Insect Damage, Aflatoxin Content, and Yield of Bt Corn in Alabama

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ABSTRACT Isoline pairs of hybrid corn, similar except for presence or absence of a Bt trait, were planted at eight sites across Alabama over three years. This study evaluated insect damage, yield, and aflatoxin levels as affected by the Bt traits, YieldGard Corn Borer (expressing *Cry1Ab*), Herculex I (expressing *Cry1F*), Genuity VT Triple PRO (expressing *Cry1A.105* and *Cry2Ab2*), Agrisure Viptera 3111 (expressing *Vip3Aa20* and *Cry1Ab*), and Genuity SmartStax (expressing *Cry1A.105*, *Cry2Ab2*, and *Cry1F*). When examined over all sites and years, hybrids with any of the included Bt traits had lower insect damage and higher yields. However, insect damage was not consistently correlated to yield. Bt traits expressing multiple proteins provided greater protection from corn earworm feeding than did traits for single proteins. Yields and aflatoxin levels were highly variable among sites although irrigated sites had higher yields than nonirrigated sites. Aflatoxins commonly accumulate in corn in the southeastern United States because of prevailing high temperatures and frequent dry conditions. Aflatoxin levels were not consistently associated with any factors that were evaluated, including Bt traits.

KEY WORDS Bt corn, maize, corn earworm, corn borer, aflatoxin

In recent years, corn hybrids have become available that differentially express insecticidal proteins from *Bacillus thuringiensis* and these are collectively called Bt corn hybrids. The first commercial Bt field corn hybrids for the southeastern United States were released in 1998 (Buntin et al. 2004). These hybrids expressed the *Cry1Ab* protein throughout the aboveground plant and were sold as YieldGard Corn Borer (MON810 event, Monsanto Co., St. Louis, MO) and Agrisure CB/LL (Bt11 event, Syngenta Crop Sciences, RTP, NC) technologies. These hybrids primarily targeted European corn borer, *Ostrinia nubilalis* (Hübner) (Buntin et al. 2004). In the mid-western United States, an area where European corn borer is the primary caterpillar pest of corn, Bt corn use has been associated with area-wide suppression of this insect pest (Hutchison et al. 2010). Economic damage from European corn borer has been reported from the northern tier of Alabama counties (K.L.F., personal communication), but this insect is not the primary caterpillar pest in Alabama.

By 2010, Bt corn hybrids were available that express proteins providing protection against other lepidopteran pests of aboveground corn that are common in the southeastern United States, including fall armyworm (*Spodoptera frugiperda* (J. E. Smith)) and corn earworm (*Helicoverpa zea* (Boddie)) (Buntin and Flanders 2012, Siebert et al. 2012). In addition to *Cry1Ab*, the proteins *Cry1F*, *Cry1A.105*, *Cry2Ab2*, and *Vip3Aa20* are now produced in Bt corn hybrids with technology trait names such as Herculex I (*Cry1F* protein; Dow AgroSciences, Indianapolis, IN, and DuPont Pioneer, Johnson, IA), Genuity VT Double PRO (*Cry1A.105* and *Cry2Ab2* proteins; Monsanto Co.), Agrisure Viptera 3111 (*Vip3Aa20* and *Cry1Ab* proteins; Syngenta), and SmartStax (*Cry1A.105*, *Cry2Ab2*, and *Cry1F* proteins; Dow AgroSciences; Buntin and Flanders 2012).

Corn earworm caterpillars are commonly found in Alabama corn (Flanders et al. 2014), where they feed on the corn whorls, silks, and ear tips including developing kernels. Fall armyworm also feed on the corn whorls, ears, and kernels in the Southeast (Buntin 2008, Siebert et al. 2012). In 1998 and 2001studies, fall armyworm and corn earworm were problematic in late plantings of corn, and Bt hybrids containing *Cry1Ab* protein were found to have 50–70% lower ear damage than did non-Bt hybrids (Buntin et al. 2004). Hybrids with the *Cry1F* protein had greater ear protection from fall armyworm but less protection from corn earworm than *Cry1Ab* hybrids (Buntin 2008). Hybrids expressing the proteins *Cry1A.104* and *Cry2Ab2* or the proteins *Cry1F*, *Cry1A.104*, and *Cry2Ab2* have shown even greater reductions in ear feeding damage from fall armyworm and corn earworm (Siebert et al. 2012). Larvae of the southwestern corn borer (*Diatraea grandiosella* (Dyar)) infest corn in northern Alabama (Flanders et al. 2013) where their greatest impact comes from feeding on the corn whorls and tunneling and girdling the corn stalks and ear shanks (Flanders et al. 2014). Southwestern corn

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Table 1. Corn hybrids used with numbers of site-years and characteristics of each

