Dynamics of Mycotoxin and *Aspergillus flavus* Levels in Aging Bt and Non-Bt Corn Residues under Mississippi No-Till Conditions

HAMED K. ABBAS,*,† CESARE ACCINELLI,‡ ROBERT M. ZABLOTOWICZ,§
CRAIG A. ABEL,‖ H. ARNOLD BRUNS,† YANHONG DONG,⊥ AND
W. THOMAS SHIER#

USDA-ARS, Crop Genetics and Production Research Unit, Stoneville, Mississippi 38776; Department
of Agro-Environmental Science and Technology, University of Bologna, 40127, Bologna, Italy;
USDA-ARS, Southern Weed Science Research Unit, Stoneville, Mississippi 38776; USDA-ARS,
Southern Insect Management Research Unit, Stoneville, Mississippi 38766; Department of Plant
Pathology, College of Food, Agricultural, and Natural Resource Sciences, University of Minnesota;
and Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota,
Minneapolis, Minnesota 55455

Mycotoxin and *Aspergillus flavus* levels in soil-surface corn debris left by no-till agriculture methods (stover, cobs, and cobs with grain) were determined during the December–March fallow period for near-isogenic Bt and non-Bt hybrid corn. By December, average mycotoxin levels in non-Bt corn were many times higher in cobs with grain than in grain harvested in September (total aflatoxins, 774 vs 211 ng/g; total fumonisins, 216 vs 3.5 μg/g; cyclopiazonic acid, 4102 vs 72.2 μg/g; zearalenone, 0.2 vs < 0.1 μg/g). No trichothecenes were detected. Levels of mycotoxins and *A. flavus* propagules were ~10- to 50-fold lower in cobs without grain and stover, respectively, for all mycotoxins except zearalenone. Mycotoxin levels in corn debris fractions decreased during winter but began to rise in March. Levels of all mycotoxins and *A. flavus* propagules were lower in harvested grain and debris from Bt than non-Bt corn, but differences were significant ($p < 0.05$) only for aflatoxins.

KEYWORDS: Aflatoxin; *Aspergillus flavus*; Bt-corn; crop residues; cyclopiazonic acid; fumonisin; zearalenone; trichothecenes

INTRODUCTION

In the southern United States corn (maize, *Zea mays* L.) is commonly grown in rotation with cotton (*Gossypium hirsutum* L.) or soybean, (*Glycine max* L. Merr.) (*1–3*). Corn is used for animal feeds, direct human consumption, and production of industrial products (i.e., biofuels, starch or alcohol beverages). Planted corn acreages in the U.S. have increased by 17% in the past 15 yrs (*4*). The Mississippi Delta has seen planted acres double in less than 5 years, in part due to a high demand for use of corn for biofuel (*5*). Corn kernels frequently can become infected with toxigenic fungi such as *Aspergillus flavus* and *Fusarium* species (*6–8*), which produce aflatoxin, cyclopiazonic acid, fumonisin, trichothecenes, and zearalenone (F-2) (*9–11*). Contamination of corn with these toxins poses serious health

risks to human and animals (*12–14*). Aflatoxins are carcinogenic compounds, which can be produced by *A. flavus*, and *A. parasiticus*, and they can be an especially serious problem for corn grain and residues produced in the southern U.S. (*12, 15, 16*). Cyclopiazonic acid, produced by many *Aspergillus* spp. and other fungi (*17*), is found in contaminated maize, beans, and nuts and can have toxicological effects on livestock (*18*). Fumonisins are potential carcinogens produced by *Fusarium verticillioides* (syn. *F. moniliforme*), which are often associated with contaminated corn (*19*). Trichothecene group B toxins, including deoxynivalenol (DON), its 3-acetyl- and 15-acetyl-derivatives, and nivalenol, cause vomiting, food refusal, and mortality and may be as important as aflatoxins and fumonisins in causing immunosuppression (*12, 20*). Trichothecene group B toxins are produced by various species of *Fusarium*, including *F. graminearum* and *F. culmorum* (*20–25*). Zearalenone, produced by *F. graminearum*, *F. culmorum*, and other *Fusarium* species, has been found in corn, cereals, hay, silage, and pasture and has been associated with estrogenic syndrome in swine around the world (*9, 26–29*).

The majority of aflatoxin research, particularly with corn, has focused on harvested grain, so that only limited information

* To whom correspondence should be addressed. Phone: 662-686-5313. Fax: 662-686-5281. E-mail: Hamed.Abbas@ars.usda.gov.
† USDA-ARS, Crop Genetics and Production Research Unit.
‡ University of Bologna.
§ USDA-ARS, Southern Weed Science Research Unit.
‖ USDA-ARS, Southern Insect Management Research Unit.
⊥ Department of Plant Pathology, University of Minnesota.
Department of Medicinal Chemistry, University of Minnesota.

Mycotoxins in Corn Residues

J. Agric. Food Chem., Vol. 56, No. 16, 2008 **7579**

exists about the fungal ecology and mycotoxins in overwintering residues. Studies in southern Mississippi have shown considerable accumulation of aflatoxin (5−5000 ng/g [ppb], mean of 427 ng/g) associated with corn cobs remaining on the soil surface, where they may pose a potential risk to Sandhill cranes (*Grus Canadensis* Pulla) (*30*). In Texas (*31*), high levels of *A. flavus* propagules survive in corn residues, especially in the cobs. Waste corn on soil near storage bins was studied by Olanya et al. (*32*), who observed that waste corn infested with *A. flavus* served as point sources of inoculum for *A. flavus* infection of corn in the surrounding agroecosystem. Despite these observations, *A. flavus* populations and mycotoxin levels in corn residue have traditionally been ignored on the assumption that after harvesting corn, the fields would be tilled and contaminated residues would not significantly increase the number of *A. flavus* propagules surviving in the soil (*31*). However, with greater than 70% of corn production in the U.S. now using some form of reduced tillage practice, the corn residue remaining on the surface could be a major factor in crop contamination in the next growing season, particularly with *A. flavus*.

Aspergillus flavus is abundant in soil, and the soil reservoir is believed to be the source of the fungal inoculum in the crop planted in the subsequent growing season. Little is known about the soil ecosystem and what factors affect the amount *A. flavus* found there and the proportion which is aflatoxigenic. No-till cultivation methods provide *A. flavus* with an additional ecosystem in which to overwinter in the form of stover, cobs, and kernels on cobs, which remain on the surface with limited contact with soil.

A research program at Stoneville, Mississippi, has been established to understand how surface corn debris left by no-till cultivation methods affects the levels and toxigenicity of mycotoxin-producing fungi in the field. It has been well-established that plant debris persists longer on the surface of the soil than in it (*33*), and thus, no-till agricultural practices should make a greater contribution to the fungal inoculum-producing capacity of the ecosystem the following growing season. In the present study, we have examined the persistence of several economically important mycotoxins (aflatoxins, fumonisins, zearalenone, cyclopiazonic acid, and trichothecenes) in corn grain and three corn debris fractions over the winter intercropping period in Mississippi. To determine if the presence of high levels of a mycotoxin persisting in corn debris favors the growth of a fungus that produces that mycotoxin, the density of *A. flavus* propagules in corn debris fractions during the intercropping period in Mississippi has been examined. The study has been carried out with Bt and non-Bt near-isolines in an experimental design that allows an assessment of the effect of Bt status on mycotoxins and fungi in this system.

