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Effet de la température, des précipitations et de la date de semis sur la contamination par les aflatoxines et les fumonisines chez les hybrides de maïs Bt et non Bt en Arkansas

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Résumé de l'article

Le maïs (*Zea mays*) est sensible à la contamination par les aflatoxines, les fumonisines et d'autres mycotoxines, surtout dans le sud-est des États-Unis. En principe, la contamination par les mycotoxines pourrait être diminuée chez les hybrides commerciaux de maïs par des saisons de croissance plus courtes en plantant à des dates qui minimisent le stress sur les plantes au moment de la période critique du remplissage des grains. Pour évaluer cette stratégie, des hybrides commerciaux Bt et non Bt ont été semés en Arkansas de la mi-avril au début de mai 2002, 2004 et 2005. Pour toutes les années, tant pour le maïs Bt que le non Bt, le grain issu des semis de la mi-avril était moins contaminé aux aflatoxines que celui semé au début de mai. De même, la contamination supérieure aux niveaux légalement acceptés a été moindre en 2005 et dans l'ensemble pour les semis de la mi-avril. Avec les semis de la mi-avril, il y avait significativement moins de contamination par les fumonisines dans le grain récolté et moins de contamination supérieure aux niveaux légalement acceptés qu'avec les semis du début de mai pour deux des trois années et dans l'ensemble, tant pour le maïs Bt que celui non Bt. La quantité de tous les sous-types de fumonisines étudiés a été diminuée. La présence simultanée d'aflatoxines et de fumonisines a fréquemment été observée. Pour tous les semis, les quantités de fumonisines des hybrides Bt ont été inférieures en moyenne à celles des hybrides non Bt. Les moindres contaminations par les aflatoxines et les fumonisines avec les semis de la mi-avril n'ont pu être expliquées par aucune des mesures du stress causé par la chaleur lors de la période du remplissage des grains.

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Effect of temperature, rainfall and planting date on aflatoxin and fumonisin contamination in commercial Bt and non-Bt corn hybrids in Arkansas

Hamed K. Abbas[1], W. Thomas Shier[2], and Rick D. Cartwright[3]

Corn (maize, *Zea mays*) is susceptible to contamination with aflatoxins, fumonisins and other mycotoxins, particularly in the southeastern USA. In principle, mycotoxin contamination could be reduced in commercial corn hybrids with shorter growing seasons by planting at dates which minimize plant stress during the critical kernel-filling period. To evaluate this strategy, commercial Bt and non-Bt hybrids were planted in Arkansas in mid-April and early May of 2002, 2004 and 2005. The mid-April planting date resulted in lower aflatoxin contamination in harvested corn each yr and in significantly less frequent contamination above a regulatory action level in 2005 and overall than did the early-May planting date in both Bt and non-Bt corn. The mid-April planting date resulted in significantly lower total fumonisin contamination in harvested corn and in less frequent contamination above a regulatory advisory level than the early May planting date in 2 of 3 yr and overall in both Bt and non-Bt corn. All fumonisin subtypes studied were reduced. Frequent co-occurrence of aflatoxin and fumonisin was observed. Fumonisin levels averaged lower in Bt hybrids than in non-Bt hybrids at all plantings. Reduced aflatoxin and fumonisin contamination with mid-April planting could not be explained by any measure of heat stress during the kernel-filling period.

Keywords: Aflatoxin, *Aspergillus flavus*, Bt and non-Bt corn hybrids, date of planting, fumonisin, maize, weather factors.

[Effet de la température, des précipitations et de la date de semis sur la contamination par les aflatoxines et les fumonisines chez les hybrides de maïs Bt et non Bt en Arkansas]

Le maïs (*Zea mays*) est sensible à la contamination par les aflatoxines, les fumonisines et d'autres mycotoxines, surtout dans le sud-est des États-Unis. En principe, la contamination par les mycotoxines pourrait être diminuée chez les hybrides commerciaux de maïs par des saisons de croissance plus courtes en plantant à des dates qui minimisent le stress sur les plantes au moment de la période critique du remplissage des grains. Pour évaluer cette stratégie, des hybrides commerciaux Bt et non Bt ont été semés en Arkansas de la mi-avril au début de mai 2002, 2004 et 2005. Pour toutes les années, tant pour le maïs Bt que le non Bt, le grain issu des semis de la mi-avril était moins contaminé aux aflatoxines que celui semé au début de mai. De même, la contamination supérieure aux niveaux légalement acceptés a été moindre en 2005 et dans l'ensemble pour les semis de la mi-avril. Avec les semis de la mi-avril, il y avait significativement moins de contamination par les fumonisines dans le grain récolté et moins de contamination supérieure aux niveaux légalement acceptés qu'avec les semis du début de mai pour deux des trois années et dans l'ensemble, tant pour le maïs Bt que celui non Bt. La quantité de tous les sous-types de fumonisines étudiés a été diminuée. La présence simultanée d'aflatoxines et de fumonisines a fréquemment été observée. Pour tous les semis, les quantités de fumonisines des hybrides Bt ont été inférieures en moyenne à celles des hybrides non Bt. Les moindres contaminations par les aflatoxines et les fumonisines avec les semis de la mi-avril n'ont pu être expliquées par aucune des mesures du stress causé par la chaleur lors de la période du remplissage des grains.

Mots clés : aflatoxine, *Aspergillus flavus*, date de semis, facteurs climatiques, fumonisine, hybrides de maïs Bt et non Bt, maïs.