Hybrid[a]	Family[b]	Bt trait	*Bt* proteins for aboveground pests	Rel. mat.[c]	Drought rating[d]	No. of sites in: 2010	2011	2012
Dyna-Gro D51RR40	DG51	None	None	111	3	0	6	8
Dyna-Gro D51SS40	DG51	Genuity Smartstax[e]	*Cry1A.105, Cry2Ab2, Cry1F*	111	3	0	6	8
NK N77P-3000GT	NK77	Agrisure 3000GT[e]	*Cry1Ab*	114	1	0	5	0
NK N77P-3111	NK77	Agrisure Viptera 3111[e]	*Vip3Aa20, Cry1Ab*	114	1	0	6	0
NK N78S-3000GT	NK78	Agrisure 3000GT[e]	*Cry1Ab*	116	2	0	3	8
NK N78S-3111	NK78	Agrisure Viptera 3111[e]	*Vip3Aa20, Cry1Ab*	116	2	0	3	8
Pioneer 33M53	P33M	None	None	115	3	2	4	8
Pioneer 33M57	P33M	Herculex I	*Cry1F*	115	3	2	4	8
Pioneer 33V14	P33V	None	None	115	3	1	3	8
Pioneer 33V16	P33V	YieldGard corn borer	*Cry1Ab*	115	3	2	3	8
Pioneer 31P40	P31P	None	None	119	4	5	3	8
Pioneer 31P42	P31P	Herculex I	*Cry1F*	119	4	6	3	8
DeKalb DKC 67–86	DK67	None	None	117	5	7	6	8
DeKalb DKC 67–88	DK67	Genuity VT triple PRO[e]	*Cry1A.105, Cry2Ab2*	117	5	8	6	8
Southern States SS 775 RR2	SS	None	None	116	5	5	0	0
Southern States SS 749 VT3 PRO	SS	Genuity VT triple PRO[e]	*Cry1A.105, Cry2Ab2*	115	3	6	0	0

[a] All hybrids in tests were tolerant to glyphosate.
[b] Family designation is arbitrary and used only in this work.
[c] Relative maturity based on company information.
[d] Drought rating on a scale of 1–9, where 1 = excellent and 9 = poor; based on company information.
[e] Hybrid also had Bt event(s) for control of belowground pests (i.e., western and northern corn rootworms). Western corn rootworm has been found in northern Alabama but is only a pest of continuous corn. All of the corn in these tests was in the first year of corn following rotation to other crops so this pest was not an issue. See Buntin and Flanders (2012) for more information on these Bt corn products.

borer can also cause kernel damage, but this is not significant in comparison with that attributed to corn earworm and fall armyworm.

Non-Bt acreage serves as a refuge to reduce the risk of selecting Bt-resistant insect pests. The amount of non-Bt refuge acreage required by the Environmental Protection Agency depends on the type of Bt protein (aboveground or belowground activity) and the number of proteins of each type. For the cotton-producing areas of the United States, Bt corn products that express multiple above-ground proteins can be planted on 80% of a grower's acreage as compared with 50% for products that express single proteins.

In addition to reducing yields, corn ear damage by European corn borer, corn earworm, and fall armyworm have been associated with increased aflatoxin contamination (Lillehoj et al. 1978, Smith and Riley 1992). Aflatoxins are naturally occurring highly carcinogenic compounds produced by the closely related fungi *Aspergillus flavus* and *Aspergillus parasiticus* (hereafter referred to as *A. flavus* or aflatoxigenic fungi). In the southeastern United States, high temperatures and dry conditions favor kernel colonization by aflatoxigenic fungi (Diener and Davis 1977) and subsequent aflatoxin contamination. This is problematic, as aflatoxin contamination is closely regulated by governmental agencies such as the USDA Federal Grain Inspection Service (U.S. Department of Agriculture [USDA] 2009). For example, corn destined for human consumption in the United States will be rejected at buying points if found to contain >20 ppb aflatoxin, while grain exceeding 300 ppb aflatoxin contamination cannot be fed to cattle at feedlots. Therefore, grower profits can be directly and substantially affected by aflatoxin contamination.

Insect damage to corn husks, ears, and kernels provide entry points for the aflatoxin-producing fungi; thus, practices that reduce insect damage, including planting Bt corn hybrids, could reduce aflatoxin contamination in corn. This was observed by Williams et al. (2005) in a study with paired Bt and non-Bt hybrids (isolines) in which the *Cry1Ab* protein was effective in reducing both southwestern corn borer damage and aflatoxin concentrations in the corn grain. Bruns and Abbas (2006) also found, in one of three study years, that Bt hybrids had lower aflatoxin levels compared with non-Bt hybrids. It is not clear, however, if Bruns and Abbas (2006) used genetically similar hybrid isolines with and without Bt genes.

Adapted-Bt corn hybrids are now commercially available for the Southeast with various proteins that are effective against lepidopteran insects that feed on above-ground plant parts. Stakeholders need to know whether the additional cost of a particular Bt corn trait will pay off in increased yield or kernel quality. The value of the Bt hybrid is only realized when there is insect pressure, which varies by year and location. This study was done to compare Bt hybrids with near-isogenic non-Bt hybrids, especially involving Bt traits expressing multiple proteins. In addition to evaluating for reduction of insect damage and yield improvement from the Bt products, we evaluated aflatoxin levels.

Materials and Methods

Corn hybrids were selected based on adaptation to southeastern United States conditions and the availability of near isogenic, commercially available hybrid pairs with and without a Bt trait (Table 1). In the case of the NK hybrids in this study, a single (expressing *Cry1Ab* protein) and multiple (expressing *Vip3Aa20* and *Cry1Ab* proteins) Bt trait comprised the hybrid pairs. For the purpose of presentation and analysis, the near isogenic pairs of cultivars were designated as a 'family' (Table 1).

Corn was planted at several sites across Alabama in 2010, 2011, and 2012 (Table 2). In 2010, trials at Prattville and Belle Mina included two planting dates which provided differential environments relative to crop production and aflatoxin development. At any site in each year, additional hybrids may have been included, but only those hybrids for which data were obtained for 5 or more site-years were retained for analysis. A complete listing of all hybrids tested can be found in Flanders et al. (2011a,b, 2012, 2013).