MATERIALS AND METHODS

Field and Crop Management. Two corn hybrids, one with the Cry1Ab expressing event MON810 (Pioneer brand 34B24) and its conventional non-Bt near-isoline (Pioneer brand 34B23), were planted in a replicated experiment at Elizabeth, Mississippi, on May 12, 2006, and harvested on September 8, 2006. The soil at this site is a Dundee silt clay loam (fine-silty, mixed, thermic Aeric Ochraqualfs) containing 32% sand, 60% silt, and 8% clay with 0.5% organic carbon. The corn received only a preplanting application of 179 kg/ha of N applied as NH_4NO_3, to achieve a yield potential of 12.5 t/ha grain. On the basis of soil analysis from a commercial laboratory (Petit Soil Testing Laboratory, Leland, MS), no supplemental P and K fertilizer was needed. The experimental design in the field was a randomized complete block design replicated in five blocks. Plots received supplemental furrow irrigation only once at the V9 stage to mitigate drought stress.

Herbicides and fertilizer (application based on soil tests) were applied according to standard cultural practices in corn for a continuous production system in northern Mississippi. Row spacing was 1 m and each experimental unit (plot) was 32 rows wide and 30.5 m long. Each plot of corn was bordered by at least 30.5 m of soybeans on all sides. Plants were machine-harvested at maturity in three eight-row strips, bulk sampled, and oven-dried for analysis for mycotoxin levels and *Aspergillus* propagules.

Corn Residues. Corn residues were collected from the same experimental corn field using the same three sample zones per plot monthly from December, 2006, to March, 2007. The corn residues were separated into 3 plant part groups: (1) stover (leaves and stalks); (2) cobs without grain; (3) cobs with grain (**Figure 1**). Each group of tissue was a composite of samples from five locations within the subplot, which were stored separately in large no. 20 paper bags. Samples were dried at 50 °C for 72 h and ground sufficiently to pass a 20 mesh using a Romer mill (Union, MO), and subsamples were stored at (−20 °C) for later chemical and (4 °C) biological determinations.

Enumeration of *Aspergillus flavus* Propagules in Soil, Corn, and Corn Residues. The size of the *A. flavus* population in soil was estimated using the method described by Horn et al. (*34*), using modified dichloronitroaniline rose bengal agar supplemented with 3% NaCl for increased selectivity (mDRBA). For the enumeration of *A. flavus* in corn or corn residue samples, 1.0 g was suspended in a 100 mL agar solution (2 g/L), shaken for 30 min, and serially diluted as needed; duplicate samples were plated onto mDRBA. Plates were incubated at 37 °C for 5 days, and colonies were counted. Colonies of *A. flavus* were randomly picked (40 colonies per plot) and transferred to β-cyclodextrin (0.3%) potato dextrose agar at 28 °C for 5 days in the dark. Aflatoxin-producing isolates were identified following exposure to UV light at 365 nm. Colonies that developed the characteristic blue fluorescence were counted as toxigenic isolates. Prior research has indicated a greater than 90% agreement with HPLC analysis and fluorescence on β-cyclodextrin potato dextrose agar (*35*). Aflatoxin production was further confirmed by observing color change of isolates exposed to aqueous ammonium hydroxide (27% v/v) for 30 min (*35*). Density of *A. flavus* colony forming units (cfu) was calculated on a soil dry weight basis and all cfu data was transformed on a log (10) scale. The detection limit of *A. flavus* was log 1.9 cfu/g substrate (*36*).

Chemicals and Reagents. All solvents were HPLC grade from Fisher Scientific (Pittsburgh, PA). Standards for all mycotoxins that were assayed and other chemicals were purchased from Sigma (St. Louis, MO).

Analyses of Trichothecenes and Zearalenone. Trichothecene and zearalenone analyses used the same extraction, cleanup, derivatization, and injection procedures, and the same gas chromatography−mass spectrometry (GC-MS) instrumentation but with different oven temperature programs and different ions monitored (*37*). Briefly, a 4 g subsample of ground cobs or ground cobs with grain was extracted with 32 mL of acetonitrile/water (84:16 v/v) while 2 g of ground stover was extracted with 20 mL of acetonitrile/water (84:16, v/v). The sample was placed on a shaker for 2 h, and then 5 mL of extract was passed through a column packed with C18 and aluminum oxide (1:3). A 2 mL aliquot of the filtrate was evaporated to dryness under nitrogen and derivatized by the silylating reagent [*N*-trimethylsilylimidazole (TMSI) to trimethylchlorosilane (TMCS), 100:1] (Pierce Chemical Co., Rockford, IL). A 1 mL solution of TMS derivatives was filtrated through a Whatman grade 589/2 filter paper disk in a 1 mL plastic syringe, and a 1 μL aliquot was analyzed by GC-MS on a GCMS-QP2010 instrument (Shimadzu Corporation, Kyoto, Japan) using as the oven temperature program 150 °C for 1 min and then 30 °C /min to 280 °C, holding 5 min (*37*). The trichothecenes measured were as follows: deoxynivalenol (DON) at fragment ions of *m/z* 235.15, 259.20, and 422.30; 3-acetyl-DON at fragment ions of *m/z* 377.25 and 392.25; 15-acetyl-DON at fragment ions of *m/z* 193.15 and 392.25; and nivalenol (NIV) at fragment ions of *m/z* 289.20 and 379.25. A high-pressure injection method (300.0 kPa, 1 min) was used with the instrument's standard splitless injector. Stream splitting was not necessary due to the small sample size. Zearalenone was analyzed by GC-MS, because the required instrumentation and method were available. Linear velocity of flow
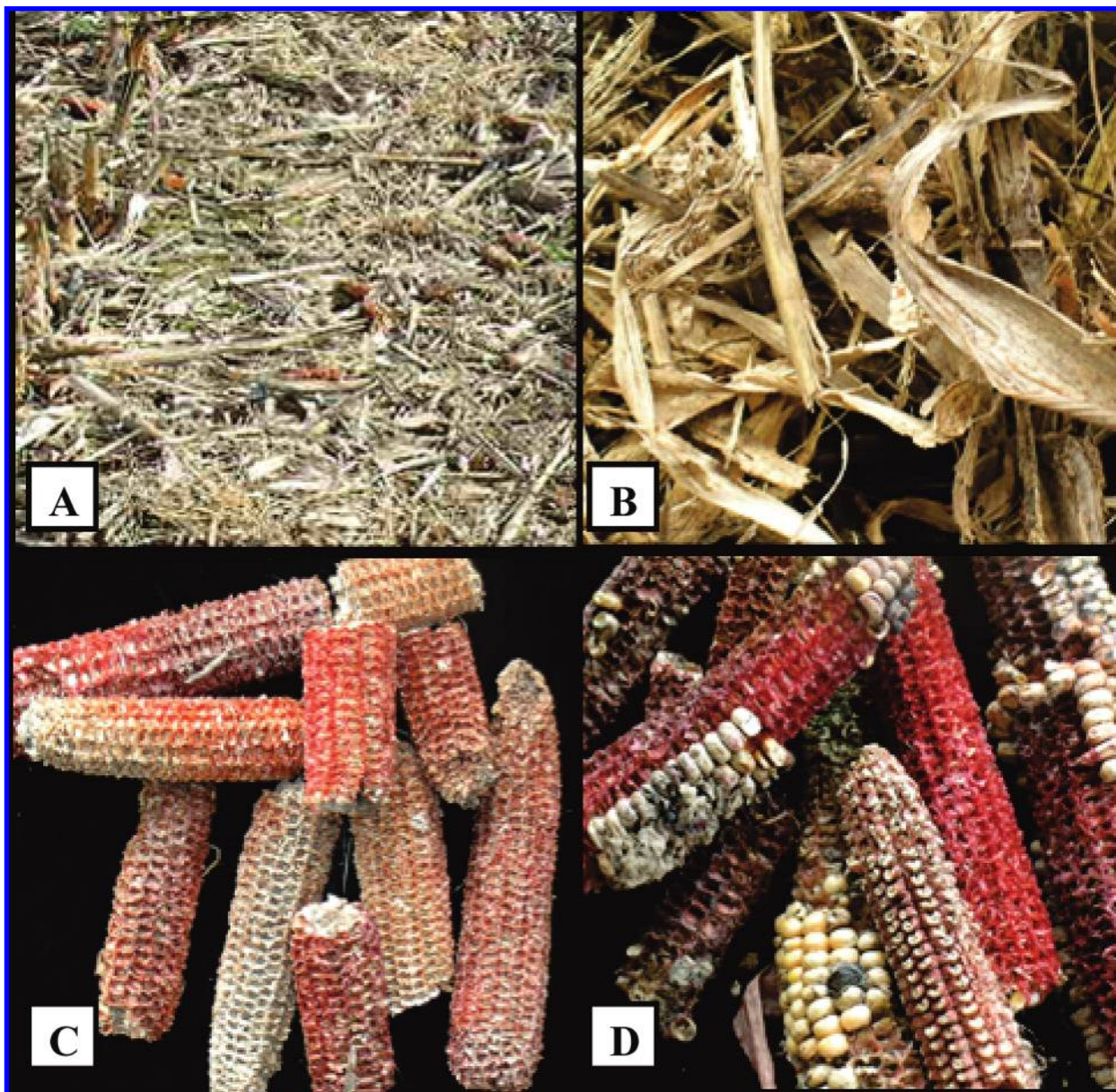
Figure 1. Illustrations of (**A**) corn residue coverage in December, (**B**) stover (leaf and stalk), (**C**) cobs without grain, and (**D**) cobs containing grain. Note that the amount of residual grain on cobs sampled in the study is typically 40% of the grain initially on the cob, and fungal colonization of the grain is apparent.

