Aflatoxin Accumulation in Commercial Corn Hybrids Artificially Inoculated with *Aspergillus flavus* in 2008 and 2009

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INTRODUCTION

Aflatoxin is a naturally occurring toxin produced by the fungus *Aspergillus flavus* and is the most potent carcinogen found in nature (Castegnaro and McGregor 1998; Park and Liang 1993; Pittet 1998). In the United States, aflatoxin contamination of corn grain occurs sporadically in the Midwest, but it is a chronic problem in the Southeast (Payne 1992; Widstrom 1996). In 1998, a major aflatoxin epidemic in corn occurred in Mississippi and throughout the Southeast. Higher than normal temperatures, drought conditions, insect damage, and other factors contributed to high concentrations of aflatoxin contamination in corn grain.

The U.S. Food and Drug Administration established action levels that limit the sale of grain with aflatoxin levels exceeding 20 parts per billion (ppb) (Park and Liang 1993). Grain exceeding 20 ppb cannot be shipped across state lines and can only be used for livestock feed. Once corn is contaminated with aflatoxins, very few detoxification and utilization options are available.

The best control strategy is to limit the amount of aflatoxin that may accumulate in developing corn ears. Cultural practices that alleviate stress, such as using adapted varieties, irrigation, planting dates, and optimal fertilization, can minimize preharvest aflatoxin contamination most years (Jones et al. 1981; Larson 1997; Payne 1992). However, no control strategy is completely effective when environmental conditions, such as those seen in 1998, are extremely stressful to the plant and favorable for fungal growth.

The best aflatoxin control method is through host plant resistance to *A. flavus* infection and subsequent aflatoxin accumulation. The USDA Agricultural Research Service (ARS) at Mississippi State, Mississippi, has released four corn germplasm lines (Mp420, Mp313E, Mp715, Mp717) with high levels of resistance to aflatoxin accumulation (Scott and Zummo 1990, 1992; Williams and Windham 2001, 2006). The commercial corn seed industry has requested seed of these lines to incorporate resistance to aflatoxin accumulation into corn hybrids. In field studies conducted in 1998, aflatoxin accumulation was determined in a set of commercial corn hybrids and in resistant single-cross hybrids produced by the USDA-ARS (Windham and Williams 1999). In two separate tests, developing ears were either injected or sprayed with *A. flavus* spores. The aflatoxin levels in the commercial hybrids were extremely high regardless of the inoculation method. Aflatoxin levels were lowest in the resistant single-cross parents.

Since our initial evaluation of commercial hybrids in 1998, the commercial seed industry has developed corn hybrids that include additional technology traits for herbicide and insect resistance or tolerance. Transgenic hybrids expressing genes that encode insecticidal proteins with the property of those found in the bacterium *Bacillus thuringiensis* (Bt) have been included in experiments to determine whether controlling insects would suppress mycotoxin levels in grain at harvest. USDA-ARS researchers found that aflatoxin levels were lower in transgenic hybrids compared with conventional corn hybrids when infested with the southwestern corn borer and inoculated with A. flavus spores (Williams et al. 2002, 2005b). Little information is available on the level of resistance to aflatoxin accumulation in these currently available commercial corn hybrids when artificially inoculated with A. flavus. The objective of our study was to evaluate commercially available corn hybrids for resistance to aflatoxin accumulation when artificially inoculated with A. flavus in the field.

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Experiments were conducted at the Brown Loam Branch Experiment Station in Raymond, Mississippi, and the R.R. Foil Plant Science Research Center at Mississippi State University in 2008 and 2009. In 2008, 92 commercial corn hybrids and 8 single-cross hybrids were grown at Mississippi State, and 77 commercial corn hybrids were grown at Raymond. In 2009, 35 commercial corn hybrids and 5 resistant single-cross hybrids were grown at both locations. Hybrids were planted in single-row, 5.1-meter plots spaced 0.96 meter apart and were grown in a randomized complete block design with four replications. Planting dates were April 24, 2008, and April 28, 2009, at Mississippi State and March 25, 2008, and April 24, 2009, at Raymond. Seedlings were thinned to 20 plants per plot. Plots at Mississippi State received supplemental irrigation during the growing season to limit drought stress.

