Food Additives and Contaminants, December 2007; 24(12): 1367–1373

Taylor & Francis
Taylor & Francis Group

Mycotoxin occurrence and *Aspergillus flavus* soil propagules in a corn and cotton glyphosate-resistant cropping systems

K. N. REDDY[1], H. K. ABBAS[2], R. M. ZABLOTOWICZ[1], C. A. ABEL[3], & C. H. KOGER[4]

[1]*US Department of Agriculture, Agriculture Research Service, Southern Weed Science Research Unit, Stoneville, MS 38776, USA,* [2]*US Department of Agriculture, Agriculture Research Service, Crop Genetics and Production Research, PO Box 345, Stoneville, MS 38776, USA,* [3]*US Department of Agriculture, Agriculture Research Service, SIMRU, Stoneville, MS 38776, USA, and* [4]*Mississippi State University, DREC, Stoneville, MS 38776, USA*

(*Received 12 December 2006; revised 17 May 2007; accepted 5 June 2007*)

Abstract
The effects of cotton–corn rotation and glyphosate use on levels of soil-borne *Aspergillus flavus*, aflatoxin and fumonisin contamination in corn and cotton seed were determined during 2002–2005 in Stoneville, Mississippi (USA). There were four rotation systems (continuous cotton, continuous corn, cotton–corn and corn–cotton) for both glyphosate-resistant (GR) and non-GR cultivars–herbicide system arranged in a randomized complete block design with four replications. *Aspergillus flavus* populations in surface (5-cm depth) soil, sampled before planting (March/April), mid-season (June) and after harvest (September), ranged from 1.47 to 2.99 log (10) cfu g^{-1} soil in the four rotation systems. Propagules of *A. flavus* were higher in the continuous corn system compared to the continuous cotton system on three sample dates, and cotton rotated with corn decreased *A. flavus* propagules in three of nine sample dates. Propagules of *A. flavus* were significantly greater in plots with GR cultivars compared to non-GR cultivars in three samples. In cotton seed, aflatoxin and fumonisin levels were similar ($\leq$4 μg kg^{-1} and non-detectable, respectively) regardless of rotation and glyphosate. In corn grain, aflatoxin was above the regulatory level ($\geq$20 μg kg^{-1}) only in GR cultivar in 2004 and 2005. Fumonisin was higher in non-GR cultivar (4 mg kg^{-1}) regardless of rotation in 2004; however, in 2002, 2003 and 2005, aflatoxin and fumonisin levels were similar regardless of rotation and glyphosate. These results indicate the potential for increased aflatoxin and fumonisin levels (1 of 4 years) in corn; however, climatic conditions encountered during this study did not allow for mycotoxin production. In laboratory incubation studies, fairly high concentrations of glyphosate were required to inhibit *A. flavus* growth; however no short-term effect of soil treatment with glyphosate on *A. flavus* populations were observed. These data suggest that altered populations of *A. flavus* or higher aflatoxin concentrations in corn grain were due to indirect effects of the GR cropping system.

Keywords: Aspergillus, *crop rotation,* Fusarium, *genetically modified crops, herbicide, glyphosate*

Introduction

Aspergillus species of section *Flavi*, especially *A. flavus* Link, *A. parasiticus* Speare and *A. nomius* Kurtzman, Horn and Hesseltine are responsible for producing aflatoxin, a potent carcinogen. Aflatoxin is a particular problem with crops such as corn, cotton, peanuts and tree nuts before or after harvesting (Diener et al. 1987; Payne 1992; Abbas 2003; CAST 2003; Robens and Brown 2004). Fumonisins are also potent toxins produced by *Fusarium* species, including *F. verticillioides* (Sacc.) Nirenberg [syn. *F. moniliforme* Sheldon], *F. proliferatum* (Matsushima) Nirenberg, *F. nygamai* Burgess & Trimboli, and *F. subglutinans* Wollenw. & Reinking (Abbas et al. 1999; Rheeder et al. 2002). Corn crops contaminated by these fungi and their respective mycotoxins can cause toxicological problems in livestock (CAST 2003; Robens and Brown 2004). In the USA, federal guidelines by the FDA prohibit the use of corn with aflatoxin greater than 20 μg kg^{-1} (van Egmond and Jonker 2004) and fumonisin greater then 2 mg kg^{-1} (US FDA 2001). Farmers incur significant economic losses if they are unable to

Correspondence: Hamad K. Abbas. E-mail: Hamed.Abbas@ars.usda.gov

ISSN 0265–203X print/ISSN 1464–5122 online
DOI: 10.1080/02652030701509964

sell their crops due to high concentration of these mycotoxins (Robens and Cardwell 2003). Hot and dry conditions favour growth of these fungi and subsequent mycotoxin production (Diener et al. 1987; Payne 1992). In 1998, drought and high temperatures in Mississippi contributed to significant levels of both aflatoxin and fumonisin in corn (Abbas et al. 2002).