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INTRODUCTION

In the Southern United States, corn (maize, *Zea mays* L.) is commonly grown in rotation with cotton (*Gossypium hirsutum* L.) or soybeans [*Glycine max* (L.) Merr.] (Curl 1963; Gazaway *et al.* 2000; Robinson *et al.* 1967; Rupe *et al.* 1997). Corn is used for animal feed, direct human consumption, fermentation for fuel ethanol and production of alcoholic beverages (Watson 1988). Corn kernels frequently become infested with the toxigenic fungi *Aspergillus flavus* Link and *Fusarium* spp. (Abbas *et al.* 2002; Cardwell *et al.* 2000; Chulze *et al.* 1996; Marin *et al.* 1998; Payne 1992; Zummo and Scott 1992), which produce the aflatoxin and fumonisin mycotoxins, respectively (Abbas 2005; Chamberlain *et al.* 1993; CAST 2003; Diener *et al.* 1987; NTP 1999; Picco *et al.* 1999; Rheeder *et al.* 2002). Because these toxins pose serious health risks to humans and animals (CAST 2003; Cullen and Newberne 1994) crop quality and value can be affected (CAST 2003; Robens and Cardwell

2003). Many approaches to reducing mycotoxin levels in corn have been investigated. These methods include crop management techniques which reduce stress (heat, drought, nutrient, population density, insect damage) on corn plants (Bruns 2003; Lauer 2001). These strategies are most likely to be effective if applied during the critical period when kernel filling occurs between silking (~80 d after planting) and blacklayer (~130 d after planting). The effects of these stress factors on aflatoxin and fumonisin levels in various types of corn hybrids require more study in order to provide a basis for recommendations to growers (Abbas 2005; Robens and Brown 2004). For example, Bt corn hybrids are often recommended to minimize insect damage, which can predispose plants to fungal infection and mycotoxin contamination (Buntin *et al.* 2001; Lauer 2001; Wiatrak *et al.* 2004; Wu *et al.* 2005). Little information exists on the use of Bt hybrids for reducing aflatoxin and fumonisin levels in harvested corn crops (Abbas *et al.* 2002, 2006; Hammond *et al.* 2004).

Table 1. Summary of mean temperatures (T) and precipitation observed by the National Climatic Data Center at Marianna, Arkansas, during the kernel-filling period

Year	Segment of kernel-filling period[1]	T max (avg. max air temp) (°C)[2]	T min (avg. min air temp) (°C)[3]	Total DD20[4]	Total MaxD20[5]	Total MinD20[6]	Rainfall (cm)[7]
2002	Early	32.5	22.2	+154.4	+262.5	+46.6	13.4
	Common	32.5	22.6	+217.8	+362.5	+77.2	7.5
	Late	32.6	20.7	+140.0	+264.6	+20.5	0.4
	Early total	32.5	22.4	+372.2	+625.0	+123.8	20.9
	Late total	32.5	21.8	+357.8	+627.1	+97.7	7.9
2004	Early	30.8	21.3	+127.2	+226.8	+29.4	3.2
	Common	28.7	17.3	+86.9	+252.3	+5.5	4.7
	Late	29.9	18.5	+88.5	+207.9	+12.8	0.4
	Early total	29.6	19.0	+214.1	+479.1	+34.9	7.9
	Late total	29.2	17.8	+175.4	+460.2	+18.3	5.1
2005	Early	30.9	21.4	+129.5	+228.9	+32.1	7.9
	Common	32.8	21.6	+207.8	+371.2	+52.3	6.0
	Late	31.0	19.3	+108.7	+231.0	+12.8	5.2
	Early total	32.0	21.5	+337.3	+600.1	+84.4	13.9
	Late total	32.0	20.6	+316.5	+602.2	+65.1	11.2
30-yr norm	Early	33.0	21.6	+153.6	+273.0	+33.6	6.8
	Common	32.8	20.8	+197.2	+371.2	+22.7	6.8
	Late	30.8	18.5	+97.0	+226.8	0.0	5.0
	Early total	32.9	21.1	+350.8	+644.2	+56.3	13.6
	Late total	32.0	19.8	+294.2	+598.0	+22.7	11.8

[1] Early (April 13 planting date) = July 4 to 24 (21 days); Common = July 25 to August 22 (29 days); Late (May 4 planting date) = August 23 to September 12 (21 days).

[2] Significant correlation with the 30-yr norm was observed in 2002 (regression analysis, $P < 0.05$).

[3] Significant correlation with the 30-yr norm was observed in each yr (regression analysis, $P < 0.05$).

[4] DD20 = degree growing units = (T max + T min)/2 - 20°C. Total DD20 is the sum of DD20s over the indicated period. Significant correlation with the 30-yr norm was observed in each yr (regression analysis, $P < 0.05$).

[5] Total MaxD20 = the sum of T max - 20°C values in which T > 20°C during the indicated period. Significant correlation with the 30-yr norm was observed in each yr (regression analysis, $P < 0.05$).

[6] Total MinD20 = the sum of T min - 20°C values in which T > 20°C during the indicated period.

[7] Cumulative rainfall in cm during the indicated period. Significant correlation with the 30-yr norm was observed in 2004 and 2005 (regression analysis, $P < 0.05$).

Development of corn hybrids with altered photoperiods that permit a shortened growing period and the ability to germinate better in cooler soils has allowed the Corn Belt to expand progressively northward. The warmer climate in the south provides a longer growing season and permits a wider selection of planting dates than at the northern edge of the Corn Belt, where there is little flexibility in planting date (Lauer 2001; Sprague and Larson 1966). In principle, the availability of commercial hybrids with shorter growing seasons should make it possible to vary planting dates in the south, as part of an effort to reduce mycotoxin contamination (Bruns 2003; Bruns and Abbas 2006). It should be possible to select a planting date which locally will place the critical kernel-filling period between silking and blacklayer, at a time when typical weather conditions are those expected to minimize stress on corn plants. Numerous studies have associated plant stress of various types, including heat, drought, nutrient deficiency, crowding and insect predation, with reduced resistance to fungal infection and contamination of harvested grain with mycotoxins (Payne 1992). Our previous studies in Mississippi (Abbas *et al.* 2002) and Arkansas (Abbas *et al.* 2006) indicated a clear, consistent association of heat stress with aflatoxin contamination in harvested corn. The same studies also indicated an association of heat stress with fumonisin contamination in harvested corn, but fumonisin levels were less affected by heat stress than were aflatoxin levels, and in 1 yr they were elevated in the absence of heat stress.