The *A. flavus* isolate NRRL 3357, which is known to produce aflatoxin in corn grain (Scott and Zummo 1988; Windham and Williams 1998, 2002; Windham et al. 2010), was used as inoculums in both tests. Inoculum was increased on sterile corn cob grits in 500-milliliter flasks, each containing 50 grams of grits and 100 milliliters of water, and then incubated at 28°C. Conidia were washed from the grits using sterile distilled water containing 20 drops of Tween 20 per liter and filtered through four layers of sterile cheesecloth. The concentration of conidia was determined with a hemacytometer and adjusted with sterile distilled water to 90 million per milliliter. Inoculum not immediately used was refrigerated at 4°C. Hybrids were inoculated 7 days after midsilk (50% of the plants in a plot had silks emerged) using the side-needle technique (Windham et al. 2005; Zummo and Scott 1989). The top ear of each plant was inoculated with a 3.4-milliliter suspension containing 300 million *A. flavus* conidia.

Ears were hand harvested around 63 days after midsilk, bulked by row, dried at 38°C for 7 days, and machine shelled. Bulk grain samples from each row were poured into a sample splitter twice to mix the grain. Samples were ground using a Romer mill (Union, Missouri). Aflatoxin contamination in a 50gram subsample from each plot was determined using the Vicam Aflatest (Watertown, Massachusetts). This procedure can detect aflatoxins (B₁ B₂ G₁ G₂) at concentrations as low as 1 ppb.

Means for aflatoxin concentration were transformed [ln (y +1)] before statistical analyses. Tests of significance were performed on transformed means before converting back to the original scale. Means were compared using Fisher's Protected Least Significant Difference Test at P = 0.05.

RESULTS AND DISCUSSION

Aflatoxin contamination was extremely high in 2008 at both locations (Table 1). At Mississippi State, aflatoxin levels ranged from 17 ppb for Mp313E x Mp717 to 10,800 ppb for Golden Acres 2831. The lowest level of aflatoxin contamination of a commercial hybrid was in Terral TV24R83 (77 ppb). This hybrid had aflatoxin concentrations statistically similar (P = 0.05) to the resistant single-cross hybrids, Mp04:97 x Mp313E and Mp04:97 x NC388. At Raymond, aflatoxin levels ranged from 285 ppb for Dyna-Gro DG58P45 to 6,005 ppb for NK Brand NKN68-B8.

In 2009, aflatoxin contamination was much lower compared with the level observed in 2008 (Table 2). At Mississippi State, aflatoxin levels ranged from 5 ppb for Mp313E x NC388 to 1,992 ppb for Pioneer Brand P32B34. Commercial hybrids that had low levels of aflatoxin contamination were Terral TVX28R92(E) and Golden Acres 28Z89. Aflatoxin levels in these two hybrids were statistically similar (P = 0.05) to the resistant single-cross hybrids Mp313E x Mp717 and Mp04:97 x NC388. At Raymond, aflatoxin contamination ranged from 47 ppb for Mp04:97 x NC388 to 3,060 ppb for Belle 1646VT3. Aflatoxin contamination in the commercial hybrids Terral TVX28R92(E) and Golden Acres 28Z89 was statistically similar (P = 0.05) to the resistant single-cross hybrids Mp313E x Mp717 and Mp313E x Mo18W.

Environmental stress can significantly increase preharvest aflatoxin levels in corn grain (Widstrom 1996; Windham et al. 2009). Ambient temperatures, rainfall, soil type, and a number of other factors may contribute to the physiological stress of a corn plant and affect the amount of aflatoxin levels in harvested grain. Aflatoxin levels averaged over both years for hybrid Terral TV24R83 were 93 ppb at Mississippi State and 1,231 ppb at Raymond. This hybrid had aflatoxin levels comparable to the resistant single-cross hybrids grown at Mississippi State, but it had very high levels of aflatoxin contamination at Raymond. The tests at Mississippi State received supplemental irrigation, while the tests at Raymond were grown under dryland conditions. Soil type also may have been a factor in the level of contamination. The soil at Mississippi State is a heavy clay soil with a higher water-holding capacity than the soil found at Raymond. The level of aflatoxin contamination of Terral TV24R83 observed under different environmental conditions at these two locations demonstrates that corn hybrids may react differently to A. flavus infection and subsequent aflatoxin accumulation.