Glyphosate is a non-selective, broad spectrum, systemic postemergence herbicide that has been used extensively throughout the world over the past 30 years (Franz et al. 1997). It blocks biosynthesis of aromatic amino acids by inhibiting the enzyme 5-enolpyruvylshikimate-3-phophate synthase (EPSPS) in the shikimate pathway (Franz et al. 1997; Duke et al. 2003). Glyphosate is used widely for weed control in glyphosate-resistant (GR) crops without concern for crop injury. Glyphosate-resistant corn and cotton were created by stable integration of a transgene coding for an insensitive EPSPS enzyme that is resistant to glyphosate (Padgette et al. 1995). Farmers in the USA have rapidly adopted GR crops, planting about 65% of cotton and 36% of corn hectares to GR varieties in 2006 (Reddy and Koger 2006; USDA 2006). Fungi possess a sensitive EPSPS and are susceptible to glyphosate. The effect of continuous use of glyphosate on *Aspergillus* and *Fusarium* under GR crop production systems is unclear. Kremer et al. (2005) reported that glyphosate is accumulated in the rhizosphere of treated soybeans and is associated with increased *Fusarium* propagules in the rhizosphere of glyphosate-treated GR soybeans.

Historically, cotton has been grown in monoculture in the Mississippi Delta, but crop rotations may boost profit margins by improving yields without increasing production costs. Corn is often used in a crop rotation with cotton because it has been shown to reduce many pests that attack cotton, especially reniform nematodes (*Rotylenchulus reniformis* Linford and Oliverira) (Windham and Lawrence 1992; McDonald et al. 1999; Gazaway et al. 2000). In many studies in the southern USA, soil populations of *A. flavus* responded to different cropping systems (Griffin et al. 1981, 2001; Horn and Dorner 1998; Abbas et al. 2004a, b). However, in an Iowa field study, there was a limited effect of tillage and rotational practices on soil populations of *A. flavus* in a corn ecosystem (McGee et al. 1996). Soil populations of *F. solani* were greater in soybean or fescue compared to sorghum or wheat and may be related to the susceptibility of soybean to sudden death syndrome (Rupe et al. 1997).

Some plant diseases can be promoted or suppressed by herbicide exposure (Lévesque and Rahe 1992). Disease severity of sudden death syndrome of soybean caused by *F. solani* f. sp. *glycines* (Mart.) Sacc. was greater under glyphosate-resistant cultivars (Sanogo et al. 2001). Soybean rhizosphere populations of *F. solani* were increased following glyphosate treatment (Means 2004). Others have evaluated the sensitivity of GR cultivars of cotton and soybeans and found no significant changes in damping off caused by *Rhizoctonia solani* compared to conventional cultivars (Harikrishnan and Yang 2002; Baird et al. 2004). However, little is known of how the opportunistic phytopathogen, *A. flavus*, responds to a GR crop management system.

Because other plant diseases have been controlled by crop rotation, and fungi possess a glyphosate-sensitive EPSPS, we wanted to determine the effects of crop rotation and glyphosate on Aspergillus ear rot and aflatoxin contamination. The specific objectives of this study were (1) to determine the effects of cotton-corn rotation on *A. flavus* in soil and levels of aflatoxin and fumonisin in corn and cotton seed and (2) to assess the effects of glyphosate use, associated with GR crop production, on *A. flavus* in soil and levels of aflatoxin and fumonisin in corn and cotton seed.

Materials and methods

Field description and crop management

A field study was initiated in 2000 and monitoring of mycotoxin and *A. flavus* soil populations conducted from 2002 through 2005 at the US Department of Agriculture, Agriculture Research Service (USDA–ARS) Southern Weed Science Research Unit farm, Stoneville, MS (33°26′N). The soil was a Dundee silt loam (fine-silty, mixed, thermic Aeric Ochraqualf) with pH 6.7, 1.1% organic matter and soil textural fractions of 26% sand, 55% silt and 19% clay. Before initiation of the study, the experimental area was under GR soybean production. Field preparation consisted of disking, subsoiling, disking and bedding in the fall of 1999. The land was not tilled in subsequent years. The old seedbeds were raised (re-bedded) with no additional tillage operations in the fall after harvest and beds were smoothed as needed to plant crops in the spring.