The typical weather conditions in this region can be predicted from 30-yr daily high and low air temperature and precipitation data provided for the Marianna, Arkansas area by the US National Climatic Data Center (Table 1). Weather conditions in the region permit a range of planting dates, which place their respective kernel-filling periods under a range of temperature and precipitation conditions. The mid-April planting date places the kernel-filling period (from about 80 to 130 d after planting) during the 49 d between July 4 and August 22. The early May planting date places the kernel-filling period from July 15 to September 12. These time periods overlap from July 25 to August 22, but only corn planted early fills kernels from July 4 to August 22, and only corn planted late fills kernels from August 23 to September 12. The conclusion for Marianna 30-yr data is that early May planting will typically expose corn to more stress related to air temperature than mid-April planting [i.e. (i) high day temperature: early planting = 33.0°C on average, late planting = 30.8°C; (ii) high night temperature: early = 21.6°C, late = 18.5°C; (iii) total DD20: early = +153.6, late = +97.0; (iv) total MaxD20: early = +273.0, late = 226.0; (v) total MinD20: early = +33.6, late = 0]. Also, the Marianna 30-yr average total daily rainfall data predicts that drought stress is more likely to occur with the late planting date (early planting = 6.8 cm rainfall, late planting = 5.0 cm). In order to provide an experimental system capable of evaluating the effects of air temperature-related stress, furrow irrigation was used to attempt to reduce drought stress to insignificant levels.

The studies reported here were designed to test the hypothesis that, for corn hybrids with short growing seasons, it is possible to reduce aflatoxin and fumonisin contamination in harvested kernels by selecting a planting date which places the critical kernel-filling period at a time when weather conditions normally cause less heat stress to corn plants. Because injury sites from insect damage are an important route for infection by toxigenic fungi, Bt corn hybrids are expected to suffer less insect damage than conventional hybrids and thus yield grain with lower mycotoxin levels. In order to address this assumption, both Bt and non-Bt hybrids were included in the study. In order to reduce uncertainty resulting from yr-to-yr variation in weather, the study was conducted for 3 yr (2002, 2004 and 2005).

Table 2. Effect of planting date on aflatoxin content of corn

Type and contamination ratio[1] of hybrids	Mean total aflatoxin levels in corn from the indicated planting date (ppb)							
	2002		2004		2005		All years	
	April	May	April	May	April	May	April	May
Non-Bt ratio	1.3 ± 0.7 0/5	0.28 ± 0.13 0/5	17 ± 17 1/6	20 ± 9 3/6	5.2 ± 1.1[2] 0/6	8.5 ± 5.2 1/6	8.5 ± 6.0 1/17	10.2 ± 4.0 4/17
Bt ratio	0.18 ± 0.05 0/4	11.5 ± 11.1 1/4	1.1 ± 0.8 0/3	75.7 ± 55.5 2/3	2.1 ± 0.5[2] 0/3	12.9 ± 10.8 1/3	1.0 ± 0.4 0/10	31.2 ± 18 4/10
Total ratio	0.80 ± 0.43 0/9	5.3 ± 5.0 1/9	12.3 ± 11.5 1/9	38.7 ± 19.4 5/9	4.2 ± 0.9 0/9[3]	10.0 ± 4.7 2/9[3]	5.7 ± 3.8 1/27[4]	18.0 ± 7.2 8/27[4]

[1] Contamination ratio = the number of hybrids with aflatoxin levels above the US Food and Drug Administration (FDA) regulatory agency action level of 20 ppb for human consumption over the total number of samples examined.
[2] Significantly lower aflatoxin levels in Bt corn than in non-Bt corn (*P* < 0.05, *t*-test).
[3] Significantly less frequent contamination above the FDA action level for mid-April planting than for early May planting (*P* < 0.05, chi-square test).
[4] Significantly less frequent contamination above the FDA action level for mid-April planting than for early May planting (*P* < 0.01, chi-square test).

MATERIALS AND METHODS

Corn growing conditions

A 3-yr study was designed to examine nine commercial corn hybrids per yr, including Bt and non-Bt corn hybrids (Tables 1 and 2). The study was conducted at the Cotton Branch Experimental Station (CBES), Marianna, Arkansas, in 2002, 2004 and 2005. In 2002, five non-Bt and four Bt hybrids were planted on April 13 and May 4, and harvested on September 10. In 2004, six non-Bt and three Bt hybrids were planted on April 15 and May 6, and harvested on September 8. In 2005, six non-Bt and three Bt hybrids were planted on April 16 and May 5, and harvested on September 8. Some of both the Bt and non-Bt hybrids were continued throughout the 3 yr of the study, whereas others were replaced with new releases when they were withdrawn from commercial production. About 100 plants per hybrid were harvested. To prevent drought stress, supplemental water was provided each yr by furrow irrigation using the Arkansas Irrigation Scheduler (Vories *et al.* 2005) when a 5.08 cm (2 inch) rainfall deficit was reached. Hybrids were grown in a randomized complete block design with four replications. Plots were managed using current Cooperative Extension Service (CES) guidelines in Arkansas.

High and low air temperature and precipitation data were obtained from the National Climatic Data Center (Asheville, NC) for Marianna, Arkansas (Abbas *et al.* 2002) for the 3 yr studied, as well as the averages over the previous 30 yr. The following weather-related criteria were compared with respect to aflatoxin and fumonisin contamination levels in corn harvested from early and late plantings: average maximum air temperature in °C; average maximum air temperature in °C minus 20°C; average minimum air temperature in °C; average minimum air temperature in °C minus 20°C; total DD20; total maximum degree-days over 20°C; total minimum degree-days over 20°C; cumulative rainfall.