A number of the commercial corn hybrids included in these studies contain technology traits (Agrisure Triple Stack, Herculex I, Yield Gard Corn Borer, and Yield Gard VT Triple) that are designed to limit insect feeding on vegetative and/or reproductive parts of the plant. A number of researchers have studied the level of aflatoxin contamination in transgenic corn containing various *Bt* events (Williams et al. 2002, 2005b; Windham et al. 1999). In our recent studies, high levels of aflatoxin were commonly found in commercial hybrids containing technology traits for insect resistance or tolerance. The *A. flavus* inoculation technique used in our studies injects large numbers of spores under the husks of developing ears and has proven useful in identifying corn genotypes with resistance to aflatoxin accumulation (Williams et al. 2005a; Windham et al. 2005). However, this inoculation method may not be the best method to demonstrate the effectiveness of these transgenic hybrids in suppressing aflatoxin contamination. Williams et al. (2002, 2005b) reported that *Bt* hybrids had lower levels of aflatoxin contamination than non-*Bt* hybrids when ears were infested with the southwestern corn borer and sprayed with *A. flavus* spores. The spray inoculation method (Buckley et al. 2006) used in those studies more closely reflects the natural infection process and allowed researchers to more effectively demonstrate the benefit of the *Bt* technology in suppressing aflatoxin contamination.

The resistant single-cross hybrids used in our studies contained at least one parent (Mp313E, Mp717, Mp04:97) developed by the USDA-ARS Corn Host Plant Resistance Research Unit at Mississippi State. Four of the single-cross hybrids grown at Mississippi State in 2008 (Mp313E x Mp717) and in 2009 (Mp04:97 x Mp313E, Mp313E x Mo18W, Mp313E x NC388) had aflatoxin levels lower than the U.S. Food and Drug Administration action level of 20 ppb. However, most of the aflatoxin-resistant lines that have been identified lack desirable agronomic characteristics. USDA-ARS scientists are currently using marker-assisted selection and conventional breeding techniques to transfer aflatoxin resistance into lines with good agronomic qualities (Warburton et al. 2009).

Our studies demonstrated that the amount of aflatoxin contamination in artificially inoculated commercial corn hybrids varies. Although aflatoxin levels were very high in many of the commercial hybrids, several of them had low contamination levels comparable to resistant single-cross hybrids in some instances. Although none of the commercial hybrids had consistently low levels of aflatoxin accumulation, the corn industry appears to be making progress in developing hybrids with resistance to aflatoxin accumulation either by incorporating genes associated with resistance or by improving plant tolerance of environmental stresses that contribute to aflatoxin accumulation. These studies also demonstrate the need for continued efforts in identifying corn genotypes with resistance to aflatoxin accumulation and molecular markers associated with resistance. Selecting hybrids from among those genotypes that exhibit lower levels of aflatoxin contamination and utilizing best management practices should reduce the risk of losses to aflatoxin.