control mode was used with the following oven temperature program: 150 °C for 1 min and then 30 °C /min to 300 °C, holding 4 min. Injection, ion source, and interface temperatures were kept at 290, 220, and 300 °C, respectively, with an injection volume of 1 μL. Zearalenone was detected using selected ion monitoring (SIM) with an electron ionization energy of 70 eV. The fragment ions of m/z 333 and 462 were used for zearalenone quantitation. Limits of quantification for all these toxins are 0.05 μg/g (*37*).

Extraction of Aflatoxin, Cyclopiazonic Acid, and Fumonisin. Ground corn or corn residues (20 or 5 g, respectively) were extracted with 100 mL of 70:30 methanol/water at 150 rpm for 3 min. The extract was clarified by filtration (Whatman #1 paper) and further clarified by centrifugation if required. Aliquots were removed for aflatoxin, cyclopiazonic acid, and fumonisin analysis and stored at −20 °C until cleanup and analysis.

Determination of Aflatoxin. Sample cleanup was performed using a modification of Sobolev and Dorner (*38*). Briefly, an aliquot (800 μL) of reconstituted sample was processed using a 1.5 mL extract-clean reservoir minicolumn packed with aluminum oxide (Alltech Co., Deerfield, IL). After elution by gravity, 20 μL of the eluate was injected on a HPLC system equipped with a 150 mm × 3.9 mm i.d., 4 μm

Nova-Pak C18 column, and a 474 model fluorescence detector (Waters Corporation, Milford, MA). Separation was carried out at 30 °C, with a mobile phase consisting of water/methanol/1-butanol (60:25:1) and a flow rate of 0.9 mL/min. Detection of AFB$_1$ was achieved by photochemical post-column derivitazation (*38*) setting the detector wavelength at 365 nm (excitation) and 440 nm (emission). Limits of quantification for the aflatoxin were 5 ng/g (AFB$_1$, AFG$_1$) and 1.5 ng/g (AFB$_2$, AFG$_2$).

ELISA Analysis of Aflatoxins and Fumonisins. Commercially available quantitative ELISA assay kits (Neogen Inc., Lansing, MI) were used to calculate total aflatoxins and total fumonisins and dilutions required for HPLC or LC/MS analysis, according to the manufacturer's instructions. These estimates were used to predict dilutions required to obtain analytical limitations and secondary confirmation.

Liquid Chromatography/Mass Spectrometry (LC/MS) Analysis of Fumonisin and Cyclopiazonic Acid. Samples from each plot of the study were examined by liquid chromatography/electrospray ionization/mass spectrometry (LC/ESI/MS) to analyze fumonisin identity and determine the relative amounts of subtypes of fumonisins (FB$_1$, FB$_2$, FB$_3$, FB$_4$) and cyclopiazonic acid. Cleanup of the fumonisins was accomplished on Bond-Elute SAX columns (Varian, Harbor City,

CA) by the method of Plattner (*39*) with minor modifications. The SAX column was 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 of water containing 2% acetic acid. Samples were evaporated under a stream of nitrogen to dryness and stored at 5 °C. Prior to analysis, the dried sample was reconstituted in 1 mL of acetonitrile/water (1:1).

LC/ESI/MS analyses were carried out as described in detail by Abbas et al. (*6, 40*) 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) (M + H). MS/MS was performed on *m/z* 722 for further confirmation of FB_1. Fumonisin analysis used a MetaChem Intersil 5 μm ODS-3 column eluted with water/acetic acid (1%)/methanol (65:35:0) at 300 μL/min for 10 min, followed by a linear gradient to water/acetic acid (1%) in methanol/methanol (5:35:65), then held constant for 10 min. Between samples, the solvent was returned to water/acetic acid (1%) in methanol (65:35) within 1 min and held constant for 4 min for column equilibration. Samples suspected to contain higher concentrations were diluted and 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. Detection limits for all types of fumonisin were 0.1 μg/g.

For analysis of cyclopiazonic acid, methanol extracts of residues were diluted 1:1 with ammonium hydroxide (5%), subjected to a cleanup procedure using Oasis Max solid phase extraction columns (Waters, Milford, MA), and eluted with 2% formic acid in methanol. Analyses were carried out on a Polar RP column (Phenomex, Torrance, CA) eluted with a gradient from ammonium acetate (10 mM, pH 5.7)/methanol (30:70, v/v) to ammonium acetate (10 mM, pH 5.7)/methanol (5:95, v/v) over 5 min, then held constant for 7 min. Cyclopiazonic acid eluted at 6 min. MS specifications are reported elsewhere (*41*). The limit of detection for cyclopiazonic acid by LC/ESI/MS/MS was 12 ng/g.