Collection of corn samples

Ears used for mycotoxin analysis were randomly selected at grain maturity. Twenty ears per plot were hand-harvested; husks were removed immediately and ears were dried to < 14% moisture content in a drier at 52°C for 1 wk. Moisture content was determined with a Seedburo model GMA-128 grain moisture analyzer (Seedburo Equipment Chicago, IL). Ears were stored dry in paper bags at room temperature until processed (< 2 wk). Ears were machine-shelled, and grain samples of at least 1 kg from each row were mixed twice in a sample splitter. Samples (1 kg) were ground using a Romer mill (Union, MO) and 1/3 of each ground sample was selected for extraction as described below.

Materials

All solvents were HPLC grade from Fisher Scientific (Pittsburgh, PA). All mycotoxin standards and other chemicals were obtained from Sigma (St. Louis, MO).

Extraction and clean-up of mycotoxins from corn samples

The resistance of various hybrids to mycotoxin contamination in the field was compared in all yr as a result of natural infection with *A. flavus* and *Fusarium* spp. Extraction and clean-up of mycotoxins from corn samples were carried out as described by Abbas *et al.* (2002, 2006). Sub-samples of ground corn (20 g) were extracted with 100 mL of methanol:water 70:30, filtered and stored at 5°C until subjected to clean-up for HPLC analysis as described in detail by Sobolev and Dorner (2002). Clean-up of the fumonisins was accomplished on Bond-Elute SAX columns (Varian, Harbor City, CA) by the method of Plattner (1999) with minor modifications. The SAX column was
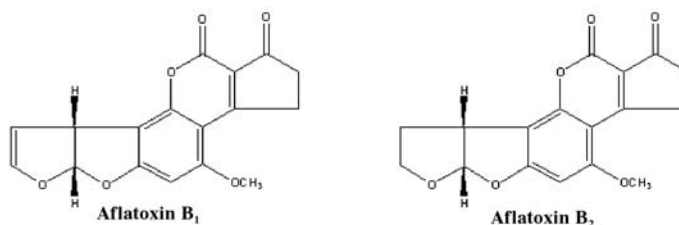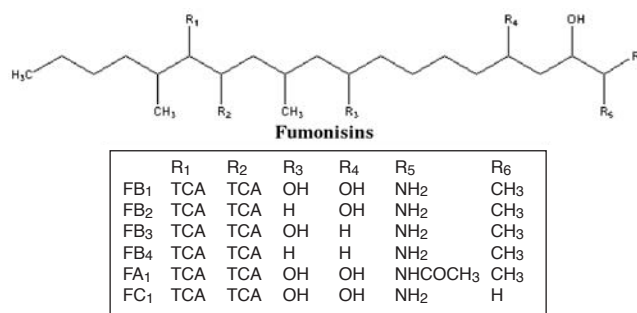

	R_1	R_2	R_3	R_4	R_5	R_6
FB_1	TCA	TCA	OH	OH	NH_2	CH_3
FB_2	TCA	TCA	H	OH	NH_2	CH_3
FB_3	TCA	TCA	OH	H	NH_2	CH_3
FB_4	TCA	TCA	H	H	NH_2	CH_3
FA_1	TCA	TCA	OH	OH	$NHCOCH_3$	CH_3
FC_1	TCA	TCA	OH	OH	NH_2	H