Brand Name	Hybrid	Technology Traits	Aflatoxin (ppb)	
			Starkville	Raymond
Golden Acres	2831	YGCB/RR	10,800 a	2,046 b-n
Croplan Genetics	6150 VT3	CRW/YGCB/RR2	10,365 a	3,698 ab
Terral	TV26R73	RR2	9,071 ab	3,577 a-d
DEKALB	DKC 63-42	CRW/YGCB/RR2	8,688 a-c	3,067 a-f
Golden Acres	26Z17	CRW/YGCB/RR2	8,495 a-d	
DEKALB	DKC 64-79	CRW/YGCB/RR2	8,423 a-d	2,608 a-i
NK Brand	NK N68-B8	YGCB/LL	7,615 a-e	6,005 a
Dyna-Gro	DG 58K81	RR2	6,984 a-f	2,817 a-h
Stine	9806VT3	CRW/YGCB/RR2	5,838 a-h	1,326 f-r
NK Brand	NK N77P	3000GT	5,737 a-i	
AgriGold	A6455VT3	CRW/YGCB/RR2	5,671 a-j	1,483 d-r
AgriGold	A6522BtRR	YGCB/RR	5,557 a-g	1,438 e-r
Dyna-Gro	DG 57V85	CRW/YGCB/RR2	5,384 a-k	1,661 b-p
Belle	1722R	RR	5,185 a-l	2,381 b-j
Terral	TVX27BR84	YGCB/RR2	4,925 a-m	1,287 f-r
AgriGold	A6479VT3	CRW/YGCB/RR2	4,881 a-m	3,454 a-e
NK Brand	NK NX7976(E)	Experimental	4,305 a-n	1,657 b-p
Terral	TVX22TR86	RR2	4,181 a-n	1,687 b-p
Crow's	4846T	YGCB/RR	4,166 a-n	1,574 b-r
Terral	TV25BR23	YGCB/RR2	3,359 b-o	1,617 b-q
Pioneer	31P42	HX1/LL/RR2	3,347 b-o	1,743 b-p
DEKALB	RX715VT3	CRW/YGCB/RR2	3,282 b-o	3,063 a-f
B-H Genetics	9078RR/PL	YGCB/RR	3,196 b-p	
B-H Genetics	8914VT3	CRW/YGCB/RR2	2,952 c-q	
DEKALB	DKC 64-24	CRW/YGCB/RR2	2,839 d-q	1,345 f-r
DEKALB	DKC 65-44	CRW/YGCB/RR2	2,628 e-r	2,146 b-l
AgriGold	A6633 VT3	CRW/YGCB/RR2	2,561 e-s	
Pioneer	32B29		2,505 f-s	
USDA-ARS	GA 209 x SC 212Ms		2,431 f-s	
Pioneer	31G71	HX1/LL/RR2	2,431 f-s	2,656 a-i

Means for aflatoxin concentration were transformed [In (y+1)] prior to statistical analysis. Tests of significance were performed on transformed means before converting back to the original scale. Means followed by the same letter do not differ at *P* = 0.05 (Fisher's Protected LSD). ^s Susceptible check.

Resistant check.