The experiment was conducted in a randomized complete block design with four replications. There were four rotation systems for each GR and non-GR cultivar. The rotation systems were continuous cotton, continuous corn, cotton–corn and corn–cotton. To enable comparison between rotations and monoculture each year, the reverse rotation sequences were included in the experiment. Each treatment consisted of eight rows spaced 102 cm apart and 45.7 m long.

Experimental area was treated with paraquat at 1.1 kg ai ha^{-1} 1–4 days prior to crop planting to kill

weed vegetation. Glyphosate-resistant and non-GR cotton and corn were planted in 102-cm wide rows. The agronomic practices used in the study have been described elsewhere (Reddy et al. 2006). Glyphosate-based program in GR cultivars and nonglyphosate herbicide-based program in non-GR cultivars were used for weed control. Herbicide treatments were applied with a tractor-mounted sprayer with TeeJet 8004 standard flat spray tips delivering 187 l ha^{-1} water at 179 kPa. Fertilizer application and insect control programs were standard for cotton and corn production in the Mississippi (Reddy 2004; Anonymous 2005). Crops were irrigated as needed. At harvest in 2002–2005, twenty corn ears and about 1 kg of seed cotton were hand-picked randomly from the second and third rows of an eight-row plot, representing the full length of each plot, for aflatoxin and fumonisin determination.

Soil sampling and enumeration of A. flavus *populations*

Soil samples were taken prior to planting (March/April), mid-season (June) and after harvest (September). Soil samples consisted of a composite of nine subsamples of 0- to 5-cm depth using a 7.5-cm (diameter) probe, approximately 2 kg at random from the middle four rows of each plot. To avoid contamination, soil sampling probes were disinfected with isopropanol between plots. Soils were sieved through a 2.25-mm mesh screen, moisture content determined gravimetrically and soils were stored at 4°C until plating, typically within 2 days of collection. *Aspergillus flavus* populations were isolated from soil using modified dichloronitroaniline rose bengal (MDRB) agar according to Horn and Dorner (1998) with improved selectivity by adding 3.0% sodium chloride (Griffin et al. 1975). Two soil dilutions were used, 0.5 and 2.5 g soil in 10 ml of water agar, and duplicate plates spread with 250 µl of suspension per plate per dilution. Fungal colony forming units (cfu) were counted following 5 days incubation at 37°C under continuous dark. Only colonies having typical *A. flavus* morphology were counted. Identifications of colonies were conducted according to Klich (2002). All colonies evaluated were typical *A. flavus* and no *A. parasiticus* or *A. nomius* were observed in the subsample assessed.

Mycotoxin analysis

Aflatoxin and fumonisin concentrations in corn were quantitatively determined using commercial ELISA kits (Neogen Corp., Lansing, MI, USA) according to Abbas et al. (2005, 2006a, 2006b). Twenty corn ears were shelled and a subsample of 200 g of kernel was ground (20 mesh) using a Romer mill (model 2A; Romer Labs Inc., Union, MO, USA). Triplicate subsamples of ground corn (20 g) were extracted in 100 ml methanol (70%) for 3 min on a high speed reciprocal shaker. Seed cotton (about 1 kg) was ginned and a subsample of 20 g seed was homogenized in 100 ml of 70% methanol for 60 s in a Waring blender. The methanol extracts were filtered (Whatman # 1 filter paper) and the filtrate analyzed by ELISA. The limit of detection in this assay was 5 µg kg^{-1} seed for aflatoxin and 0.2 µg g^{-1} seed for fumonisin.