Planting procedures and agronomic practices differed in specifics from site to site, but did not diverge from normal production practices (e.g., Mask and Mitchell 1988). Experiments were planted in the first year of corn following rotation from other crops the preceding year. Corn cropped behind another row crop typically will not suffer significant corn root worm damage, thereby eliminating this insect as a confounding effect. Generally, corn was planted in a reduced tillage system, e.g., behind a KMC (Tifton, GA) strip-till rig into a killed rye cover crop or into fallowed ground, at recommended seeding rates for rainfed and irrigated sites (Wright et al. 2011). Soil fertility and pH were adjusted at each study site according to the results of a soil fertility assay done by the Auburn University Soil Fertility Laboratory. Irrigated sites received between 224 and 252 kg/ha total N and dryland sites received between 135 and 168 kg/ha N. Planting dates by year and soil type for each study site are listed in Table 2. Weed control was according to the recommendations of the Alabama Cooperative Extension System (Flanders et al. 2014). All hybrids were tolerant to glyphosate (RoundUp) herbicide. At the Brewton, Fairhope, Headland (southern) and Tallassee (central) study sites, the experimental design was a factorial set of treatments arranged in a split plot with corn family as the main plot and isolines (Bt and non-Bt hybrids) as the split plot treatment. A randomized complete block design (RCB) was employed at Belle Mina, Crossville, Winfield (northern), and Prattville (central) study sites. Depending on the study site, the design included four to six replications. Individual plots consisted of either four (subplots in split plot design) or eight (RCB) 9.1-m rows on 0.9- or 1.1-m centers.

To determine lepidopteran feeding damage, 10 ears were arbitrarily collected from each plot at dough stage (GS R4; northern and central sites) or dent to black layer (GS R5 to R6; central and southern sites; Hanway and Ritchie 1984). Each ear was examined, and caterpillar damage to the ear tips and kernels was recorded (centimeter square per ear). Silk feeding damage with or without corresponding feeding on the kernels was often observed; this was rated from 0 to 2 where 0 = no cut silks, 1 = <50% of cut silks, and 2 = >50% of cut silks due to feeding damage.

Plots were harvested on dates listed in Table 2. Grain was harvested from each plot with a combine, plot yield was recorded (reported at 15.5% moisture), then a subsample was removed for aflatoxin assays.

Table 2. Study sites for *Bt* corn hybrids: soil type and irrigation capacity, numbers of pairs of isolines, and planting (PD) and harvest (HD) dates for each study year

Site	Soil type[a]	Irrig.	2010			2011			2012		
			No. hybrid families	PD[b]	HD[c]	No. hybrid families	PD[b]	HD[c]	No. hybrid families	PD[b]	HD[c]
Fairhope	Malbis FSL	No	2	29 Mar.	10 Aug.	5	4 April	4 Aug.	6*[d]	21 Mar.	8 Aug.
Brewton	Benndale SL	Yes	n.i.[e]	n.i.	n.i.	n.i.	n.i.	n.i.	6	21 Mar.	23 Aug.
Headland	Dothan SL	Yes	3	1 April	9 Sept.	5	8 April	29 Aug.	6	28 Mar.	28 Aug.
Tallassee	Independence (Cahaba) LFS	Yes	n.i.	n.i.	n.i.	5*	7 April	23 Aug.	6*	4 April	23 Aug.
Prattville	Lucedale SL	No	3	5 April/12 May	24 Aug./24 Aug.	5	4 April	29 Aug.	6	8 Mar.	31 Aug.
Winfield	Bama-Cuthbert-Shubuta SCL	No	n.i.	n.i.	n.i.	5	9 May	13 Sept.	6	25 April	21 Sept.
Crossville	Wynnville StL	No	3*	3 June	12 Oct.	n.i.	n.i.	n.i.	6	3 April	11 Sept.
Belle Mina	Decatur StL	Yes	3	1 April/12 May	23 Aug./15 Sept.	5	8 April	2 Sept.	6	3 April	29 Aug.

[a] FSL, fine sandy loam; SL, sandy loam; LFS, loamy fine sand; SCL, sandy clay loam; StL, silt loam.
[b] PD, planting date.
[c] HD, harvest date.
[d] Asterisk (*) indicates that no toxin data were available.
[e] n.i., not included. Study not done at site (Brewton) or data not included in current analysis due to lack of data on more than one factor (Tallassee, Winfield, Crossville).

Generally these samples weighed 0.5–1 kg (5–10% of plot yield). Grain samples were ground using a Wiley mill (Swedesboro, NJ), which reduces particle size with rotating cutting blades, using a 6-mm screen. Ten grams of ground corn from each sample was assayed for aflatoxin using the Veratox test (Neogen Corp., Lansing, MI). This ELISA-based assay is valid for 5–50 ppb total aflatoxins; when the assay indicated levels >50 ppb, the extraction was diluted and reassayed. At least 10% of all samples were assayed a second time to confirm aflatoxin content.

Data Analysis. Aflatoxin concentrations (ppb) were transformed using the natural logarithm transformation (ln(ppb+1)) for all analyses. Means of measured variables from each hybrid in each site-year were used in analysis to determine effects due to family, having any Bt trait (± Bt), and effects of specific Bt traits. Preliminary analysis showed that year had a significant ($P < 0.01$) effect on all measured variables, so analyses were separate for each year. Effects on measured variables due to hybrid family, ± Bt, and the interaction of these two (i.e., effects due to specific hybrid) were first analyzed with generalized linear mixed model analysis (PROC GLIMMIX, SAS 9.2 and 9.3) and site, site × family, site × (± Bt), and site × family × (± Bt) as random variables. Effects on measured variables due to specific Bt traits were also done for each year with site and site × (specific Bt trait) as random effects. Spearman's rank correlation coefficients (PROC CORR SPEARMAN, SAS 9.3) were calculated among all measured variables using individual site data ($n \geq 35$) in each study year. The level of statistical significance used was $P \leq 0.10$ for all analyses but lower P values, when applicable, are presented.