Brand Name	Hybrid	Technology Traits	Aflatoxin (ppb)	
			Starkville	Raymond
Croplan Genetics	6831TS	CRW/YGCB/RR2	2,366 f-s	1,835 b-o
Croplan Genetics	691RR	RR2	2,302 g-s	2,527 a-j
AgriGold	A6632 VT3	CRW/YGCB/RR2	2,274 g-s	· · · ·
AgriGold	A6489 VT3	CRW/YGCB/RR2	2,194 g-t	1,395 f-r
BioGene	BG83V08	YGCB/RR	2,064 g-u	
Belle	1626R	RR	1,963 h-u	1,730 b-p
BioGene	BG84V09	YGCB/RR	1,933 i-u	· · ·
Crow's	5291B	YGCB/RR	1,896 j-v	3,609 a-c
DEKALB	DKC 66-23	YGCB/RR2	1,833 k-w	2,778 a-h
Merchman	M-816A	YGCB/RR	1,796 k-x	1,864 b-o
JSG	80B00	Conv.	1,766 l-x	1,745 b-p
Pioneer	31P42	HX1/LL/RR2	1,755 l-x	· · ·
DEKALB	DKC 62-99	YGCB/RR2	1,714 m-y	1,498 c-r
Crow's	5304VT3	CRW/YGCB/RR2	1,658 m-y	485 t-v
erral	TV25BR71	YGCB/RR2	1620 n-y	1,672 b-p
EKALB	DKC 67-23	YGCB/RR2	1,468 n-z	2,079 b-m
DEKALB	DKC 67-87	YGCB/RR	1,455 n-z	2,758 a-h
Belle	1533Y	YGCB	1,442 n-z	2,185 b-l
Pioneer	34F96	HX1/LL/RR2	1,437 n-z	2,244 b-l
)yna-Gro	DG 57V21	CRW/YGCB/RR2	1,435 n-z	2,266 b-k
Belle	1844RY	YGCB/RR	1,359 o-z	2,931 a-g
Pioneer	33M57	HX1/LL/RR2	1,323 o-a'	1,591 b-r
DEKALB	DKC 61-69	CRW/YGCB/RR2	1,258 o-a'	1,797 b-o
Belle	1147RY	YGCB/RR	1,243 o-a'	1,212 g-r
Pioneer	31N26	RR	1,209 o-a'	· · ·
erral	TV26BR61	YGCB/RR2	1,171 o-b'	1,002 k-u
lerchman	M-314A-10	YGCB/RR	1,077 p-c'	2,150 b-l
)yna-Gro	DG 57K58	RR2	1,072 p-c'	1,645 b-p
Pioneer	33N58	HX1/LL/RR2	1,071 p-c'	1,278 f-r
Pioneer	31G96	HX1/LL/RR2	1,055 q-d'	491 s-v
DEKALB	DKC 61-19	YGCB/RR	1,031 q-d'	1,012 k-u
Golden Acres	2821 RLH	HX1/LL/RR2	996 q-d'	1,425 f-r
NK Brand	NK N78N-GT/CB/LL	GT/CB/LL	987 q-e'	2,042 b-n
3-H Genetics	8895VT3	CRW/YGCB/RR2	958 r-e'	
Garst	82R45GT	GT	944 r-e'	
)yna-Gro	DG 58P59	YGCB/RR2	915 r-f'	983 l-u
Belle	1545RY	YGCB/RR	904 r-f'	1,461 e-r
Croplan Genetics	6818 TS	CRW/YGCB/RR2	884 r-f'	2,797 a-h
Dyna-Gro	DG 57V05	CRW/YGCB/RR2	881 r-f'	1,711 b-p
Golden Acres	2841 RRB	YGCB/RR	868 s-f'	855 n-u
DEKALB	DKC 69-40	YGCB/RR	746 t-g'	665 r-v
)yna-Gro	DG 57N96	Conv.	730 u-g'	3,721 ab
erral	TV25R31	RR2	707 u-g'	429 m-v
Croplan Genetics	7505 VT3	CRW/YGCB/RR2	639 v-h'	826 o-u
yna-Gro	DG 58P60	YGCB/RR2	620 w-h'	741 p-u
ýna-Gro	DG 58P27	YGCB/RR2	614 w-h'	1,100 i-t
3-H Genetics	7005RR/HX(E)	Experimental	610 x-h'	,
Golden Acres	27Z07	CRW/YGCB/RR2	579 z-i'	1,923 b-o
)yna-Gro	DG 57P12	YGCB/RR2	508 z-i'	1,924 b-o
erral	TV26BR41	YGCB/RR2	494 z-i'	1,643 b-p
Belle	1646RY	YGCB/RR	441 a'-j'	
)yna-Gro	DG 58P45	YGCB/RR2	398 b'-j'	285 v
yna-Gro	DG 58V24	CRW/YGCB/RR2	398 b'-j'	1,184 h-s
yna-Gro	DG 58K02	RR2	388 c'-j'	1,828 b-o
Froplan Genetics	851 VT3	CRW/YGCB/RR2	356 d'-j'	1,357 f-r
B-H Genetics	7066RB(E)	Experimental	352 d'-j'	
erral	TVX28R92	RR2	330 e'-j'	1,401f-r
AgriGold	A6639VT3	CRW/YGCB/RR2	309 f'-j'	1,049 j-t
3-H Genetics	9015RR/YGCB	YGCB/RR2	307 f'-j'	, , , , ,
Golden Acres	28Z89	YGCB/RR	283 q'-j'	
JSDA-ARS	Ab 24E x GA 209 ^s		270 g'-j'	
JSDA-ARS	Mp 494 x Mp 715 ^R		261 g'-j'	

Means for aflatoxin concentration were transformed [In (y+1)] prior to statistical analysis. Tests of significance were performed on transformed means before converting back to the original scale. Means followed by the same letter do not differ at P = 0.05 (Fisher's Protected LSD). ^S Susceptible check. ^R Resistant check

Brand Name	Hybrid	Technology Traits	Aflatoxin (ppb)	
			Starkville	Raymond
USDA-ARS	Mp 313E x Mo 18W [⊩]		249 g'-j'	
Terral	TV26TR41	RR2	224 h'-k'	1,866 b-o
Dyna-Gro	DG 58K40	RR2	196 h'-k'	674 q-n
USDA-ARS	Mp 313E x NC 388 ^B		157 j'-l'	
Terral	TV24R83	RR2	77 k'-l'	876 m-u
USDA-ARS	Mp 04:97 x Mp 313E ^R		77 k'-l'	
USDA-ARS	Mp 04:97 x NC 388 ^B		58 l'	
USDA-ARS	Mp 313E x Mp 717 ^R		17 m'	
DYNA-GRO	DG57K33	RR2		1,424 f-r
USG	82C00	Conv.		2,238 b-l