Statistical Analysis. Mycotoxin concentrations in corn grain were analyzed using PROC GLM (SAS Institute) and mean separation conducted using Fisher's LSD with analysis as a randomized complete block design with five replicates. The corn residue component data, including mycotoxins, *A. flavus* propagules, and their toxigenicity, were analyzed as a split split plot design. The main unit of treatment (Bt corn versus non-Bt corn isoline) was established as a randomized complete block design with five replicates. The two subunits were plant parts and time. Each subunit treatment was a repeated triplicate measure in time or location. All data were subjected to analysis of variance using PROC MIXED (SAS Institute) to assess the effects of corn isoline (Bt or non-Bt), type of residue (stover, versus cobs, or cobs with grain), time of sampling, and the interaction of these variables. *p* Values < 0.05 were considered significant. Pearson correlation coefficient analysis was carried out using PROC MIXED (SAS Institute). All mycotoxin data did not follow a normal distribution; therefore, concentration data were transformed using a log (10) of value +1. Following data analysis, geometric means were ascertained by back-transforming log data, using SAS and the +1 subtracted. Data on *A. flavus* propagules and aflatoxigenicity was also subjected to analysis of variance using PROC MIXED (SAS Institute) to assess the effects of corn isoline, type of residue or soil and the interaction of these variables. Least significant differences (*p* = 0.05 level) used in mean separation was calculated using pdmix800 (SAS Institute).

RESULTS AND DISCUSSION

Mycotoxin Levels in Harvested Corn Grain. Table 1 gives the levels of myctoxins in corn kernels harvested at a typical time in Mississippi (September) from the Bt and non-Bt near isolines used to study mycotoxins in debris samples. The

**Table 1.** Mycotoxin Levels in Mature Bt and Non-Bt Corn Grains Harvested in September

mycotoxin (unit)	Bt corn	non-Bt corn	probability > *F*
aflatoxin B1 (ng/g)	104 ± 30[a]	200 ± 53.8	0.039
aflatoxin B2 (ng/g)	4.9 ± 1.4	10.8 ± 8.4	0.102
total aflatoxin (ng/g)	109 ± 32	211 ± 58	0.0432
total fumonisin (μg/g)	1.6 ± 0.2	3.5 ± 0.8	0.0683
cyclopiazonic acid (ng/g)	61.0 ± 14.3	72.2 ± 19.1	0.4763
zearalenones (μg/g)	<0.1	<0.1	
trichothecenes (μg/g)	<0.1	<0.1	

[a] Mean and standard error of the average of five replicates.

aflatoxin B_1 and total aflatoxin levels were 93% higher in kernels from the non-Bt corn hybrid compared to the Bt isoline expressing the cry1 protein (**Table 1**). Aflatoxin B_2 was approximately the same amount higher in kernels from the non-Bt corn hybrid compared to the Bt isoline, but the difference was not statistically significant. Aflatoxin G_1 and G_2 were found in only one of 30 samples tested (<10 ng/g). The level of aflatoxin found in the grain harvested from all plots was above the FDA regulatory limits for direct human consumption (20 ng/g) but not above the FDA action level for finishing beef cattle (*42*). Minimal irrigation was applied, and the month prior to harvest was hot with an average maximum temperature of 36 °C, and less than 4 cm of rain fell the month prior to harvest. Although the use of the Bt hybrid significantly reduced aflatoxin levels, the reduction was insufficient to meet regulatory guidelines for direct human consumption. The Bt hybrid is expected to reduce damage inflicted by the corn ear worm and thus reduce *A. flavus* contamination carried by the insect (*43*). However, aflatoxin reductions achieved by this mechanism are not always observed (*44*).

Total fumonisin contamination in harvested Bt corn was less than half that in non-Bt corn (**Table 1**), but the difference was not significant (*p* = 0.068). Many studies reported in the literature (*43–47*) have observed greater effectiveness of the Bt gene in reducing fumonisin contamination in corn than in reducing aflatoxin contamination compared to that in non-Bt hybrids. There was no significant difference in cyclopiazonic acid (CPA) levels in corn from Bt and non-Bt hybrids. A linear regression analysis was conducted to assess the relationship between aflatoxin and cyclopiazonic acid levels in grain. There was a significant positive Pearson correlation (*p* < 0.012) between levels of total aflatoxin and cyclopiazonic acid, consistent with colonization by *Aspergillus* being the major source of contamination of both mycotoxins in the corn. Zearalenone and trichothecenes were not detected in kernels harvested in September.

Mycotoxin Levels in Corn Plant Debris Fractions in December. The highest levels of all mycotoxins were observed in the initial December sampling (**Figure 2**), and for clarity, the arithmetic means and standard error of the mean are summarized in **Table 2**. Total aflatoxin contamination was over 20-fold higher in cobs with kernels than in cobs without kernels, indicating that most of this contamination is due to the enrichment of *A. flavus* in corn grain compared to the remainder of the corn plant. Aflatoxin was detected in over 80% of cobs and in 100% of cobs with grain sampled in December. The lowest aflatoxin content was found in stover (3.8 ng/g), and it was detected in fewer than 50% of the samples tested. Total aflatoxin levels were about 6-fold greater in cobs without grain from non-Bt corn compared to Bt plots. In cobs with grain, total aflatoxin was 43% greater in non-Bt plots compared to Bt plots. Aflatoxin B_1 accounted for about 93% of the total aflatoxin
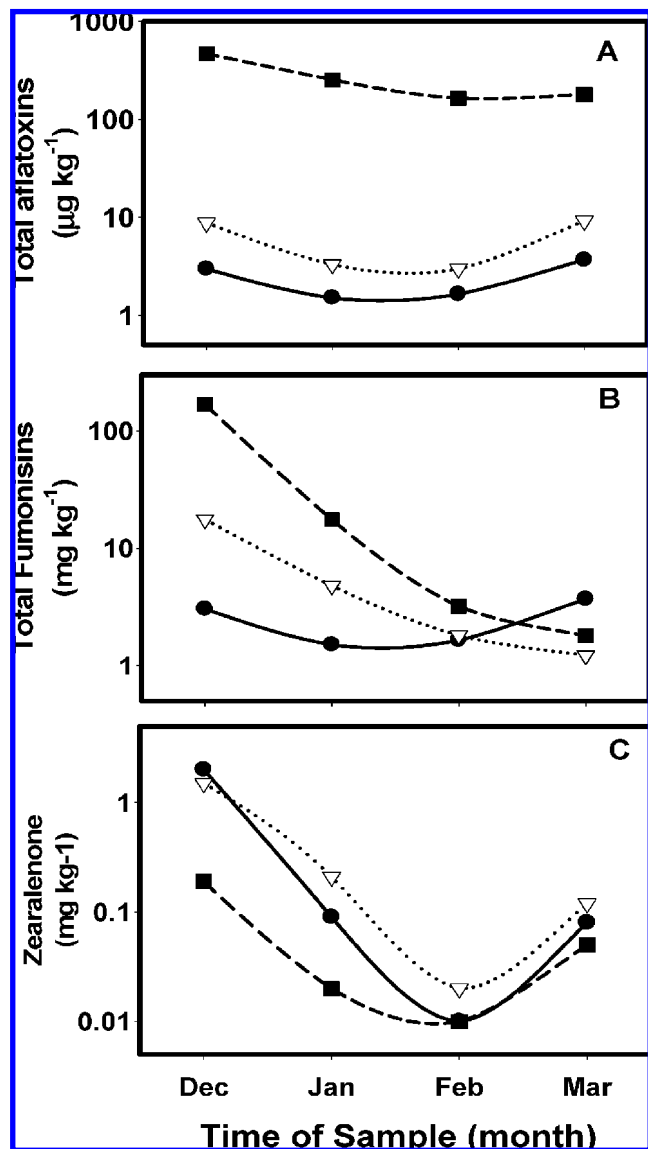
Figure 2. Levels of mycotoxins ((**A**) aflatoxin, (**B**) fumonisin, (**C**) zearalenone) remaining on the soil surface during the winter months in Mississippi in the following types of corn residues: stover (●), cobs (▽), and cobs with grain (■). Least significant ratios ($p = 0.05$) for significance among a given plant part comparing at a given month were as follows: total aflatoxin = 2.7; total fumonisins = 1.2; zearalenone = 1.2. Least significant ratios ($p = 0.05$) for significance among a given plant part comparing at a given month were as follows: total aflatoxins = 1.9; total fumonisins = 1.3; zearalenone = 1.2.