Effect of glyphosate on A. flavus *growth, and soil colonization*

The effect of various concentrations of glyphosate (0–10 mM) on the growth of two *A. flavus* strains F3W4 (aflatoxin producer) and K49 (non-toxigenic) (Abbas et al. 2006b) was evaluated in vitro. Glyphosate stock solutions were prepared from technical grade material (97% purity, Sigma-Aldrich, St. Louis, MO, USA) and filter sterilized. Stocks were added to sterile molten potato dextrose agar (PDA, Difco) or water agar (Difco Bacto agar, 20 g l^{-1}) and glyphosate was added to attain concentrations of 0–10 mM, mixed well and poured into 9 cm diameter Petri dishes. Inoculum of conidia suspension for the study was harvested from defined 7-day cultures grown on defined Czapek media in sterile water containing 2.0% Tween 20 and adjusted to a concentration of 10^6 conidia ml^{-1}. The conidia suspension (100 µl) was spotted in the center of five replicate plates and incubated at 30°C in the dark. Radial growth was recorded in four directions for each plate at 3, 7 and 14 days after inoculation. After 14 days of growth, the plates (agar and fungus mass) were homogenized in 70% methanol (100 ml solvent per Petri dish) and aflatoxin concentration determined using ELISA.

Triplicate samples of soil (10 g) collected from these experimental plots, containing high levels of *A. flavus* (3.2 cfu g^{-1} soil), were treated with 1.0 ml of water or 0.5, 5.0 or 10.0 mM of technical grade glyphosate or the commercial glyphosate formulation used in the field study. The soils were incubated in the dark at 28°C and cfu of *A. flavus* were determined as previously described at 0, 3, 7 and 14 days after treatment.

Statistical methods

Data were subjected to analysis of variance using PROC GLM (SAS 2001). Treatments means were separated at the 5% level of significance using Fisher's protected LSD test. Data were presented separately for each year as aflatoxin and fumonisin production is more dependent on environmental conditions.

Table I. Soil *Aspergillus flavus* propagules associated with a Dundee silt loam soil as affected by rotation system, glyphosate and sampling date from 2002 to 2005.

	Propagule density of *Aspergillus* (log_{10} colony-forming units g^{-1} soil)[a]								
	2002		2003		2004		2005		
Rotation system/cultivar type	June	September	March	September	March	September	March	June	September
Rotation system									
Corn–Corn	2.67ab	2.91ab	2.57a	2.49a	2.53a	2.36a	1.95a	1.66a	2.40a
Cotton–Cotton	2.80a	2.83c	2.51a	2.53a	1.96c	2.64a	1.59a	1.47a	1.70c
Cotton–Corn	2.57b	2.85b	2.53a	2.52a	2.24b	2.53a	1.78a	1.56a	2.00b
Corn–Cotton	2.55b	2.99a	2.56a	2.58a	2.24b	2.53a	1.70a	1.80a	2.10b
Cultivar type									
GR	2.60a	3.14a	2.50a	2.56a	2.35a	2.52a	1.69a	1.58a	2.10a
Non-GR	2.71a	2.65b	2.58a	2.48b	2.13b	2.51a	1.82a	1.66a	2.03a
ANOVA[b]									
Rotation system	0.0154	0.0049	0.7827	0.7357	0.0476	0.0643	0.112	0.308	0.027
GR	0.0610	<0.001	0.0722	0.0432	0.0431	0.7880	0.199	0.520	0.665
System × GR	0.3185	0.3527	0.1990	0.3474	0.3102	0.2766	0.0081	0.096	0.373

[a]Log_{10} colony forming units *Aspergillus* g^{-1} soil. Mean of four replicates determined by serial dilution and plating on modified dichloronitroaniline rose bengal (MDRB) agar amended with 3% sodium chloride; colonies were counted after a 5-day incubation.
[b]Contribution of rotation system, herbicide regime and rotation system × herbicide regime interaction to differences among various treatments.
Values followed by the same letter in a given column and rotation system or cultivar type do not differ significantly at the 95% confidence level using Fisher's least significant difference test.Abbreviations: GR, glyphosate-resistant; Non-GR, non-glyphosate-resistant.