Figure 1. Chemical structures of aflatoxins B_1 and B_2, and various fumonisin analogs. TCA = tricarballylic acid (propane-1,2,3-tricarboxylic acid).
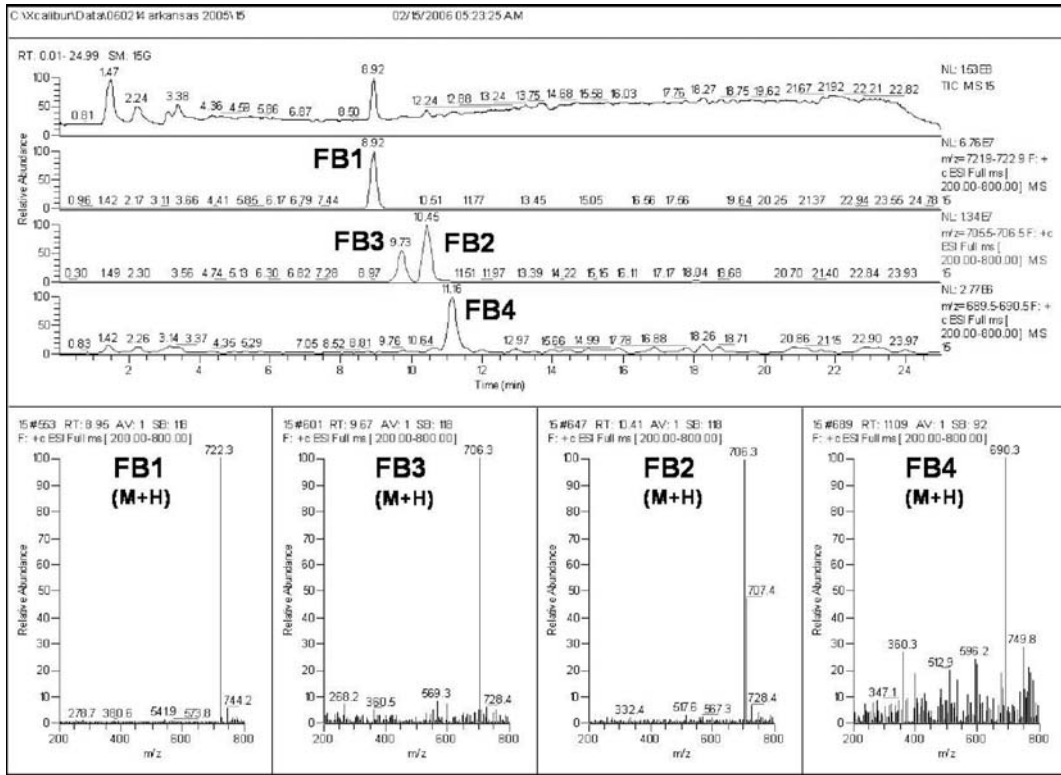
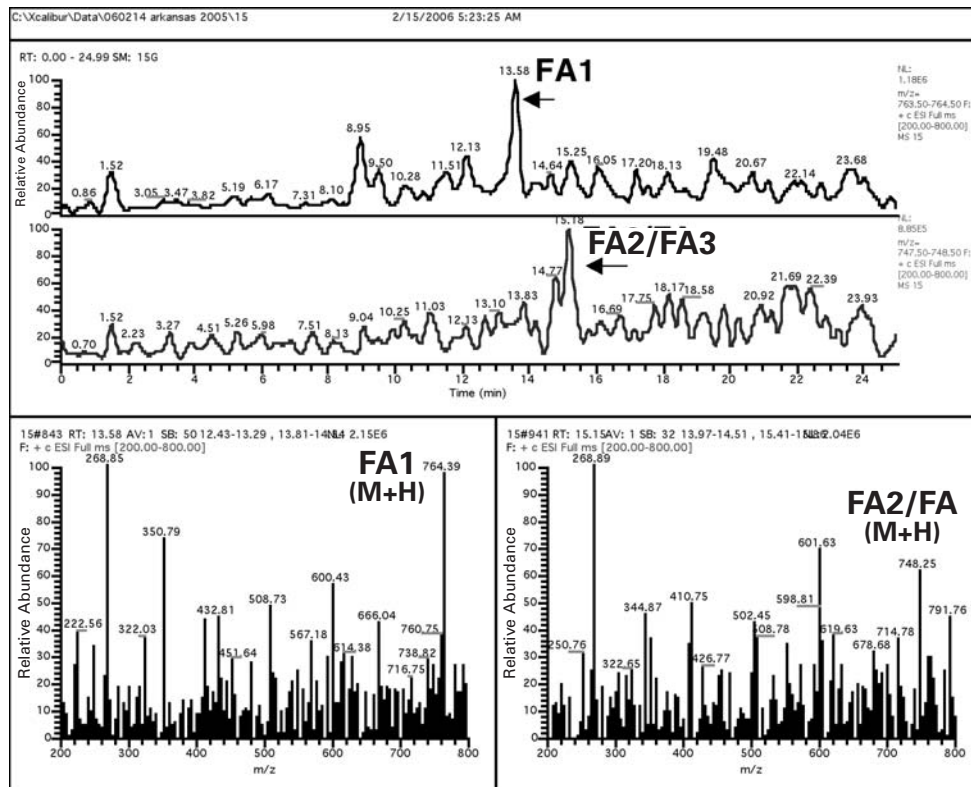Figure 2. Chromatograms and mass spectrums of various analogs of fumonisins (FB$_1$, FB$_2$, FB$_3$, and FB$_4$).

Figure 3. Chromatogram and mass spectrum of fumonisins FA$_1$ plus FA$_2$ (FA$_1$/FA$_2$).
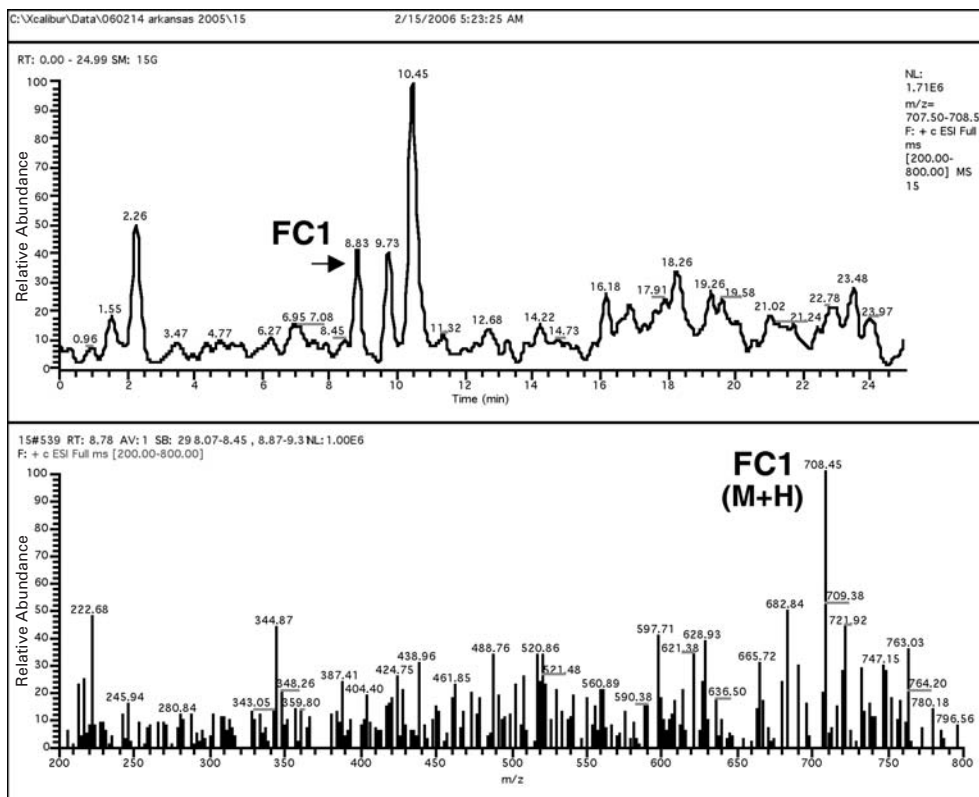
Figure 4. Chromatogram and mass spectrum of fumonisin C_1 (FC_1).

conditioned by applying 5 mL of methanol followed by 5 mL of methanol:water (3:1). A 10 mL aliquot of the extracted corn sample was applied followed by a 3 mL methanol wash step, followed by elution with 10 mL water containing 2% acetic acid. Samples were evaporated under a stream of nitrogen and stored at 5°C until further analysis by liquid chromatography/electrospray ionization/mass spectrometry (LC/ESI/MS). The cleaned sample was reconstituted in 1 mL acetonitrile:water (1:1) and diluted if necessary.