Table 2. Mycotoxin Levels in Non-Bt and Bt Corn Debris Fractions in December

corn type	mycotoxin type (unit)	mycotoxin level of debris fractions ± SEM[a]		
		stover	cobs	cobs with grain
non-Bt	aflatoxin B$_1$ (ng/g)	3.8 ± 2.5	96 ± 87	720 ± 181
	aflatoxin B$_2$ (ng/g)	<0.1	8.7 ± 8.7	54 ± 17
	total aflatoxins (ng/g)	3.9 ± 2.5	105 ± 96	774 ± 195
	fumonisin B$_1$ (μg/g)	1.8 ± 1.1	14.6 ± 2.6	129 ± 21
	fumonisin B$_2$ (μg/g)	0.4 ± 0.2	7.3 ± 1.7	65 ± 21
	fumonisin B$_3$ (μg/g)	0.2 ± 0.2	2.0 ± 0.6	10.1 ± 2.6
	fumonisin B$_4$ (μg/g)	<0.1	3.0 ± 0.4	12.4 ± 2.4
	total fumonisins (μg/g)	2.4 ± 1.3	26.8 ± 4.9	216 ± 39
	cyclopiazonic acid (ng/g)	12.0 ± 7.8	139 ± 47	4102 ± 1168
	zearalenone (μg/g)	1.3 ± 0.2	1.3 ± 0.2	0.2 ± 0.1
	trichothecenes	nd[b]	nd[b]	nd[b]
Bt	aflatoxin B$_1$ (ng/g)	3.3 ± 1.7	15.2 ± 9.0	517 ± 249
	aflatoxin B$_2$ (ng/g)	<0.1	1.3 ± 1.3	25 ± 14
	total aflatoxins (ng/g)	3.3 ± 1.7	16.5 ± 9.8	542 ± 263
	fumonisin B$_1$ (μg/g)	0.7 ± 0.3	9.8 ± 3.8	122 ± 27
	fumonisin B$_2$ (μg/g)	0.2 ± 0.1	4.0 ± 1.6	46.2 ± 11.4
	fumonisin B$_3$ (μg/g)	0.1 ± 0.1	1.7 ± 0.6	8.2 ± 2.2
	fumonisin B$_4$ (μg/g)	<0.1	2.0 ± 0.8	14.0 ± 4.1
	total fumonisins (μg/g)	0.9 ± 0.4	17.5 ± 6.5	190 ± 45
	zearalenone (μg/g)	2.7 ± 0.8	2.4 ± 0.5	0.3 ± 0.2
	cyclopiazonic acid (ng/g)	11.4 ± 7.1	156 ± 91	2780 ± 915
	trichothecenes	nd[b]	nd[b]	nd[b]

[a] SEM = standard error of the mean of five replicates. [b] nd = none detected.

significant difference in Bt versus non-Bt corn hybrids. Fumonisin B$_1$ accounted for ~60% of the total fumonisin observed in all three classes of corn residues (**Table 2**). The distribution of zearalenone in corn residues was different from that of aflatoxin and fumonisin in that the lowest zearalenone levels were observed in cobs with grain compared to cobs or stover (**Table 2**). The average zearalenone level in December was 2 μg/g in cobs and 2.5 μg/g in stover with a maximum level of 7.5 μg/g compared to 0.24 μg/g average concentration in cobs with grain. Cyclopiazonic acid levels were determined in residues in December only. Cyclopiazonic acid was observed in about 30% of stover, 80% of cobs, and 100% of cobs with grain. The highest levels of cyclopiazonic acid were found in cobs with grain (3441 ng/g), intermediate in cobs (147 ng/g), and lowest in stover (12 ng/g). Cyclopiazonic acid levels were similar in non-Bt and Bt corn residues ($p < 0.22$). No trichothecenes (DON and its derivatives) were detected in any residue samples throughout the study.

Aflatoxin Persistence in Corn Residue Fractions. Aflatoxin occurrence in various residues was affected by sample month, plant part, and corn isoline with a significant interaction between month and plant part (**Table 3**). Aflatoxin levels were highest in December, and the rates of dissipation varied with the toxin and plant part (**Figure 2A**). Levels of total aflatoxin found in March in cobs with grain represented ~30% of that found in December. Recent studies (*48*) have indicated that the half-life of aflatoxin B$_1$ is <5 days in soil collected from this study site. The most common metabolic pathway for aflatoxin B$_1$ is conversion to aflatoxin B$_2$ (*49*), which may be mediated by *A. flavus* itself or by other microorganisms. Because the relative ratios of aflatoxn B$_1$ to B$_2$ remained constant throughout the study (data not shown), this is an unlikely fate of aflatoxin in corn residues. However, biodegradation by bacterial monoxygenases is an important pathway for aflatoxin destruction in other microorganisms (*50, 51*).

Several types of observations have led to concerns that kernel corn left in fields is a potential source of mycotoxin contamination for browsing wildlife. Aflatoxin has been associated with liver dysfunction and immunosuppression in certain species of

and B$_2$ about 6%. Aflatoxin G$_1$ was found in only 2 of 240 samples, and aflatoxin G$_2$ was not detected in any samples (data not shown). A similar aflatoxin B$_1$ to aflatoxin B$_2$ ratio was found in all three types of corn residues, and the ratio was consistent for all months sampled (data not shown) and in Bt and non-Bt hybrids. The levels of aflatoxin found in cobs with grain in December (**Table 2**) were 4- to 5-fold higher than levels found in grain at harvest (**Table 1**). Cobs with grain typically had between 10 and 50% of the original kernels remaining on the cobs (**Figure 1D**). Thus, as the kernels on the cobs aged on the soil surface, additional aflatoxin production by *Aspergillus* spp. occurred.

As observed with aflatoxin, the highest levels of fumonisins were found in cobs with grain in December (average total = 190−216 μg/g). Fumonisin levels were much lower in cobs without grain and in stover residues; however, there was no

Table 3. Analysis of Variance of Total Aflatoxins, Total Fumonisins, and Zearalenone Concentrations Determined in Aging Corn Residues as Affected by Sample Date, Plant Part, and Corn (Bt) Isoline

	total aflatoxin		total fumonisin		zearalenone	
source of variance	F value	p > F	F value	p > F	F value	p > F
month	4.97	0.0074	203.49	<0.0001	51.92	<0.0001
plant part	73.48	<0.0001	450.47	<0.0001	13.86	0.0015
month × plant part	5.29	<0.0001	176.43	<0.0001	41.08	<0.0001
corn isoline (Bt)	5.83	0.0230	0.19	0.6857	1.46	0.2540
month × isoline	0.12	0.9467	3.46	0.0513	5.07	0.0136
plant part × isoline	1.46	0.2829	1.67	0.2196	0.55	0.5924
month × plant part × isoline	0.88	0.5108	3.83	0.0011	1.64	0.1353

birds. Aflatoxins are toxic to wild turkeys at 100 ng/g (*52*). However, some bird species selectively consume grain that is more highly contaminated than the average for the sample, presumably by selecting broken kernels (*53*). The higher levels of aflatoxins in corn left in the field in December than at harvest observed in this study is in agreement with Couvillion et al. (*29*), who surveyed corn near breeding grounds for sandhill cranes and observed aflatoxin levels 5-fold higher in cobs on the soil surface than in cobs remaining on the stalk in southern Mississippi. The data obtained in the present study supports these concerns by confirming the higher mycotoxin levels in winter using more reliable analytical techniques (HPLC rather than TLC) than used by Couvillion et al. (*29*).