Results and discussion

Enumeration of A. flavus *soil populations*

Propagule densities of *A. flavus* in surface 5-cm soil ranged from 1.47 to 2.99 log (10) cfu g^{-1} soil in four rotation systems (Table I). *Aspergillus flavus* propagules were higher in the continuous corn system compared to the continuous cotton system in September 2002, March 2004 and September 2005. Cotton rotated with corn tended to decrease *A. flavus* propagules on three of nine sample dates. Propagules of *A. flavus* were significantly greater in plots with GR cultivars compared to non-GR cultivars in September 2002 and 2003, and March 2004. Both corn and cotton are susceptible to colonization by *A. flavus* and, in this field study, *A. flavus* propagules were higher in continuous corn compared to continuous cotton on three of nine sample dates. In a commercial field study assessing the effects of various cropping sequences in the Mississippi Delta, *A. flavus* propagules were greatest in soil following corn compared to either cotton or wheat (Abbas et al. 2004a). Likewise, Griffin (1981) observed greater *A. flavus* propagules associated with soil following corn or peanuts compared to fallow soil, and Horn and Dorner (1998) found greater propagules associated with peanuts compared to cotton. Corn produces remarkably more crop residues compared to cotton and the corn residues are rich in carbohydrates, which are capable of sustaining both *A. flavus* conidia and sclerotia. Thus, the corn systems would be expected to harbour greater *A. flavus* levels compared to continuous cotton. This current study is in agreement with results by McGee et al. (1996), who reported a limited effect of crop rotations on soil *A. flavus* populations under Midwestern US conditions.

Mycotoxin concentrations in corn and cotton

In cotton seed, aflatoxin and fumonisin levels were low ($\leq 4\,\mu g\,kg^{-1}$ and non-detectable, respectively) regardless of rotation and glyphosate (data not shown). In corn grain, aflatoxin was above the regulatory level ($\geq 20\,\mu g\,kg^{-1}$) only in GR cultivar in 2004 and 2005 (Table II). Fumonisin was higher in non-GR cultivar ($4\,mg\,kg^{-1}$) regardless of rotation in 2004; however, in 2002, 2003 and 2005, the mycotoxins levels were similar regardless of rotation and glyphosate. Although the highest abundance of *A. flavus* was observed during the summer and fall of 2005, there was only a limited effect on aflatoxin production in corn or cotton.

Mycotoxins contamination is influenced by the level of infestation by toxigenic fungi and conduciveness of environmental stress for expression of the mycotoxin synthesis pathway. The hot and dry weather in 1998 contributed to wide spread contamination of corn with aflatoxin and fumonisin in Mississippi (Abbas et al. 2002). Overall, normal rainfall and cooler temperatures in 2002–2005 compared to 1998 may have been unfavorable for infestation of toxigenic fungi and mycotoxin production (Mississippi State University and Extension Service; http://ext.msstate.edu/anr/drec/stations.cgi).

Table II. Aflatoxin and fumonisin in glyphosate-resistant and non-glyphosate-resistant corn grown continuously and in rotation with cotton at Stoneville, Mississippi, 2002–2005.[a]

		Aflatoxin ($\mu g\,kg^{-1}$)				Fumonisin ($mg\,kg^{-1}$)			
Cultivar type	Rotation system	2002	2003	2004	2005	2002	2003	2004	2005
Non-GR	Continuous corn	0a	1a	0b	6a	2a	16a	4a	2a
	Corn–cotton rotation	1a	1a	0b	5a	2a	15a	4a	2a
GR	Continuous corn	0a	1a	34a	5a	2a	17a	1b	2a
	Corn–cotton rotation	1a	1a	20ab	20a	4a	13a	1b	6a
Pr>F		0.630	0.981	0.027	0.485	0.421	0.986	0.004	0.277
LSD		–	–	24	–	–	–	2	–

[a]Means within a column followed by the same letter are not significantly different at the 5% level as determined by Fisher's protected LSD test.
GR, glyphosate-resistant; Non-GR, non-glyphosate-resistant.

In addition, nutrition may affect crop sensitivity to aflatoxin accumulation (Payne et al. 1989; Tubajika et al. 1999). Relatively large quantities of nitrogen fertilizer were applied to corn ($202\,kg\,ha^{-1}$) and cotton ($134\,kg\,ha^{-1}$) (Reddy et al. 2006), therefore, nitrogen availability should not be a susceptibility factor.

Although mycotoxin contamination was minimal in the 4 years of this study, there was no evidence that rotation of corn with cotton had any affect on reducing inoculum potential in soil and there was no consistent effect of glyphosate on propagule density of *A. flavus*.