^s Susceptible check.
^R Resistant check

Brand Name	Hybrid	Technology Traits	Aflatoxin (ppb)	
			Starkville	Raymond
Pioneer	P32B34	YGCB/RR2	1,992 a	2,277a-c
DEKALB	DKC 66-94	RR2	1,383 ab	1,109 a-i
Dyna-Gro	DG 58K02	RR	1,323 a-c	1,044 a-i
Pioneer	P33F87	HX1/LL/RR2	1,207 a-d	1,610 a-e
Pioneer	P 31D59	HX1/LL/RR2	1,033 a-e	816 c-j
DEKALB	DKC 63-84	YGCB/RR2	1,019 a-e	2,914 ab
Pioneer	33N58	HX1/LL/RR2	865 a-e	1,106 a-i
Croplan Genetics	851 VT3	CRW/YGCB/RR2	747 a-f	1,025 a-i
Terral	TV 25TR59	CRW/YGCB/RR2	744 a-f	1,812 a-e
Croplan Genetics	CPL 6986 VT3	CRW/YGCB/RR2	646 a-f	925 b-i
Terral	TV25R31	RR	606 a-f	1,974 a-d
DEKALB	DKC64-47	RR2	600 b-f	871 c-j
Terral	TV26TR41	CRW/YGCB/RR2	568 b-g	1,459 a-f
Dyna-Gro	DG 58P60	YGCB/RR	534 b-g	2,317 a-c
Pioneer	31G96	HX1/LL/RR2	525 b-g	500 f-k
Terral	TV 25TR29	CRW/YGCB/RR2	461 b-h	1,957 a-d
Golden Acres	2841 RRB	YGCB/RR	454 b-h	648 d-j
Dyna-Gro	DG 58V24	YGCB/RR	423 b-h	642 d-j
Dyna-Gro	DG 58P59	YGCB/RR	402 c-h	765 c-i
Terral	TV26BR61	YGCB/RR	397 d-h	1,441 a-g
B-H Genetics	9015RR/YGCB	YGCB/RR	371 d-h	789 c-j
DEKALB	DKC 67-88VT3P	CRW/RR2/BT	359 e-i	988 a-j
Dyna-Gro	DG 58K40	RR	323 e-j	679 d-j
Belle	Belle 1646VT3	CRW/YGCB/RR2	270 f-j	3,060 a
Crow's	5304VT3	CRW/YGCB/RR2	248 f-j	2,789 ab
B-H Genetics	7066RB(E)	Experimental	236 f-j	389 i-l
Dyna-Gro	DG 58V69	CRW/YGCB/RR2	228 f-j	323 j-k
DEKALB	DKC 68-06	YGCB/RR2	227 f-j	167 k-m
DEKALB	DKC 69-40	CRW/YGCB/RR2	181 g-k	620 e-j
AgriGold	A6639VT3	CRW/YGCB/RR2	146 ĥ-l	741 c-j
Croplan Genetics	7505 VT3	CRW/YGCB/RR2	144 h-l	1,310 a-h
Terral	TV24R83	RR	109 i-l	1,587 a-e
Dyna-Gro	DG 58P45	YGCB/RR	108 j-l	619 e-j
Terral	TVX28R92(E)	Experimental	65 k-m	416 h-l
Golden Acres	28Z89	RR	45 l-n	459 g-l
USDA-ARS	Мр 313E x Мр 717 ^в		31 mn	164 k-m
USDA-ARS	Mp 04:97 x NC388 ^R		29 mn	47 n
USDA-ARS	Mp 04:97 x Mp 313E ^B		13 no	93 mn
USDA-ARS	Mp 313E x Mo18W ^B		7 0	146 l-n
USDA-ARS	Mp 313E x NC 388 ^R		50	57 mn

Means for aflatoxin concentration were transformed [In (y+1)] prior to statistical analysis. Tests of significance were performed on transformed means before converting back to the original scale. Means followed by the same letter do not differ at P = 0.05 (Fisher's Protected LSD). ^a Resistant check

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