute, Cary, NC.

Guo, B. Z., N. W. Widstrom, D. R. Lee, D. M. Wilson, and A. E. Coy. 2005. Prevention of preharvest aflatoxin contamination: Integration of crop management and genetics in corn, pp. 437–457. *In* H. K. Abbas (ed.), Aflatoxin and food safety. Taylor and Francis, Boca Raton, FL.

Harding, J. 1976. *Heliothis* spp.: seasonal occurrence, hosts and host importance in the Lower Rio Grande Valley. Environ. Entomol. 4: 666–668.

Hodgson, E. W., and A. J. Gassmann. 2010. New corn trait deregulated in US. Integrated Crop Management News. (https://lib.dr.iastate.edu/cropnews/453/) (accessed 12 October 2018).

Isakeit, T., K. May-Field, S. Murray, R. Minzenmayer, and J. Ripple. 2010. Biological control of aflatoxin contamination of corn in Texas using Afla-Guard, a commercial atoxigenic strain of *Aspergillus flavus*. Can. J. Plant Path. Rev. 32: 407–408.

Kiniry, J. R., and A. J. Bockholt. 1998. Maize and sorghum simulation in diverse Texas environments. Agron. J. 5: 682–687.

Murray, S. C., K. Mayfield, J. Pekar, P. Brown, A. Lorenz, T. Isakeit, G. Odvody, W. Xu, and J. Betrán. 2019. Tx741, Tx777, Tx779, Tx780, and Tx782 inbred maize lines for yield and southern United States stress adaptation. J. Plant Registr. 13: 258–269, doi:10.3198/jpr2017.07.0044crp.

National Oceanic and Atmospheric Administration (NOAA). 2017. Climate data online. (http://www.ncdc.noaa.gov/cdo-web/) (accessed 12 May 2018).

Neter, J., W. Wasserman, and M. H. Kutner. 1985. Applied linear statistical models: regression, analysis of variance, and experimental designs, Chap 10, Qualitative Independent Variables, pp. 349–376, 2nd ed. D. Richard (eds.), Irwin, Homewood, IL.

Palma, L., C. S. Hernández-Rodríguez, M. Maeztu, P. Hernández-Martínez, I. R. de Escudero, B. Escriche, D. Muñoz, J. Van Rie, J. Ferré, and P. Caballero. 2012. Vip3C, a novel class of vegetative insecticidal proteins from *Bacillus thuringiensis*. Applied Environ. Microbiol. 78: 7163–7165.

Payne, G. A., and N. W. Widstrom. 1992. Aflatoxin in maize. Critical Rev. Plant Sci. 10: 423–440.

Reay-Jones, F. P. F., and D. D. Reisig. 2014. Impact of corn earworm injury on yield of transgenic corn producing Bt toxins in the Carolinas. J. Econ. Entomol. 107: 1101–1109.

Ruiz de Escudero, I., N. Banyuls, Y. Bel, M. Maeztu, B. Escriche, D. Muñoz, P. Caballero, and J. Ferré. 2014. A screening of five *Bacillus thuringiensis* Vip3A proteins for their activity against lepidopteran pests. J. Invert. Path. 117: 51–55.

Smith, M. S., and T. J. Riley. 1992. Direct and interactive effects of planting date, irrigation, and corn earworm (Lepidoptera: Noctuidae) damage on aflatoxin production in preharvest field corn. J. Econ. Entomol. 85: 998–1106.

U.S. Department of Agriculture (USDA). 2017. Crop production report 2017. USDA, Beltsville, MD.

U.S. Department of Agriculture – Economic Research Service (USDA-ERS). 2018. Recent trends in GE adoption. USDA, Beltsville, MD. (https://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us/recent-trends-in-ge-adoption.aspx) (accessed 14 June 2018).

U.S. Food and Drug Administration (U.S. FDA). 1994. Section 683.100. Action levels for aflatoxins in animal feeds. Rockville, MD. (www.fda.gov/ora/compliance_ref/cpg/cpgvet/cpg683-100.html) (Accessed April 8, 2019).

Vickery, R. A. 1929. Studies on the fall armyworm in the Gulf Coast District of Texas. Technical Bulletin No. 138. U.S. Department of Agriculture, Washington, D.C.

Wahl, N., S. C. Murray, T. Isakeit, M. Krakowsky, G. L. Windham, W. P. Williams, B. Guo, X. Ni, J. Knoll, W. Xu, *et al*. 2016. Identification of resistance to aflatoxin accumulation and yield potential in maize hybrids in the Southeast Regional Aflatoxin Trials (SERAT). Crop Sci. 57: 202–215.

Weaver, M. A., H. K. Abbas, M. J. Brewer, L. S. Pruter, and N. S. Little. 2017. Integration of biological control and transgenic insect protection for mitigation of mycotoxins in corn. Crop Protect. 98: 108–115.

Wiatrak, P. J., D. L. Wright, J. J. Marois, and D. Wilson. 2005. Influence of planting date on aflatoxin accumulation in Bt, non-Bt, and tropical non-Bt hybrids. Agron. J. 97: 440–445.

Wicklow, D. T., D. M. Wilson, and T. C. Nelsen. 1993. Survival of *Aspergillus flavus* sclerotia and conidia buried in soil in Illinois or Georgia. Phytopathology 83: 1141–1147.

Williams, W. P., G. L. Windham, P. M. Buckley, and C. A. Daves. 2002. Aflatoxin accumulation in conventional and transgenic corn hybrids infested with southwestern corn borer (Lepidoptera: Crambidae), J. Agric. Urban Entomol. 19: 227–236.

Williams, J. H., T. D. Phillips, P. E. Jolly, J. K. Stiles, C. M. Jolly, and D. Aggarwal. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am. J. Clin. Nutr. 80: 1106–1122.

Williams, W. P., G. L. Windham, and P. M. Buckley. 2005. Enhancing maize with resistance to *Aspergillus flavus* infection and aflatoxin accumulation, pp. 395–405. *In* H. K. Abbas (ed.), Aflatoxin and food safety. Taylor and Francis, Boca Raton, FL.

Williams, W. P., G. L. Windham, M. D. Krakowsky, B. T. Scully, and X. Ni. 2010. Aflatoxin accumulation in Bt and non-Bt maize testcrosses. J. Crop Improv. 24: 392–399.

Windham, G. L., W. P. Williams, and F. M. Davis. 1999. Effects of the Southwestern corn borer on *Aspergillus flavus* kernel infection and aflatoxin accumulation in maize hybrids. Plant Dis. 83: 535–540.

Wu, F. 2014. Global impacts of aflatoxin in maize: trade and human health. World Mycotoxin J. 8: 137–142.

Xinzhi, N., J. P. Wilson, D. G. Buntin, B. Guo, M. D. Krakowsky, R. D. Lee, T. E. Cottrell, B. T. Scully, A. Huffaker, and E. A. Schmelz. 2011. Spatial patterns of aflatoxin levels in relation to ear-feeding insect damage in pre-harvest corn. Toxins 3: 920–931.