Effect of glyphosate on A. flavus *growth and soil colonization*

Similar effects of glyphosate on the radial growth of *A. flavus* strains K49 and F3W4 were observed (Figure 1). On rich media (PDA), partial and temporal inhibition was only observed at the highest concentration tested (10 mM). However, on water agar devoid of nutrients, radial growth of both strains was reduced by about 50 and 80% at 5 and 10 mM glyphosate, respectively. Aflatoxin was only detected in extracts of strain F3W4 grown on PDA; however, levels of aflatoxin were 2371 and $1920\,\mu g\,kg^{-1}$ in extracts from 5 and 10 mM glyphosate, respectively, compared to $10\,127\,\mu g\,kg^{-1}$ in the 0 mM glyphosate control. No aflatoxin production was detected on water agar regardless of strain or glyphosate treatment. A similar level of inhibition of growth of *A. flavus* by glyphosate was found, as in the bacteria *Bradyrhizobium japonicum* (Moorman et al. 1992) and two phytopathogenic fungi *Fusarium oxysporum* and *Rhizoctonia solani* (Larson et al. 2006). A greater degree of inhibition on dilute media (water agar) compared to chemically defined media (PDA) indicates that inhibition of aromatic amino acids derived from the shikimic acid pathway was the likely cause of inhibition. Furthermore,

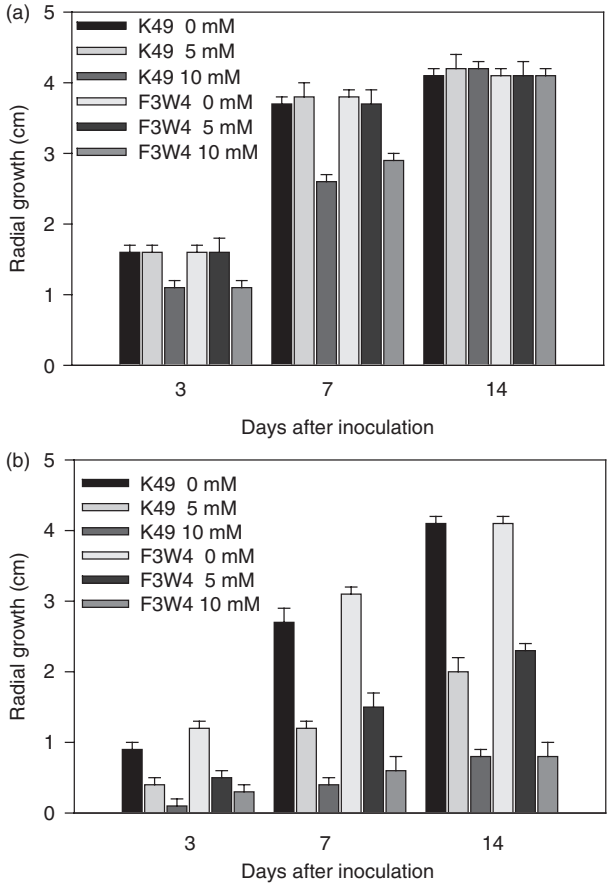

Figure 1. Effects of three glyphosate concentrations on the radial growth of two *Aspergillus flavus* strains (K49 and F3W4) grown on potato dextrose agar (A) and water agar (B). Mean and standard deviation of five replicates.

cultures grown in the presence of glyphosate were yellowish-brown in colour, agreeing with accumulation of hydroxybenzoic acids. However, the concentrations of glyphosate required for inhibition are well beyond what would be applied under field conditions. In soil, no effect of glyphosate was observed on

the recovery of *A. flavus* cfu from soil regardless of rate, formulation or time after treatment. Glyphosate is readily bound by soil colloids and less material would be bioavailable to interfere with growth (Accinelli et al. 2005). Indirect effects of the glyphosate cropping system on soil microflora may be responsible for some alterations of *A. flavus* cfu observed in the field study and altered aflatoxin concentrations in corn grain.

Acknowledgements

We thank Bobbie Johnson, Earl Gordon, Terry Newton and Albert Tidwell for field and laboratory technical assistance.

References

Abbas HK. editor. 2003. Special issue on Aflatoxin and Food Safety. Part 1. Journal of Toxicology – Toxin Reviews 22:139–459.

Abbas HK, Cartwright RD, Xie W, Mirocha CJ, Richard JL, Dvorak TJ, Sciumbato GL, Shier WT. 1999. Mycotoxin production by *Fusarium proliferatum* isolates from rice with *Fusarium* sheath rot disease. Mycopathologi 147:97–104.

Abbas HK, Williams WP, Windham GL, Pringle Jr.JC , Xie W, Shier WT. 2002. Aflatoxin and fumonisin contamination of commercial corn (*Zea mays*) hybrids in Mississippi. Journal of Agricultural and Food Chemistry 50:5246–5254.