ELISA analysis of aflatoxins and fumonisins
Commercially available quantitative ELISA assay kits (Neogen Inc., Lansing, MI) were used to measure total aflatoxins and total fumonisins according to the manufacturer's instructions.

Liquid chromatography/mass spectrometry (LC/MS) analysis of mycotoxins
Selected samples from each yr of the study were examined by LC/ESI/MS to confirm mycotoxin identity and determine the relative amounts of subtypes of fumonisins (FB_1, FB_2, FB_3, FB_4, FA_1, FA_2, FA_3 and FC_1) and aflatoxins (AFB_1 and AFB_2) (Fig. 1). LC/ESI/MS analyses were carried out as described in detail by Abbas *et al.* (2002) on a Thermo Finnigan LCQ Advantage, coupled to a Thermo Finnigan Surveyor MS and a Thermo Finnigan Surveyor MS Pump (Thermo Electron Corporation, West Palm Beach, FL) with minor modifications for fumonisin analyses. Samples were run using 10 μL partial loop injections analyzed in full-scan mode plus at the following mass

ranges: FB_1: 722 (M+H); FB_2 and FB_3: 706 (M+H); FB_4: 690 (M+H); FA_1, FA_2, FA_3: 764, 748, 748 (M+H); and FC_1: 708 (M+H) (Figs. 2, 3, 4). MS/MS was performed on m/e 722 for further confirmation of FB_1. Fumonisin analysis was conducted using a MetaChem Intersil 5μ ODS-3 column eluted with water:1% acetic acid:methanol (65:35:0) at 300 μL min^{-1} for 10 min followed by a linear gradient to water:1% acetic acid in methanol:methanol (5:35:65), then held constant for 10 min. Between samples the solvent was returned to water:1% acetic acid in methanol:methanol (65:35:0) within 1 min and held constant for 4 min for column equilibration. Samples suspected to contain higher concentrations were diluted, followed by a wash step, which was incorporated after gradient completion to eliminate sample carry-over. Quantitation of FB_1, FB_2 and FB_3 was carried out by the external standard method, whereas other fumonisin subtypes were calculated as a percentage of FB_1.

Statistical analysis
Regression analysis was used for comparing weather-related measurements in the years being considered. Student's unpaired *t*-test and analysis of variance were used for comparing mycotoxin levels with respect to planting date and Bt status. In all cases, the statistical package included in Microsoft Excel 97 software was used. Contamination frequencies were compared using the chi-square test with Yates's correction. Outliers were rejected according to the two standard deviation rule (Ratcliff 1993).

RESULTS AND DISCUSSION

The aflatoxin levels in corn harvested in 2002, 2004 and 2005 are summarized in Table 2. Observed aflatoxin levels were highly variable, ranging from below the limit of detection to 184.3 ppb, so that average aflatoxin levels were usually dominated by one or two heavily contaminated samples. LC/MS analyses showed that aflatoxin B1 was the only aflatoxin found in all samples. The mid-April planting date resulted in numerically lower average aflatoxin contents in harvested corn than early May planting in each yr and in total for the 3 yr, but the difference was not significant (*t*-test, $P > 0.05$) in any case, even if samples with unusually high levels were rejected from the data as outliers according to the two standard deviation rule. The mid-April planting resulted in a lower frequency of aflatoxin levels in harvested corn being above the US Food and Drug Administration (FDA) action level for human consumption 20 ppb (van Egmond and Jonker 2004) each yr, and the difference was significant (chi-square test, $P < 0.01$) for all hybrids in 2005 and for all yr considered together. Aflatoxin levels in harvested corn from Bt hybrids were numerically lower than from non-Bt hybrids in early plantings each yr and in total, although the differences were only significant (*t*-test, $P < 0.05$) for the 2005 early planting. In contrast, aflatoxin levels in harvested corn from Bt hybrids in the early May planting were higher every yr and in total, but the differences were not significant (*t*-test, $P < 0.05$) in any case, even if samples with unusually high levels were rejected from the data as outliers according to the two standard deviation rule. A similar pattern of differences was observed when the frequency of aflatoxin contamination in excess of the FDA action level was compared in harvested corn from Bt hybrids and non-Bt hybrids, but the differences were not significant for either early or late plantings.

The fumonisin contents of the same samples of corn harvested in 2002, 2004 and 2005 are summarized in Table 3. Fumonisin levels observed in harvested corn were less variable than aflatoxin levels, ranging from 0 to 39.0 ppm. The mid-April planting of either Bt hybrids or non-Bt hybrids resulted in lower fumonisin levels in harvested corn for all yr combined and in 2 out of 3 individual yr. The differences were significant (*t*-test, $P < 0.05$) in 2002 and for all yr combined for Bt hybrids, non-Bt hybrids, and all hybrids considered together. Corn grown in Arkansas is seldom harvested with fumonisin levels below the FDA advisory level for direct human consumption of 2 ppm total fumonisins (Abbas *et al.* 2006), so the FDA advisory level for feeding corn to swine (20 ppm) was used to define heavy contamination. Early May planting of corn resulted in a higher frequency of heavy fumonisin contamination of harvested corn for all yr combined, and in each individual yr except 2005, when no incidences of heavy contamination occurred. The increased frequency of heavy contamination was significant (chi-square test) in all yr combined ($P < 0.01$) and in 2004 ($P < 0.05$). Average fumonisin levels were consistently higher in corn harvested from non-Bt hybrids than in corn from Bt hybrids at both early and late plantings in each of the 3 yr studied (Table 3), and the differences were significant (*t*-test, $P < 0.05$) only for all plantings combined (non-Bt hybrids = 14.3 ± 1.8 ppm; Bt hybrids = 9.0 ± 0.0 ppm) and for all early plantings combined. Only in 2004 were fumonisin levels significantly higher (*t*-test, $P < 0.05$) in corn harvested from non-Bt hybrids when combined and early plantings were considered. These observations are generally consistent with previous observations in Arkansas (Abbas *et al.* 2006) that fumonisin levels in corn harvested from Bt hybrids were significantly lower than in non-Bt corn.