Levels of Other Mycotoxins in Corn Plant Debris Fractions during Winter. Fumonisin occurrence in various residues was affected by sample month, plant part, and the interaction of month and plant part, but there was no effect of corn isoline (**Table 3**). Unlike with aflatoxins, the fumonisin levels declined about 10-fold between December and January (**Figure 2B**). By March the average total fumonisin level in cobs with kernels was <1% of that found in December, and they were not detected in most samples of cobs without grain and stover. Initially FB_1 was about 60% of total fumonisins and twice as abundant as FB_2, but by late winter, FB_2 levels in cobs with kernels were equal or greater in occurrence than FB_1 (data not shown). Presumably the additional hydroxyl group on FB_1 facilitates its degradation (*54*). FB_3 was rarely observed in any winter residue samples. Generally, consumption of feed containing fumonisins at greater than 30 μg/g over a prolonged period can cause toxic symptoms in animals and birds (*9, 12, 19, 55*). In corn cobs with grain, average concentrations above this level were found only in the December sample. Thus, the potential for long-term exposure of foraging livestock or wildlife to fumonisin should be minimal.

Zearalenone occurrence in various residues was affected by sample month, plant part, and the interaction of month and plant part with no effect of corn isoline (**Table 3**). Zearalenone was also degraded more rapidly than aflatoxins (**Figure 2C**). The maximum concentrations of greater than 1 μg/g were observed only in the December sample, and the high level was found in stover and cobs without grain, which are less likely to be consumed. Zearalenone was detected in about 90% of samples in December, whereas it was detected in less than 12 and 6% of January and February samples, respectively (data not shown). However, in March samples, zearalenone levels and the frequency of detectable levels increased in cobs and in stover compared to earlier in stover. The levels of most mycotoxins rose in March, presumably reflecting renewed synthesis in response to rising temperatures and moisture levels, combined with little alteration in nonbiogenic decomposition rates. The response was strongest for zearalenone, the production of which is favored by cooler temperatures (*27, 28*) than those usually

Table 4. Occurrence of *Aspergillus flavus* Propagules in Soil and Corn Residue Fractions in Bt and Non-Bt Corn during Winter Months in Mississippi

variable	type	occurrence of *A. flavus* (log$_{10}$ cfu/g)	% aflatoxigenic isolates
month	December	4.43[a]	45.4
	January	4.32	35.9
	February	4.28	25.2
	March	4.52	40.0
LSD (0.05)		0.12	9.1
plant part	stover	4.05[b]	34.8
	cobs	4.36	27.0
	cobs with grain	5.65	35.6
	soil	3.49	49.1
LSD (0.05)		0.14	6.1
corn isoline (Bt)	Bt	4.38[c]	40.0
	non-Bt	4.40	33.2
LSD (0.05)		0.13	5.8

source of variance	significance (p)	significance (p)
month	<0.0004	<0.0005
plant part	<0.0001	<0.0001
month × plant part	<0.0001	0.0003
corn isoline (Bt)	0.7615	0.0113
month × isoline	0.0915	0.7511
plant part × isoline	0.8341	0.1343
month × plant part × isoline	0.7415	0.5003

[a] Mean of all three plant parts or soil for the given month. [b] Mean for a given source (plant part or soil) averaged across all four months. [c] Mean for all plant parts and soil averaged for all months for a given corn isoline.

encountered in the more subtropical regions of the southern U.S. Zearalenone levels were similar in Bt and non-Bt corn residues ($p > 0.25$). Zearalenone can be toxic to animals such as pigs, sheep, and cattle if present at levels greater than 1 μg/g, but it is less toxic to poultry (*29*). Considering that the higher levels are not associated with cobs containing grain, which would be preferably grazed by animals or wildlife, the levels of zearalenone found in corn residues may have minimal risk to these fauna.

***Aspergillus flavus* Propagules in Soil and Corn Residues.** *Aspergillus flavus* propagules were readily isolated from all soil and corn residue samples using semiselective plating techniques (**Table 4**). A similar level of *A. flavus* propagules was observed in grain harvested from both Bt and non-Bt isolines, ~log 4.9 cfu/g with ~40% of the isolates being aflatoxigenic (data not shown). All corn residues contained higher levels of *A. flavus* propagules than soil, consistent with previous observations at harvest (*35, 56*). In December samples, propagules of *A. flavus* were found in cobs with grain at >10-fold higher levels than in cobs without grain and at >50-fold higher levels than in stover. Although there were significant differences in *A. flavus* populations at various times during the winter, these differences

7584 *J. Agric. Food Chem.,* Vol. 56, No. 16, 2008

Abbas et al.

were not as great as the enrichment in cobs with grain compared to other samples. There was no significant difference in the total number of *A. flavus* propagules in Bt and non-Bt corn debris fractions and soil, but there was a significantly higher percentage of aflatoxigenic *A. flavus* in Bt (40%) compared to non-Bt (33%) corn debris residues and soils ($p > 0.01$). Soils amended with Bt crop residues have been reported (*57*) to cause different responses in the microbial community contained in the soil, and the different responses were related to differences in composition of Bt crop residues, including starch, protein, soluble carbon, or lignin content. Although total propagule counts were not affected by the Bt genotype of the residues, altered composition of the corn residues may have affected the community structure of the *A. flavus* population in corn residues. The highest percent of aflatoxigenic *A. flavus* isolates was observed in soil, whereas cobs had the lowest percent of aflatoxigenic *A. flavus*, while stover and cobs with grain had similar intermediate percentages. These data may indicate that different populations of *A. flavus* are colonizing soil than are colonizing various corn parts. It has been suggested by Wicklow (*58*) that *A. flavus* overwinters on crop residues as sclerotia, which germinate when environmental conditions are suitable. In Texas field studies, populations of *A. flavus* associated with corn cobs (*31*) were in a similar magnitude as those found in the present study in Mississippi. Companion studies (*48*) demonstrated that aflatoxin biosynthesis genes are capable of being expressed in soil sampled in March, indicating that the *A. flavus* soil population is active even in winter months. Additional study is needed to determine if the ecosystem provided by corn debris on the soil surface results in more aflatoxin contamination of the subsequent crop, because of a larger *A. flavus* inoculum available or because of the altered percentage of aflatoxin-producing *Aspergillus* spp.

Using more sophisticated analytical techniques, this study supports previous research (*30*) indicating that high levels of aflatoxins can remain in corn debris. In addition, the present research also indicates that other mycotoxins such as fumonisins may be present at high levels in residues after harvest, although they do degrade more rapidly than aflatoxin. In contrast, lower levels of zearalenone were found in cobs containing grain, which may be more likely to be ingested by wildlife. This study also indicates that in December corn debris samples containing relatively high levels of multiple toxins are observed. Multiple toxins may have an additive toxic effects on the organism that is exposed to them (*9*). This research also confirms that corn residues contribute to maintaining the reservoir of *A. flavus* propagules on the soil surface and can provide an inoculum for *A. flavus* infestation of future years crops. These risks can be minimized by implementing tillage to bury corn residues, but this would be against the concept of sustainable agriculture intended to improve soil quality by leaving crop residues on the surface. Thus, the risks of contamination by aflatoxin and other mycotoxins need to be balanced against gains in soil quality.