Abbas HK, Zablotowicz RM, Locke MA. 2004a. Spatial variability of A*spergillus flavus* soil populations under different crops and corn grain colonization and aflatoxins. Canadian Journal of Botany 82:1768–1775.

Abbas HK, Zablotowicz RM, Weaver MA, Horn BW, Xie W, Shier WT. 2004b. Comparison of cultural and analytical methods of determination of aflatoxin production by Mississippi Delta *Aspergillus* isolates. Canadian Journal of Microbiology 50:193–199.

Abbas HK, Weaver MA, Zablotowicz RM, Horn BW, Shier WT. 2005. Relationships between aflatoxin production, sclerotia formation and source among Mississippi Delta *Aspergillus* isolates. European Journal of Plant Pathology 112:283–287.

Abbas HK, Cartwright RD, Xie W, Shier WT. 2006a. Aflatoxin and fumonisin contamination of corn (maize, *Zea mays)* hybrids in Arkansas. Crop Protection 25:1–9.

Abbas HK, Zablotowicz RM, Bruns HA, Abel CA. 2006b. Biocontrol of aflatoxin in corn by inoculation with non-aflatoxigenic *Aspergillus flavus* isolates. Biocontrol Science and Technology 16:437–449.

Accinelli C, Koskinen WC, Seebinger JD, Vicari A, Sadowsky MJ. 2005. Effects of incorporated corn residues on glyphosate mineralization and sorption in soil. Journal of Agricultural and Food Chemistry 53:4110–4117.

Anonymous. 2005. Fertilization and insect control guidelines for corn and cotton. Mississippi State University Extension Service, Mississippi State, MS, USA. Available: http://www.msucares.com/pubs/publications/. Accessed: 5 October 2005.

Baird R, Batson W, Watson C, Hightower P. 2004. Evaluation of transgenic cotton varieties and a glyphosate application on seedling disease incidence. Mycopathologia 158:363–368.

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

Diener UL, Cole RJ, Sanders TH, Payne GA, Lee LS, Klich MA. 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. Annual Review of Phytopathology 25:249–270.

Duke SO, Baerson SR, Rimando AM. 2003. Herbicides: glyphosate. In: Plimmer JR, Gammon DW, Ragsdale NN, editors. Encyclopedia of Agrochemicals. Wiley: New York. Available: http://www.mrw.interscience.wiley.com/eoa/articles/agr119/frame.html. Accessed: 5 October 2005.

Franz JE, Mao MK, Sikorski JA. 1997. Glyphosate: A Unique Global Herbicide. American Chemical Society Monograph 189. American Chemical Society: Washington, DC.

Gazaway WS, Akridge JR, Mclean K. 2000. Impact of various crop rotations and various winter cover crops on reniform nematode in cotton. Proceedings of Beltwide Cotton Conference 1:162–163.

Griffin GJ, Ford RH, Garren KH. 1975. Relation of *Aspergillus flavus* colony growth on three selective media to recovery from naturally infested soil. Phytopathology 65:704–707.

Griffin GJ, Garrner KH, Taylor JD. 1981. Influence of crop rotation and minimum tillage on the populations of *Aspergillus flavus* group in peanut field soil. Plant Disease 65:898–900.

Griffin GJ, Smith EP, Robinson TJ. 2001. Population patterns of *Aspergillus flavus* group and *Aspergillus niger* group in field soil. Soil Biology Biochemistry 33:253–257.

Harikrishnan R, Yang XB. 2002. Effects of herbicides on root rot and damping-off caused by *Rhizoctonia solani* in glyphosate-tolerant soybean. Plant Disease 86:1369–1373.

Horn BW, Dorner JW. 1998. Soil populations of *Aspergillus* species from section *Flavi* along a transect through peanut-growing regions of the United States. Mycologia 90:767–776.

Klich MA. 2002. Identification of common *Aspergillus* species. Centralbureau voor SchimmelculturesUtrecht, Netherlands. 116p.

Kremer RJ, Means NE, Kim S. 2005. Glyphosate affects soybean root exudation and rhizosphere micro-organisms. International Journal of Environmental Analytical Chemistry 85:1165–1174.

Larson RL, Hill AL, Fenwick A, Kniss AR, Hanson LE, Miller SD. 2006. Influence of glyphosate on Rhizoctonia and *Fusarium* root rot in sugar beet. Pest Management Science 62:1182–1192.