Table 3. Effect of planting date on total fumonisin content of corn

Type and contamination ratio[1] of hybrids	Mean total fumonisin levels in corn from the indicated planting date (ppm)							
	2002		2004[3]		2005		All years[3]	
	April	May	April[3]	May	April	May	April[3]	May
Non-Bt	7.1 ± 4.6[2]	23.9 ± 5.4[2]	11.4 ± 0.9	20.9 ± 6.7	12.8 ± 1.7	9.8 ± 1.6	10.6 ± 1.5[2]	17.9 ± 3.1[2]
ratio	1/5	3/5	0/6	3/6	0/6	0/6	1/17	6/17
Bt	1.2 ± 0.6[2]	21.1 ± 7.1[2]	5.9 ± 1.1	7.5 ± 3.0	9.6 ± 2.6	7.5 ± 1.6	5.1 ± 1.4[2]	13.0 ± 3.5[2]
ratio	0/4	2/4	0/3	0/3	0/3	0/3	0/10	2/10
Total	4.5 ± 2.6[2]	22.7 ± 4.1[2]	9.5 ± 1.1	16.4 ± 5.0	11.7 ± 1.5	9.0 ± 1.2	8.6 ± 1.2[2]	16.0 ± 2.4[2]
ratio	1/9	5/9	0/9[4]	3/9[4]	0/9	0/9	1/27[5]	8/27[5]

[1] Contamination ratio = the number of hybrids with fumonisin levels above the US Food and Drug Administration (FDA) regulatory agency advisory level of 20 ppm for feeding swine over the total number of samples examined.
[2] Significantly lower fumonisin levels in corn from mid-April planting than from early May planting ($P < 0.05$, *t*-test).
[3] Significantly lower fumonisin levels in Bt corn than in non-Bt corn ($P < 0.05$, *t*-test).
[4] Significantly less frequent contamination above the FDA swine advisory level for corn from mid-April planting than from early May planting ($P < 0.05$, chi-square test).
[5] Significantly less frequent contamination above the FDA swine advisory level for corn from mid-April planting than from early May planting ($P < 0.01$, chi-square test).

LC/MS analyses showed that fumonisin subtypes B_1, B_2, B_3, B_4, A_1 and C_1 were present in most samples tested (Figs. 2, 3, 4; Table 4), whereas fumonisin subtypes A_2, A_3 and C_4 were not detected. Individual fumonisin subtypes were examined to determine if any alterations in the total amount of fumonisins could be the result of a relatively large alteration in one or two individual subtypes, or if all subtypes were being altered in similar directions by similar amounts. There was no significant difference (t-test, $P > 0.05$) between early and late planting in the percentage of any fumonisin subtype measured, indicating that all fumonisin subtypes were altered in a similar manner. Similarly, there were no consistent significant differences (t-test, $P > 0.05$) between percents of fumonisin subtypes in Bt and non-Bt corn, which could account for differences in total fumonisin levels between Bt and non-Bt corn. The study also observed frequent co-occurrence of both aflatoxin and fumonisin in corn samples.

The hypothesis that reducing plant stress during the kernel-filling period will result in reduced mycotoxin levels in harvested corn was investigated in this study by determining if certain measures of heat and drought correlated with mycotoxin levels. Table 5 summarizes measures of heat and drought for the three sets of early and late kernel-filling periods, and the observed aflatoxin and total fumonisin levels in corn harvested from all hybrids maturing during those periods. Aflatoxin contamination in all hybrids correlated significantly with all five measures of heat stress examined (with T max: $R^2 = 0.68$, $P < 0.05$; with

T min: $R^2 = 0.81$, $P < 0.05$; with DD20: $R^2 = 0.76$, $P < 0.05$; with MaxD20: $R^2 = 0.67$, $P < 0.05$; with MinD20: $R^2 = 0.70$, $P < 0.05$, regression analysis), but in each case the correlation with heat stress was inverse. The association of higher aflatoxin contamination with lower measures of air temperature is contrary to most previous studies (Abbas *et al.* 2002, 2006; Bruns 2003; Diener *et al.* 1987; Payne 1992). These observations suggest that for corn in Arkansas, some factor(s) other than the measures of heat stress used in this study are of primary importance in determining aflatoxin contamination levels in harvested corn. Total fumonisin contamination in all hybrids did not correlate significantly with any of the five measures of heat stress examined ($P > 0.05$, regression analysis).

The following conclusions can be drawn from this study. (1) The mid-April planting date resulted in lower levels of aflatoxin contamination in harvested corn each yr, and in significantly less frequent incidences of contamination levels in excess of the regulatory action level in 2005 and overall, than did the early May planting date in both Bt and non-Bt corn. (2) The mid-April planting date resulted in significantly lower levels of total fumonisin contamination in harvested corn, and in less frequent incidences of contamination levels in excess of the regulatory advisory level in 2 of 3 yr and overall in both Bt and non-Bt corn. (3) Aflatoxin B_1 was the only subtype of aflatoxin observed, whereas fumonisin subtypes B_1, B_2, B_3, B_4, A_1 and C_1 were present in most samples tested, but