LITERATURE CITED

(1) Bruns, H. A.; Pettigrew, W. T.; Meredith, W. R.; Stetina, S. R. Corn yields benefit in rotation with cotton. *Crop Manage.*, in press (doi: 10.1094/CM-2007-0424-01-RS).

(2) Reddy, K. N.; Locke, M. A.; Koger, C. H.; Zablotowicz, R. M.; Krutz, L. J. Cotton and corn rotation under reduced tillage management: impacts on soil properties, weed control, yield, and net return. *Weed Sci.* **2006**, *54*, 768–774.

(3) Pettigrew, W. T.; Meredith, M. R., Jr.; Bruns, H. A.; Stetina, S. R. Effects of a short-term corn rotation on cotton dry matter partitioning, lint yield, and fiber quality production. *J. Cotton Sci.* **2006**, *10*, 244–251.

(4) National Agricultural Statistics Service, Agricultural Statistics Board, U.S. Department of Agriculture. Corn Objective Yield Survey Data, 1992−2006. USDA.mannlib.cornell.edu/usda/current/Cornobj/Cornobj-05-21-2007.pdf.

(5) Mojovic, L.; Nikolic, S.; Rakin, M.; Vukasinovic, M. Production of bioethanol from corn meal hydrolyzate. *Fuel* **2006**, *85*, 1750–1755.

(6) Abbas, H. K.; Williams, W. P.; Windham, G. L.; Pringle, J. C., Jr.; Xie, W.; Shier, W. T. Aflatoxin and fumonisin contamination of commercial corn (*Zea mays*) hybrids in Mississippi. *J. Agric. Food Chem.* **2002**, *50*, 5246–5254.

(7) Payne, G. A. Aflatoxins in maize. *Crit. Rev. Plant Sci.* **1992**, *10*, 423–440.

(8) Zummo, N.; Scott, G. E. Interaction of *Fusarium moniliforme* and *Aspergillus flavus* on kernel infection and aflatoxin contamination in maize ears. *Plant Dis.* **1992**, *76*, 771–773.

(9) Binder, E. M.; Tan, L. M.; Chin, L. J.; Handl, J.; Richard, J. Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. *Anim. Feed Sci. Technol.* **2007**, *137*, 265–282.

(10) Mirocha, C. J.; Mackintosh, C. G.; Mirza, U. A.; Xie, W.; Xu, Y.; Chen, J. Occurrence of fumonisin in forage grass in New Zealand. *Appl. Environ. Microbiol.* **1992**, *58*, 3196–3198.

(11) Rheeder, J. P.; Marasas, W. F. O.; Vismer, H. F. Production of fumonisin analogs by *Fusarium* species. *Appl. Environ. Microbiol.* **2002**, *68*, 2101–2105.

(12) Council for Agriculture Science and Technology. Mycotoxins risks in plant, animal, and human systems, Task Force Report 139; CAST: Ames, IA, 2003.

(13) Lewis, L.; Onsongo, M.; Njapau, H.; Schurz-Rogers, H.; Luber, G.; Kieszak, S.; Nyamongo, J.; Backer, L.; Dahiye, A. M.; Misore, A.; DeCock, K.; Rubin, C. The Kenya Aflatoxicosis Investigation Group. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. *Environ. Health Perspect.* **2005**, *113*, 1779–1783.

(14) Peraica, M.; Radic, B.; Lucic, A.; Pavlovic, M. Toxic effects of mycotoxins in humans. *Bull. W. H. O.* **1999**, *77*, 754–766.

(15) *Aflatoxin and Food Safety*; Abbas, H. K., Ed.; Taylor & Francis Group: Boca Raton, FL, 2005; p 585.

(16) Diener, U. L.; Cole, R. J.; Sanders, T. H.; Payne, G. A.; Lee, L. S.; Klich, M. A. Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Ann. Rev. Phytopathol.* **1987**, *25*, 249–270.

(17) Trucksess, M. W.; Mislivec, P. B.; Young, K.; Bruce, V. R.; Page, S. W. Cyclopiazonic acid production by cultures of *Aspergillus* and *Penicillium* species isolated from dried beans, corn meal, macadamia nuts and pecans. *J. AOAC Intl.* **1987**, *70*, 123–126.

(18) Bryden, W. L. Occurence and biological effects of cyclopiazonic acid. In *Emerging Problems Resulting from Microbial Contamination*; Mixe, K., Richard, J. L., Eds.; National Institute of Hygienic Science: Tokyo, 1991; pp 127−147.

(19) NTP (National Toxicology Program). *Toxicology and carcinogenesis studies on fumonisin B1 in F344/N rats and B6CF1 mice (feed studies)*; Technical Report Series, n. 496. NIH publication no. 99-3955.; U.S. Department of Health and Human Services, National Institutes of Health: Research Triangle Park, NC, 1999.

(20) Ueno, Y. Historical background of trichothecenes problems. In *Trichothecenes−Chemical, Biological and Toxicological aspects*; Ueno, Y., Ed.; Elsevier: Amsterdam, The Netherlands, 1983; pp 1−6.

(21) Abbas, H. K.; Mirocha, C. J.; Meronuk, R. A.; Pokorny, J. D.; Gould, S. L.; Kommedahl, T. Mycotoxins and *Fusarium* species associated with infected ears of corn in Minnesota. *Appl. Environ. Microbiol.* **1988**, *54*, 1930–1933.

Mycotoxins in Corn Residues

J. Agric. Food Chem., Vol. 56, No. 16, 2008 **7585**

(22) Abbas, H. K.; Mirocha, C. J.; Kommedahl, T.; Vesonder, R. F.; Golinski, P. Production of trichothecene and non-trichothecene mycotoxins by *Fusarium* species isolated from maize in Minnesota. *Mycopathologia* **1989**, *108*, 55–58.

(23) Windels, C. E. Economic and social impacts of *Fusarium* head blight: changing farms and rural communities in the Northern Great Plains. *Phytopathology* **2000**, *90*, 17–21.

(24) Yoshizawa, T.; Matsuura, Y.; Tsuchiya, Y.; Morooka, N.; Kitani, K.; Ichinoe, M.; Kurate, H. On the toxigenic *Fusaria* invading barley and wheat in southern Japan. *J. Food Hyg. Soc.* **1979**, *20*, 21–26.

(25) Mirocha, C. J.; Abbas, H. K.; Windels, C. E.; Xie, W. Variation in deoxynivalenol, 15-acetyl-DON, 3-acetyl-DON and zearalenone production by *Fusarium graminearum* isolates. *Appl. Environ. Microbiol.* **1989**, *55*, 1315–1316.

(26) Abbas, H. K.; Mirocha, C. J.; Tuite, J. Natural occurrence of deoxynivalenol, 15-acetyl-deoxynivalenol, and zearalenone in refusal factor corn stored since 1972. *Appl. Environ. Microbiol.* **1986**, *51*, 841–843.

(27) Mirocha, C. J.; Christensen, C. M.; Nelson, G. H. F-2 (zearalenone) estrogenic mycotoxin from Fusarium. In *Microbial Toxins*; Kadis, S., Ciegler, A., Aji, S. J., Eds.; Academic Press: New York, 1971; Vol. 7, pp 107−138.

(28) Mirocha, C. J.; Christensen, C. M. Oestrogenic mycotoxins synthesized by *Fusarium*. In *Mycotoxins*; Purchase, C. H. F., Ed.; Elsevier: Amsterdam, The Netherlands, 1974; p 129−148.