Environmental (=site) effects were examined by setting site as a fixed variable in generalized linear mixed model analysis and hybrid as the random variable. Rainfall and irrigation (where applicable) amounts were obtained from AL Mesonet stations and on-site measurements, respectively, for each study site in each year from planting date to harvest. Rainfall and irrigation were summed over cropping duration (planting to harvest date) for total water on the crop. Spearman's rank correlation coefficients were calculated over all sites and years among all measured variables with season-long temperatures and total water ($n = 20$).

Results

Over all sites in this study, season-long temperatures averaged 26.1°C in 2010 compared with 25.3°C and 24.2°C in 2011 and 2012, respectively. Rainfall amounts were generally lowest in 2010 (averaging 34.9 cm across study sites), and were lower in 2011 (averaging 42.3 cm) than in 2012 (47.2 cm). Season-long temperatures across these sites normally average 25°C with 50.3 cm rainfall; thus, 2010 was warmer and 2012 was cooler than normal, and all 3 yr had lower than normal rainfall. However, conditions did differ from site to site within each year; in addition, corn was irrigated at some locations in each year.

Yield. Results for individual cultivars by site in each year have been published (Flanders et al. 2011a,b, 2012, 2013) and show that hybrid yields were inconsistent across sites. In the current analysis, over all sites and cultivars, average yields were 6.92, 7.13, and 8.14 mt/ha in 2010, 2011, and 2012, respectively.

Family did not significantly ($P > 0.18$) affect yield in any study year (Table 3). Yield was significantly affected by ± Bt ($P < 0.08$) in 2011 and 2012 (Table 3) when hybrids with any Bt trait had 7.2 and 4.1%, respectively, higher yields than those without Bt (Table 4). Although not significant, hybrids with any Bt trait also had higher yields in 2010 (3.6%) than non-Bt hybrids (Table 4).

Table 3. Significance (*P*) of effects for measured variables in each year

Variable	Source of variation	2010	2011	2012
Yield	Family	0.5679	0.1889	0.3951
	± Bt	0.5979	0.0049	0.0751
	2-way	0.9716	0.2779	0.3392
	Specific Bt traits	0.4665	0.0119	0.0750
	Site	<0.0001	<0.0001	<0.0001
Ear damage	Family	0.3629	0.4360	0.0291
	± Bt	0.0552	0.0100	<0.0001
	2-way	0.1552	0.1603	0.0014
	Specific Bt traits	0.0127	0.0665	<0.0001
	Site	0.0057	0.0009	<0.0001
Silk damage	Family	0.4702	0.3195	0.0386
	± Bt	0.4070	<0.0001	0.0002
	2-way	0.9169	0.0009	0.1869
	Specific Bt traits	0.2462	<0.0001	0.0006
	Site	0.1818	0.0101	<0.0001
Aflatoxin	Family	0.1284	0.9474	0.2279
	± Bt	0.7976	0.2812	0.8894
	2-way	0.3912	0.8181	0.6858
	Specific Bt traits	0.0522	0.8572	0.9570
	Site	<0.0001	<0.0001	<0.0001

Table 4. Yields (mt/ha) for hybrid families, ± Bt, and specific Bt traits, over all sites, in each study year

Factor value	2010	2011	2012
DKC67–8	7.03a[a]	7.57a[a]	8.45a[a]
P31P	6.81a	7.37a	8.34a
SS7	7.02a	n.i.	n.i.
P33M	6.58a	6.99a	8.38a
P33V	5.45a	7.15a	7.99a
N77P	n.i.[b]	7.29a	n.i.
N78S	n.i.	7.22a	8.07a
DG51	n.i.	6.42a	7.80a
No Bt	6.39a[a]	6.84a[a]	8.02a[a]
With Bt	6.77a	7.33b	8.35b
No Bt	6.53a[a]	6.84cd[a]	8.01b[a]
Herculex I	6.96a	7.49abc	8.55a
YieldGard corn borer	5.44a	7.06bcd	8.00ab
Genuity VT triple PRO	7.21a	7.94a	8.53ab
Agrisure viptera 3111	n.i.	7.63ab	8.27ab
Genuity smartstax	n.i.	6.38d	7.74b

n.i. Factor not included in study in that year.

[a] Letters following means in each column, when different, indicate significant differences at $P \leq 0.10$.

[b] n.i. Family or specific Bt trait not included in study in that year.

Table 5. Caterpillar feeding damage as cm^2 of destroyed kernels and silk damage for hybrid families[a] and ± Bt in each year

Family	2010		2011		2012	
	cm^2	Silk damage[b]	cm^2	Silk damage[b]	cm^2	Silk damage[b]
DKC67–8	16.7a[c]	1.29a[c]	6.6a[c]	1.18a[c]	3.5b[c]	0.76ab[c]
P31P	18.4a	1.46a	7.8a	1.41a	4.0ab	0.94a
P33M	20.0a	1.41a	6.2a	1.14a	4.5a	0.93a
P33V	18.1a	1.27a	5.1a	1.35a	3.8ab	0.91a
DG51	n.i.[d]	n.d.	6.2a	1.09a	3.5b	0.61b
SS7	16.0a	1.04a	n.i.	n.i.	n.i.	n.i.
No Bt	20.0a[c]	1.40a[c]	7.8a[c]	1.49a[c]	4.9a[c]	0.99a[c]
With Bt	15.7b	1.19a	5.0b	0.98b	2.8b	0.68b

[a] Analysis did not include NK families which were composed of two isolines with Bt trait(s).
[b] Silk damage rated on a scale of 0 (no damage) to 2 (all silks cut).
[c] Letters following means in each column, when different, indicate significant differences at $P \leq 0.10$.
[d] n.i. Family not included in study in that year.