Lévesque CA, Rahe JE. 1992. Herbicide interactions with fungal root pathogens, with special reference to glyphosate. Annual Review of Phytopathology 30:579–602.

McDonald BA, Zhan J, Burdon JJ. 1999. Genetic structure of *Rhynchosporium secalis* in Australia. Phytopathology 89:639–645.

McGee DC, Olanya OM, Hoyos GM, Tiffany LH. 1996. Populations of *Aspergillus flavus* in the Iowa cornfield ecosystems in years not favorable for the aflatoxin contamination of corn grain. Plant Disease 80:742–746.

Means, N.E. 2004. Glyphosate effects on fungal populations in the soybean rhizosphere [M.S. thesis]. University of Missouri, p 130.

Moorman TB, Becerril JM, Lydon JM, Duke SO. 1992. Production of hydroxybenzoic acid by *Bradyrhizobium japonicum* strains after treatment with glyphosate. Journal of Agricultural and Food Chemistry 40:289–293.

Padgette SR, Kolacz KH, Delannay X, Re DB, LaVallee BJ, Tinius CN, Rhoades WK, Otero YI, Barry GF, Eicholtz DA, et al. 1995. Development, identification, and characterization of a glyphosate-tolerant soybean line. Crop Science 35:1451–1461.

Payne GS. 1992. Aflatoxin in maize. Critical Reviews in Plant Science 10:423–440.

Payne GA, Kamprath EJ, Adkins CR. 1989. Increased aflatoxin contamination in nitrogen stressed corn. Plant Disease 73:556–559.

Reddy KN. 2004. Weed control and species shifts in bromoxynil- and glyphosate-resistant cotton (*Gossypium hirsutum*) rotation systems. Weed Technology 18:131–139.

Reddy KN, Koger CH. 2006. Herbicide—resistant crops and weed management. In: Singh HP, Batish DR, Kohli RK, editors. Handbook of sustainable weed management. New York: Food Products Press Imprint of the Haworth Press. pp 549–580.

Reddy KN, Locke MA, Koger CH, Zablotowicz RM, Krutz LJ. 2006. Cotton and corn rotation under reduced tillage management: impacts on soil properties, weed control, yield, and net return. Weed Science 54:768–774.

Rheeder JP, Marasas WFO, Vismer HF. 2002. Production of fumonisin analogs by *Fusarium* species. Applied and Environmental Microbiology 68:2101–2105.

Robens J, Cardwell K. 2003. The costs of mycotoxin management to the USA: Management of aflatoxins in the United States. Journal of Toxicology – Toxin Reviews 22:139–152.

Robens JF, Brown RL, editors. 2004. Special Issue: Aflatoxin/fumonisin elimination and fungal genomics workshops. Mycopathologia 157:393–505.

Rupe JC, Robbibs JT, Gbur Jr EE. (1997). Effect of crop rotations on soil population densities of *Fusarium solani* and *Heterodera glycinea* and on the development of sudden death syndrome of soybean. Crop Protection 16:575–580.

Sanogo S, Yang XB, Lundeen P. 2001. Field response of glyphosate-tolerant soybean to herbicides and sudden death syndrome. Plant Disease 85:773–779.

SAS 2001. SAS® Proprietary Software Release 8.2, Windows version 5.1.2600. Cary, NC: SAS Institute Inc.

Tubajika KM, Mascagni HJ Jr., Damann KE, Russin JS. 1999. Nitrogen fertilizer influence on aflatoxin contamination of corn in Louisiana. Journal of Agricultural and Food Chemistry 47:5257–5260.

USDA 2006. United States Department of Agriculture, National Agricultural Statistics Service. Available: http://usda.mannlib.-cornell.edu/usda/current/Acre/Acre-09–12-2006.pdf. Accessed: 5 December 2006.

US FDA 2001. United States Food and Drug Administration, Guidance for Industry: Fumonisin levels in human foods and animal feeds. 9 November 2001. Washington, DC: FDA. Available: www.cfsan,fda.gov/~dms/fumongu2.html. Accessed: 20 September 2005.

van Egmond HP, Jonker MA. (2004). Worldwide regulations on aflatoxins – the situation in 2002. Journal of Toxicology – Toxin Reviews 23:273–293.

Windham GL, Lawrence GW. 1992. Host status of commercial maize hybrids to *Rotylenchulus reniformis*. Journal of Nematology 24:(supplementary) 745–748.