(29) Zinedine, A.; Soriano, J. M.; Molto, J. C.; Manes, J. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. *Food Chem. Toxicol.* **2007**, *45*, 1–18.

(30) Couvillion, C. E.; Jackson, J. R.; Ingram, R. P.; Bennett, L. W.; McCoy, C. P. Potential natural exposure of Mississippi sandhill cranes to aflatoxin B1. *J. Wildl. Dis.* **1991**, *27*, 650–656.

(31) Jamie-Garcia, R.; Cotty, P. J. *Aspergillus flavus* in soils and corn cobs in South Texas: Implications for management of aflatoxins in corn-cotton rotations. *Plant Dis.* **2004**, *88*, 1366–1371.

(32) Olanya, O. M.; Hoyos, G. M.; Tiffany, L. H.; McGee, D. C. Waste corn as a point source of inoculum for *Aspergillus flavus* in the corn agroecosystem. *Plant Dis.* **1997**, *81*, 576–581.

(33) Palm, C. J.; Seidler, R. J.; Schaller, D. L.; Donegan, K. K. Persistence in soil of transgenic plant-produced *Bacillus thuringensis* var. *Kurstaki* delta-endotoxin. *Can. J. Microbiol.* **1996**, *42*, 1258–1262.

(34) Horn, B. W.; Dorner, J. W. Soil populations of *Aspergillus* species from section Flavi along a transect through peanut-growing regions of the United States. *Mycologia* **1998**, *90*, 767–776.

(35) Abbas, H. K.; Zablotowicz, R. M.; Weaver, M. A.; Horn, B. W.; Xie, W.; Shier, W. T. Comparison of cultural and analytical methods for determination of aflatoxin production by Mississippi Delta *Aspergillus* isolates. *Can. J. Microbiol.* **2004**, *50*, 193–199.

(36) Zablotowicz, R. M.; Abbas, H. K.; Locke, M. A. Population ecology of *Aspergillus flavus* and other fungi associated with Mississippi Delta soils. *Food Addit. Contam.* **2007**, *24*, 1102–1108.

(37) Mirocha, C. J.; Kolaczkowski, E.; Xie, W.; Yu, H.; Jelen, H. Analysis of deoxynivalenol and its derivatives (batch and single kernel) using gas chromatography/mass spectrometry. *J. Agric. Food Chem.* **1998**, *46*, 1414–1418.

(38) Sobolev, V. S.; Dorner, J. W. Cleanup procedure for determination of aflatoxins in major agricultural commodities by liquid chromatography. *J. AOAC Int.* **2002**, *85*, 642–645.

(39) Plattner, R. D. HPLC/MS analysis of *Fusarium* mycotoxins, fumonisins and deoxynivalenol. *Nat. Toxins* **1999**, *7*, 365–370.

(40) Abbas, H. K.; Cartwright, R. D.; Xie, W.; Shier, W. T. Aflatoxin and fumonisin contamination of corn (maize, *Zea mays*) hybrids in Arkansas. *Crop Prot.* **2006**, *25*, 1–9.

(41) Abbas, H. K.; Zablotowicz, R. M.; Bruns, H. A. Modeling the colonization of maize by toxigenic and non-toxigenic *Aspergillus flavus* strains: implications for biological control. *World Mycotoxin J.*, in press.

(42) van Egmond, H. P.; Schothorst, R. C.; Jonker, M. A. Regulations relating to mycotoxins in food perspectives in a global and European context. *Anal. Bioanal. Chem.* **2007**, *389*, 147–157.

(43) Dowd, P. Biotic and abiotic factors limiting efficacy of Bt corn in indirectly reducing mycotoxin levels in commercial fields. *J. Econ. Entomol.* **2001**, *94*, 1067–1074.

(44) Pazzi, F.; Lener, M.; Colombo, L.; Monastra, G. Bt maize and mycotoxins: The current state of research. *Ann. Microbiol.* **2006**, *56*, 223–230.

(45) Hammond, B. G.; Campbell, K. W.; Pilcher, C. D.; Degooyer, T. A.; Robinson, A. E.; McMillen, B. L.; Spangler, S. M.; Riordan, S. G.; Rice, L. G.; Richard, J. L. Lower fumonisin mycotoxin levels in the grain of Bt corn grain in the United States in 2002−2003. *J. Agric. Food Chem.* **2004**, *52*, 1390–1397.

(46) Wu, F.; Miller, J. D.; Casman, E. A. Bt corn and mycotoxin reduction: an economic perspective. In *Aflatoxin and Food Safety*; Abbas, H. K., Ed.; CRC Press, Taylor & Francis Group: Boca Raton, FL, 2005; Chapter 23, pp 459−482.

(47) Wu, F. Mycotoxin reduction in Bt corn: potential economic, health and regulatory impacts. *Transgenic Res.* **2006**, *15*, 277–289.

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

(49) Huynh, V. L.; Lloyd, A. B. Synthesis and degradation of aflatoxins by *Aspergillus parasiticus*. I. Synthesis of aflatoxin B1 by young mycelium and its subsequent degradation in aging mycelium. *Aust. J. Biol. Sci.* **1984**, *37*, 37–43.

(50) Ciegler, A.; Lillehoj, E. B.; Peterson, R. E. Microbial detoxification of aflatoxin. *Appl. Microbiol.* **1966**, *14*, 934–939.

(51) Hormisch, D.; Brost, I.; Kohring, G. W.; Gifthorn, F.; Krooppenstedt, E.; Farber, P.; Holzapfel, W. H. *Mycobacterium fluoranthenivorans* sp. Nov., a fluoranthene and aflatoxin B1 degrading bacterium from contaminated soil of a former coal gas plant. *Syst. Appl. Microbiol.* **2004**, *27*, 653–660.

(52) Quist, C. F.; Bounous, D. I.; Kilburn, J. V.; Nettles, V. F.; Wyatt, R. D. The effect of dietary aflatoxin on wild turkey poults. *J. Wildl. Dis.* **2000**, *36*, 436–444.

(53) Perez, M.; Henke, S. E.; Fedynich, A. M. Detection of aflatoxin-contaminated grain by three grainivorous bird species. *J. Wildl. Dis.* **1991**, *37*, 358–361.

(54) Wetzstein, H.-G.; Stadler, M.; Tichy, H.-V.; Dalhoff, A.; Wolfgang Karl, W. Degradation of ciprofloxacin by Basidiomycetes and identification of metabolites generated by the brown rot fungus *Gloeophyllum striatum*. *Appl. Environ. Microbiol.* **1999**, *65*, 1556–1563.

(55) Conkova, E.; Laciakova, A.; Kovac, G.; Seidel, H. Fusarial toxins and their role in animal diseases. *Vet. J.* **2003**, *165*, 214–220.

(56) Bilgrami, K. S.; Choudhary, A. K. Impact of habitats on toxigenic potential of *Aspergillus flavus*. *J. Stored Prod. Res.* **1993**, *29*, 351–355.

(57) Icoz, I.; Stotzky, G. Fate and the effects of insect-resistant Bt crops in soil ecosystems. *Soil Biol. Biochem.* **2008**, *40*, 559–586.

(58) Wicklow, D. T.; Wilson, D. W.; Nelson, T. C. Survival of *Aspergillus flavus* sclerotia and conidia buried in soil in Illinois or Georgia. *Phytopathology* **1993**, *83*, 1141–1147.