Yield was affected by specific Bt traits in 2011 and 2012 ($P < 0.08$; Table 3). In both years, non-Bt hybrids or those with the Genuity SmartStax trait had lower yields than those with Genuity VT Triple PRO or Agrisure Viptera 3111 traits (Table 4).

Caterpillar Damage: Ear and Silk. Feeding damage on ears (cm^2) has been reported for each cultivar at each site and year (Flanders et al. 2011a,b, 2012, 2013). These previous reports show that hybrids with any Bt trait consistently had lower ear damage than did cultivars without Bt, although the differences due to ± Bt were not always significant among locations. In the current analysis, feeding damage on ears over sites and hybrids averaged 16.6, 5.3, and 3.6 cm^2 in 2010, 2011, and 2012, respectively. In a preliminary analysis of ear and silk damage, the NK families (NK78S and NK77P in 2011; NK78S in 2012) had substantially lower levels of damage (>35% lower ear damage and >30% lower silk damage) than other families. Both isolines in the NK families had Bt traits; therefore, data from these families were excluded for analysis of family and ± Bt effects on ear and silk damage.

Ear damage (cm^2) was shown to differ significantly ($P < 0.03$) due to family, ± Bt, and the interaction of family × (± Bt) in 2012, and due to ± Bt in 2010 and 2011 ($P < 0.06$; Table 3). The two-way interaction was likely significant in 2012 due to statistically similar ear damage levels for all hybrids in the P33M and P31P families (with and without Herculex I) while in other families Bt hybrids had statistically lower ear damage levels than non-Bt hybrids (data not shown). In 2012, the DG51and DKC67–8 families had lower ear damage than the P33M family; other families had intermediate ear damage (Table 5). Hybrids with any Bt trait had 21.5, 35.9, and 42.9% lower ear damage than those without Bt in 2010, 2011 and 2012, respectively (Table 5).

Family had a significant effect on silk damage only in 2012 ($P = 0.04$; Table 3) when the DG51 family had lower scores for silk damage than the P31P, P33M, or P33V families (Table 5). Silk damage was not affected by family, ± Bt, or the two-way interaction of these factors in 2010; in 2011 and 2012, ± Bt significantly affected silk damage ($P < 0.0003$), and the family × (± Bt) interaction was also significant in 2011 (Table 3). The two-way interaction was significant likely due to statistically similar silk damage levels for Bt and non-Bt hybrids in the P33M, P33V, and P31P families while in other families the Bt hybrids had statistically lower silk damage levels than non-Bt hybrids (data not shown). In 2011 and 2012, those hybrids with any Bt trait had 34 and 31% lower silk damage, respectively, than hybrids without a Bt trait (Table 5); this was also a trend in 2010 when Bt hybrids had 15% lower silk damage.

Analysis on the effects of specific Bt traits included all families (i.e., NK families were retained). Specific Bt traits significantly affected ($P < 0.07$) caterpillar feeding damage on ears over all sites in all three years (Table 3). In all study years, ear damage was lower on hybrids with Genuity VT Triple PRO, Genuity SmartStax, or Agrisure Viptera 3111 traits than on non-Bt hybrids; hybrids with YieldGard Corn Borer or Herculex I had intermediate levels of ear damage (Fig. 1).

A significant effect of specific Bt traits was seen on silk damage ($P < 0.0003$) in 2011 and 2012 (Table 3). In both of these years, hybrids with the Agrisure Viptera 3111 and Genuity SmartStax traits consistently had lower silk damage than non-Bt hybrids or those hybrids with YieldGard Corn Borer or Herculex I traits (Fig. 1). Hybrids with the Genuity VT Triple PRO trait had intermediate levels of silk damage (Fig. 1).

Aflatoxin Concentrations. Aflatoxin concentrations averaged 72, 109, and 21 ppb over all cultivars and sites in 2010, 2011, and 2012, respectively, in this analysis. Aflatoxin concentrations associated with hybrids at specific sites have been reported for 2011 and 2012 (Flanders et al. 2012, 2013). No consistent effects due to family or ± Bt were noted in these reports and this was also true in the current analysis, in that neither family nor ± Bt significantly affected aflatoxin levels ($P > 0.12$; Table 3). Specific Bt traits affected aflatoxin levels only in 2010 (Table 3), when hybrids with the Genuity VT Triple PRO trait had higher aflatoxin contamination (19.1 ppb) than hybrids with other traits (ppb <11).