Table 4. Relative amounts of individual fumonisin types in corn from early and late planting dates during the 3 yr investigated

| Fumonisin subtype | Type of corn hybrid | Mean percent fumonisin type (± SEM) | | | | | | |
| | | 2002 | | 2004 | | 2005 | | All Samples |
		April	May	April	May	April	May	
FB$_1$	Non-Bt	58.0 ± 1.7*	59.3 ± 1.2*	71.2 ± 1.4	68.2 ± 2.1	69.7 ± 1.0	72.3 ± 6.0	66.9 ± 1.5
	Bt	46.6 ± 6.6*	46.9 ± 12.1*	68.2 ± 0.8	79.5 ± 6.8	69.9 ± 1.7	70.3 ± 1.3	61.9 ± 3.9
	Total	52.9 ± 1.7	53.7 ± 2.4	70.2 ± 0.6	72 ± 0.3	69.8 ± 1.9	71.6 ± 1.3	65.0 ± 1.7
FB$_2$	Non-Bt	25.5 ± 0.6	25.2 ± 1.0	17.2 ± 1.0	17.2 ± 2.0	17.3 ± 0.5	20.7 ± 2.7	20.2 ± 0.8
	Bt	25.1 ± 3.5	23.5 ± 5.4	18.1 ± 1.0	18.4 ± 3.1	18.7 ± 0.8	16.6 ± 3.9	20.5 ± 1.0
	Total	25.3 ± 0.7	24.4 ± 1.2	17.5 ± 0.7	17.6 ± 1.3	17.8 ± 0.7	19.3 ± 1.9	20.3 ± 0.6
FB$_3$	Non-Bt	10.7 ± 1.4	9.4 ± 0.2	6.8 ± 0.4	6.2 ± 0.7	7.8 ± 0.3	7.6 ± 0.6	7.9 ± 0.4*
	Bt	11.9 ± 1.2	10.3 ± 0.4	6.8 ± 1.4	7.6 ± 1.7	8.1 ± 1	8.6 ± 0.8	9.1 ± 0.6*
	Total	11.2 ± 0.9	9.8 ± 0.2	6.8 ± 0.5	6.6 ± 0.7	7.9 ± 0.4	7.9 ± 0.5	8.4 ± 0.3
FB$_4$	Non-Bt	6.9 ± 0.7	6.1 ± 0.2	3.3 ± 0.3*	3.1 ± 0.5	3.4 ± 0.2	3.8 ± 0.6	4.3 ± 0.3
	Bt	5.7 ± 1.2	6.8 ± 0.4	5.1 ± 0.3*	3.8 ± 0.4	2.7 ± 0.6	3.0 ± 0.3	4.7 ± 0.4
	Total	6.3 ± 0.6	6.4 ± 0.2	3.9 ± 0.4	3.4 ± 0.4	3.2 ± 0.3	3.5 ± 0.4	4.4 ± 0.2
FA$_1$	Non-Bt	0 ± 0	0 ± 0	0.9 ± 0.4	1.3 ± 0.3	1.2 ± 0.4	0.5 ± 0.2	0.7 ± 0.1
	Bt	0 ± 0	0 ± 0	1.0 ± 1	1.1 ± 0.8	0.9 ± 0.4	0.7 ± 0.7	0.6 ± 0.2
	Total	0 ± 0	0 ± 0	0.96 ± 0.37	1.2 ± 0.3	1.1 ± 0.3	0.6 ± 0.3	0.6 ± 0.1
FC$_1$	Non-Bt	0 ± 0	0 ± 0	1.2 ± 0.3	1.8 ± 0.3	1.0 ± 0.5	0.4 ± 0.2	0.8 ± 0.2*
	Bt	0 ± 0	0 ± 0	0.4 ± 0.4	0.7 ± 0.4	0.6 ± 0.3	0.4 ± 0.4	0.3 ± 0.1*
	Total	0 ± 0	0 ± 0	0.9 ± 0.3	1.4 ± 0.3	0.9 ± 0.3	0.4 ± 0.2	0.6 ± 0.1

* Significantly different amounts of the fumonisin subtype in Bt and non-Bt corn ($P < 0.05$, t-test).

Table 5. Summary table of weather-related factors and aflatoxin and total fumonisin contents in harvested corn during the kernel-filling periods studied

Year	Planting time	Mean aflatoxin level (ppb)	Mean total fumonisin level (ppm)	T max (avg. max air temp)* (°C)	T min (avg. min. air temp)* (°C)	Total DD20*	Total MaxD20*	Total MinD20*	Rainfall
2002	Early	0.80 ± 0.43	4.5 ± 2.6	32.5	22.4	+372.2	+625.0	+123.8	20.9
	Late	5.3 ± 5.0	22.7 ± 4.1	32.5	21.8	+357.8	+627.1	+97.7	7.9
2004	Early	12.3 ± 11.5	9.5 ± 1.1	29.6	19.0	+214.1	+479.1	+34.9	7.9
	Late	38.7 ± 19.4	16.4 ± 5.0	29.2	17.8	+175.4	+460.2	+18.3	5.1
2005	Early	4.2 ± 0.9	11.7 ± 1.5	32.0	21.5	+337.3	+600.1	+84.4	13.9
	Late	10.0 ± 4.7	9.0 ± 1.2	32.0	20.6	+316.5	+602.2	+65.1	11.2

* Significant inverse correlation with mean aflatoxin levels ($R^2 > 0.66$; $P < 0.05$, regression analysis).

subtypes A_2, A_3 and C_4 were not observed. (4) The observed changes in total fumonisin levels were consistent with similar changes occurring in the levels of all subtypes and with small changes in selected subtypes. (5) Frequent co-occurrence of both aflatoxin and fumonisin in corn samples was observed. (6) Fumonisin contamination in corn harvested from Bt hybrids was lower than from non-Bt hybrids at every planting, but the reductions were significant only sporadically (i.e. 1 in 3 yr) for fumonisin and aflatoxin. (7) The lower levels of aflatoxin and fumonisin contamination observed with the mid-April planting could not be explained by greater heat stress during the kernel-filling period. Either the role of heat stress during the kernel-filling period is less important in determining mycotoxin contamination levels than was assumed for the purposes of this study, or other factors not measured in this study are the primary determinants of mycotoxin levels in Arkansas corn.

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