Correlations. Yield was negatively correlated with aflatoxin concentrations in each of three years (2010, $R = -0.78$, $P < 0.0001$, $n = 40$; 2011, $R = -0.71$, $P < 0.0001$, $n = 50$; 2012, $R = -0.49$, $P < 0.0001$, $n \geq 64$;

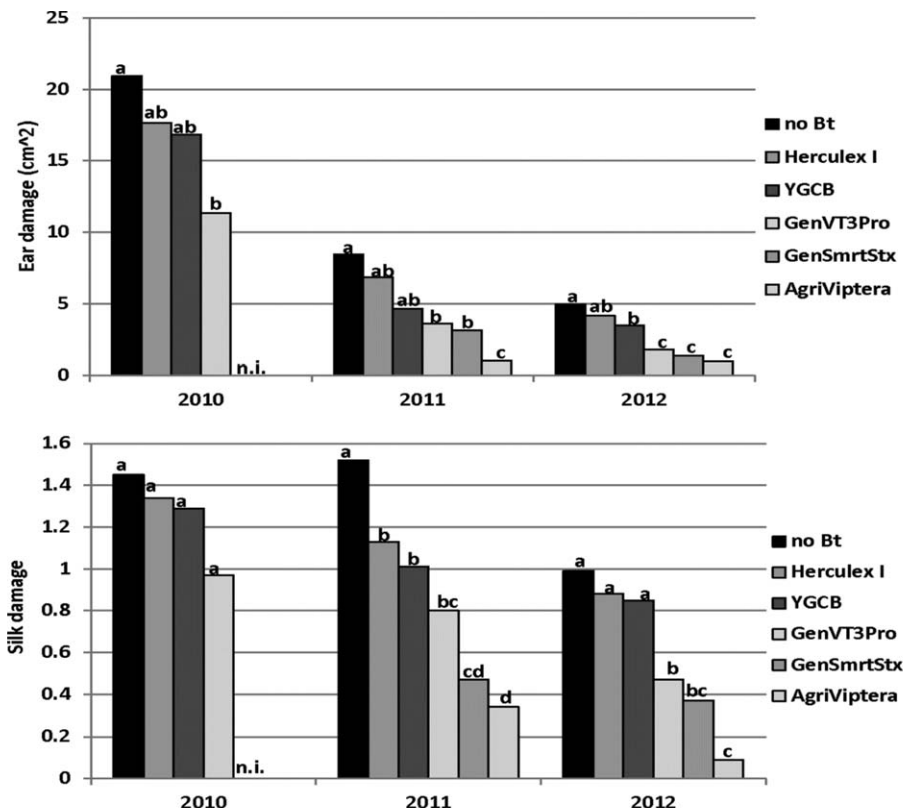

Fig. 1. Ear damage (cm^2; upper box) and silk damage (lower box) on hybrids with specific Bt traits. Different letters above bars within each year indicate significant differences due to Bt trait ($P < 0.05$). "n.i." indicates that Genuity SmartStax and Agrisure Viptera 3111 were not included in 2010.

Fig. 2) and when calculated over all three years ($R = -0.68$, $P < 0.0001$, $n = 168$). In 2010, yield was correlated to silk damage ratings ($R = -0.38$, $P = 0.015$); however, this is a weak correlation and not a consistent trend in other years. In addition, there was a significant correlation of ear damage with silk damage in two study years (2011, $R = 0.70$, $P < 0.0001$; 2012, $R = 0.85$, $P < 0.0001$), and for all 3 yr ($R = 0.67$, $P < 0.001$, $n = 155$). Because corn earworm feeds on both silks and kernels on the ear, these correlations were expected. In 2012, ear and silk damage were each correlated to aflatoxin concentrations ($R > 0.42$, $P < 0.0001$). No other variables were significantly correlated, including ear damage (cm^2), to yield (Fig. 3).

Environmental Effects. Yield, ear damage (cm^2), and aflatoxin concentrations differed significantly ($P < 0.006$) due to site in each year; silk damage differed due to site in 2011 and 2012 ($P \leq 0.01$; Table 3). Site effects on yield, ear damage, and aflatoxin content had lower P values than other factors evaluated. These results were expected given differences in weather events, soil types, and agronomic practices at each site. For example, yields were consistently higher at sites with irrigation; no significant correlations ($P > 0.18$) were found between total water and aflatoxin concentrations or ear and silk damages (data not shown). Average season-long temperatures were not correlated to yield, aflatoxin levels, or silk damage ($P > 0.44$), while a significant correlation was found between ear damage and average temperature ($R = 0.52$, $P = 0.0266$; Fig. 4). However, this relationship between season-long temperature and ear damage was largely due to substantially higher temperature and ear damage in 2010 than in 2011 or 2012.

Ear damage differed among sites from year to year. For example, in 2010, ears from Fairhope had the highest damage ratings, 25 cm^2, compared with $\leq$18.5 cm^2 at other sites in that year. However, in 2012, highest ear damage was noted at Headland (10.5 cm^2) compared with <5 cm^2 at other sites. There were no consistent year-to-year site effects with ear damage due to region of the state or irrigation at sites. Differences in ear damage among sites are likely due to differences in insect pressure at each site which is highly variable from year to year. Fairhope had higher damage than other sites in 2010 likely due to fall armyworm infestations (Flanders et al. 2011a,b). In 2011, Winfield and Tallassee had higher ear damage than Headland and Fairhope. In 2012, Winfield and Prattville had higher ear damage than Crossville and Brewton.

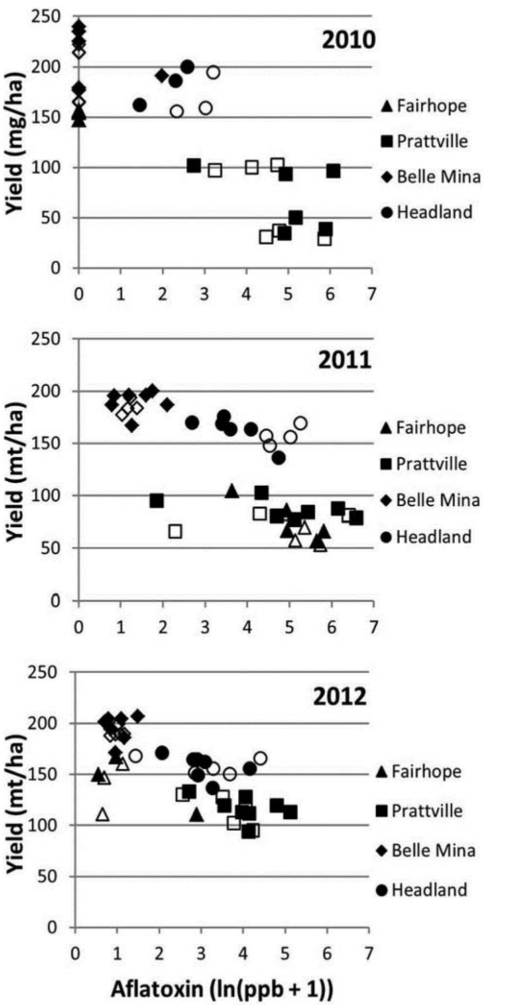

Fig. 2. Average yields and corresponding aflatoxin contents for non-Bt and Bt hybrids from select study sites in each of three years. Open markers represent non-Bt hybrids, while closed markers represent Bt hybrids. Study sites not on graph were not included in each of the three study years. Over all study sites, R = −0.78, −0.71, and −0.49 for 2010, 2011, and 2012, respectively, and $P < 0.0001$ in every year.

Discussion

In this study, we sought to determine the impact of the use of Bt corn hybrids on yields and aflatoxin concentrations in Alabama. Buntin et al. (2004) had noted that Bt hybrids only have a yield advantage over non-Bt hybrids when there are lepidopteran corn pests present. Differences in insect pressure at each site-year of this study would be expected to affect yield and ear damage. The higher levels of ear damage seen in 2010 than in the subsequent two study years can be attributed to higher than usual fall armyworm populations at central and southern Alabama sites, while north Alabama sites were heavily infested with southwestern corn borer, European corn borer, or both (Flanders et al. 2011a, b). Conversely, in 2012,

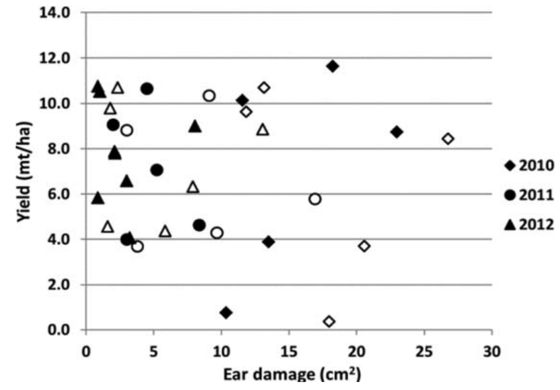

Fig. 3. Average yields and corresponding average ear damage (cm^2) from each study site in three study years (R = −0.12 and $P = 0.10$). Open markers are non-Bt hybrids; closed markers are Bt hybrids.

corn earworm and fall army worm infestations were consistently lighter than in past years (Flanders et al. 2013), which led to lower levels of ear damage than in preceding years. Corn earworm infestations were moderate in 2011 (Flanders et al. 2012), as were ear damage levels. These general, regional trends in insect pressure were not reflected in yields.

Yields were consistently higher for hybrids with any Bt trait compared with non-Bt hybrids in this study. Even in 2012, when corn earworm and fall armyworm pressure was low, a 4% yield advantage was noted for hybrids with any Bt trait compared with non-Bt hybrids. The yield advantage in 2012 may be, in part, attributable to a heavy infestation of southwestern corn borer at Crossville, which lead to a substantial yield advantage (24%) for Bt hybrids compared with non-Bt hybrids even though ear feeding damage was low (averaging 0.7 and 1.6 cm^2 for non-Bt and Bt hybrids, respectively) at this location (Flanders et al. 2013). Buntin (2008) had similarly found improved yields with Bt hybrids but only in late-planted corn with high fall armyworm pressure. Cox et al. (2009) noted that Bt hybrids with corn borer resistance traits yielded more than non-Bt isolines but only at sites

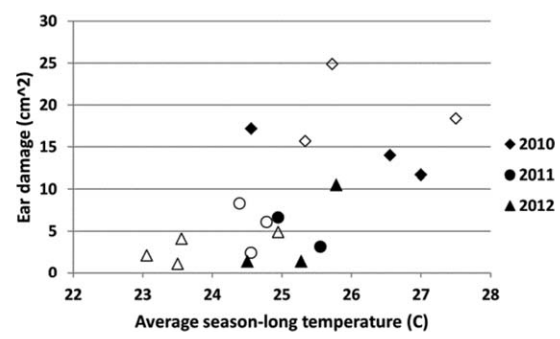

Fig. 4. Average ear damage (cm^2) graphed against season-long temperature from individual sites in each year (R = 0.52, $P = 0.0266$). Sites that supplied irrigation have solid markers.

where lodging had occurred, which may have been attributed to western corn rootworm. We did not look at all possible sources of damage due to lepidopteran pests (e.g., early season caterpillar pests or lesser cornstalk borers) in this study. It may be that some yield increase attributed to the Bt hybrids was through control of these other insects.

Hybrids with Genuity VT Triple PRO (in three study years) and Agrisure Viptera 3111 (in 2011 and 2012) traits out-yielded non-Bt hybrids by an average of 10.9 and 7.4%, respectively. In both 2011 and 2012, hybrids with the Herculex I trait had significantly higher yields than the hybrid with the Genuity SmartStax trait. Only a single hybrid in our trials, in the DG51 family, had the Genuity SmartStax trait and low yields could be due to family characteristics (e.g., relative maturity, adaptation to region) rather than this particular Bt trait.

Kernel feeding damage was lower on hybrids with any Bt trait compared with non-Bt hybrids in 2011 and 2012, with a similar trend in 2010. In the later 2 yr of this study, ears from hybrids with Bt traits for multiple proteins (Genuity VT Triple PRO, Genuity SmartStax, and Agrisure Viptera 3111) consistently had lower insect (both ear and silk) damage than hybrids with Herculex I or no Bt. In 2010, the only multiple protein trait among the hybrids was Genuity VT Triple PRO, and ears from hybrids with this trait tended to have the lowest levels of damage. These results are consistent with observations by Siebert et al. (2012).

Corn genetic background was represented by family in this study. Differences such as corn ear size and husk cover can be found among corn hybrid families and might impart resistance to corn earworm damage (Rector et al. 2002, Ni et al. 2007). We did not find differences in kernel feeding damage ratings among families that included both Bt and non-Bt isolines.

Aflatoxin concentrations were not consistently affected by hybrid family, ± Bt, or specific Bt traits across each of our study years. These results generally agree with observations by Wiatrak et al. (2005), Bruns and Abbas (2006) and Abbas et al. (2007), from studies with Bt hybrids. In the 2005 and 2006 studies, in two out of three study years, similar levels of aflatoxins were found in Bt and non-Bt hybrids. Our results differ from those of Williams et al. (2005) who found that Bt hybrids had reduced aflatoxin concentrations compared with non-Bt hybrids; however, these results were from plots in which corn ears had been inoculated with *A. flavus* and manually infested with southwestern corn borer. Plots not infested with southwestern corn borer did not have reduced aflatoxin levels in two study years (Williams et al. 2005). Lillehoj et al. (1980) observed that elevated toxin levels are associated with heavy insect damage, suggesting that only when non-Bt hybrids are heavily infested with ear-feeding insects will reduced aflatoxin levels be realized in their Bt isolines. Among our study years, ear damage levels were highest in 2010, particularly at Fairhope (data not shown herein, see Flanders et al. 2011a); however, in 2010, no aflatoxins were detected in samples from Fairhope. In addition, in general, aflatoxin concentrations were highest in 2011, which was intermediate in our three study years for ear and silk damage due to caterpillar feeding.

Yield was found to be significantly positively correlated to season-long water (rain plus irrigation) in each of the three years of this study. Inadequate water is known to be a limiting factor for corn yield (e.g., Wagger and Cassel 1993). However, dry conditions and lack of water are known to favor aflatoxin contamination; thus, the negative correlation of yield to aflatoxin concentrations found in the current study does not suggest a causal relationship. Jones et al. (1981) had also noted that conditions that are conducive to aflatoxin contamination are also those that cause plant stress and contribute to yield reduction. Previous studies have noted aflatoxin reduction with irrigation (Payne et al. 1986, Smith and Riley 1992, Rodriquez-del-Bosque, 1996), and in the current study, there tended to be lower levels of aflatoxins at irrigated sites. However, a significant correlation between aflatoxin content and season-long water was not found in the current study.

Several previous studies have noted that temperature, rather than moisture, is a more important variable affecting aflatoxin contamination (Widstrom et al. 1990, Hawkins et al. 2008). However, we found that season-long temperatures were not related to either yields or aflatoxin levels. This result may be because there seems to be a critical period of time (e.g., 65–85 d after planting [Hawkins et al. 2008] or 20–60 d after full silk [Widstrom et al. 1990]) during which either aflatoxigenic fungi become established or aflatoxin accumulation occurs in corn kernels. It is only during this critical time period that temperature or other weather conditions are important relative to aflatoxin concentrations. These conditions would have differed at each of our study sites and likely had a larger impact on aflatoxin content than did insect damage to ears.

Feeding damage ratings on ears were positively correlated to silk damage ratings in all 3 yr of this study. Because corn earworms feed on both silks and kernels, these correlations would be expected. In only one (2012) of 3 yr of the current study, feeding damage on silks and ears was positively related to aflatoxin content. This appears to support previous studies that had found an association between ear damage by lepidopteran pests and increased aflatoxin contamination (e.g., Lillehoj et al. 1978, Smith and Riley 1992, Ni et al. 2011). However, in the current study, this was not a consistent relationship over study years. As previously noted, weather is a critical driver for aflatoxin contamination in corn, and conditions in 2012 were more moderate than in the two preceding years. It may be that when more moderate weather conditions occur, increases in insect damage play a greater role in contributing to *A. flavus* establishment and subsequent aflatoxin levels.

We conclude that Bt corn hybrids can provide yield advantages in many situations, but did not impact aflatoxin concentrations under the conditions in this study. In addition, we found that Bt hybrids expressing multiple proteins provide greater protection from ear damage by lepidopteran pests than those with single Bt traits. Hybrids with more than one aboveground Bt protein have the additional advantage over single protein traits in that they can be planted on more of a grower's acreage (80 vs 50%). However, Bt traits should not be the sole consideration when choosing corn hybrids. Growers should pick corn hybrids that are agronomically adapted to their location and that have a good disease resistance package.

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