Aspergillus flavus in Soils and Corncobs in South Texas: Implications for Management of Aflatoxins in Corn–Cotton Rotations

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ABSTRACT

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Aspergillus flavus causes aflatoxin contamination in both cottonseed and corn. Corn–cotton rotations are common in South Texas, where reduced tillage frequently results in long-term residence of corncobs on soil surfaces. Corncobs are colonized by *A. flavus* either prior to harvest or while in the soil. This study sought to determine the potential of corncobs as sources of inoculum for cotton and corn in South Texas. *A. flavus* communities in corncob and soil samples were collected during the planting seasons of 2001 to 2003 from 29 fields extending from Calhoun and Victoria Counties in the north to the Rio Grande Valley. In order to assess persistence of *A. flavus* in corncobs, *A. flavus* communities in corncobs and soil were contrasted every 2 to 3 months in four fields throughout the 3-year study. To assess seasonal variation, similar contrasts were performed in two fields on a biweekly basis. The results indicate that corncobs are major sources of *A. flavus* inoculum. Corncobs from the previous season contained, on average, over 190 times more *A. flavus* propagules than soil from the same field, and 2-year-old corncobs still retained 45 times more propagules than soil. There was no significant difference in the incidence of *A. flavus* strain S on corncobs and soil. The quantity of *A. flavus* in corncobs decreased with corncob age ($r^2 = 0.54$; $P = 0.002$).

Additional keywords: pith, placenta, sclerenchyma

Aspergillus flavus is the main causal agent of aflatoxin contamination in several agricultural products, including cottonseed and corn (2,11,15,22). Aflatoxins are toxic fungal metabolites that are limited in food and feed by government regulations throughout most of the world (26,32).

A. flavus can be divided into S and L strains based on morphologic, genetic, and physiologic criteria (3,10,16,17). The S strain produces numerous small sclerotia (average diameter < 400 μ m) and high levels of aflatoxins, whereas the L strain produces fewer, larger sclerotia (average diameter > 400 μ m) and, on average, less aflatoxin (10,13,18). Aflatoxin producers from North America with the S strain morphotype produce only B aflatoxins (8). *A.*

flavus S strain isolates have been implicated as important aflatoxin producers in several areas worldwide, including Arizona (10,13,25), Texas (14,19,20), Louisiana, Mississippi, and Alabama (13) in the United States, Thailand in Southeast Asia (28), and Argentina in South America (24).

A. flavus typically lives as a saprophyte in the soil depending on organic matter to propagate and survive (1,6). Crop residues, including corncobs, are the main source of organic matter in soils where corn crops are produced and these support large populations of microorganisms (9,23,29,30). Unlike most crop residues that readily decay in agricultural soils (23), corncobs reside in soil for several years before being totally decomposed (5,7). Harvest and cultivation practices influence how long crop residues reside in soil (7). Cultivation practices vary widely in South Texas, with reduced tillage practices being very common in some areas. A high percentage of corncobs are colonized by *A. flavus* before their incorporation into soil (33), making them a potentially important source of inoculum for susceptible crops planted in fields previously cropped to corn. These infested corncobs may play a major role as reservoirs of *A. flavus* in corn–cotton rotations. The objective of this study was to examine the potential importance of corncobs both as a source of inoculum of *A. flavus* in corn–cotton crop rotations and as a refuge for the long-term maintenance of

A. flavus communities in agricultural environments in South Texas.

MATERIALS AND METHODS

In order to assess the potential importance of corncob residue in harboring *A. flavus* during corn–cotton rotations in South Texas, *A. flavus* resident in corncobs and soils was quantified during the 2001 to 2003 seasons.

Sample processing and isolation of fungi. Both the corncob and soil samples were oven dried at 48°C for 48 h before processing. Soil samples were placed inside a plastic bag, hammered to break clumps, and passed through a no. 12 sieve (1.7-mm opening). Corncob samples were ground to a fine powder in a laboratory mill (IKA Labortechnik MF10 basic S1; IKA-Werke GmbH & Co. KG, Staufen, Germany). Both the sieve and the mill were vacuumed to remove loose contaminants and washed with ethanol to kill contaminating fungal spores after processing each sample. *A. flavus* was isolated from both substrates by dilution plating onto a modified rose-Bengal agar (12). Dilution plates were incubated for 3 days at 37°C. For each sample, 12 to 16 discrete *Aspergillus* section *Flavi* colonies were subcultured on 5/2 agar (5% V8 juice and 2% agar, pH 5.2) for 5 to 7 days at 31°C and assigned either to *A. flavus* S or L strains, *A. tamarii*, or *A. parasiticus* on the basis of colony characteristics and strain morphology (10). The quantities of *A. flavus* in soil and corncob samples were calculated as the number of CFU of *A. flavus* per gram. The percent of isolates belonging to the highly aflatoxigenic S strain of *A. flavus* (percent S) was obtained by dividing the number of strain S isolates by the total number of *A. flavus* isolates and multiplying by 100.

Population structure of *A. flavus* on corncobs and soil. To determine the structure of *A. flavus* communities in soils and corncobs in South Texas, samples of soil and corncobs were collected from 29 fields before they were planted in spring 2001, 2002, and 2003. The corncobs collected were from corn crops grown either the previous season or two seasons prior. In order to compare the structures of *A. flavus* communities residing on corncobs with those in soils over time, two separate studies were performed. First, to determine the fluctuations in *A. flavus* populations between seasons, four fields were sampled

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every 2 to 3 months over three crop seasons. In the second study, the fluctuations of *A. flavus* populations over a single season were determined by sampling corn-cobs and soil biweekly during the season of 2002. For the second study, two fields, one with 1-year-old corn-cobs from the 2001 season and one with 2-year-old corn-cobs from the 2000 season, were sampled in 2002.

Corn-cob inoculation. To assess the growth and reproduction of *A. flavus* on corn-cobs after delivery to the soil, corn-cob inoculation experiments were performed. Whole corn-cobs from recently harvested fields were collected from the soil surface in July 2002. The corn-cobs were washed with a 0.05% Tween 80 solution five times. Treatments tested were (i) washed corn-cobs, (ii) washed corn-cobs autoclaved for 60 min at 121°C, (iii) washed corn-cobs autoclaved as above and inoculated with *A. flavus* AF36, and (iv) corn-cobs as they were collected from the field (control). The inoculation of the corn-cobs was performed by dipping 1 cm of both ends of the corn-cobs into a 48-nephelometric turbidity units (NTU) spore suspension of *A. flavus* strain AF36. The NTU were measured with an Orbeco-Helling digital direct-reading turbidimeter (Orbeco Analytical Systems Inc., Farmingdale, NY). The corn-cobs from the other treatments were dipped in sterile water. Three replicates of each treatment were incubated individually in wet chambers (a steam-sterilized, wide-mouth bottle containing a small bottle full of sterile water was used to provide humidity) at 37°C for 7 days (experiment 1) or 5 days (experiment 2). After incubation, the corn-cobs were washed with 0.05% Tween 80 and the washings were subjected to dilution plating on the modified rose-Bengal isolation medium (12).

Distribution of *A. flavus* within corn-cobs. *A. flavus* was isolated from separated pith, sclerenchyma, and placenta of corn-cobs obtained from a South-Texas field in 2002. Corn-cobs were dissected to separate these tissues, which were ground to a fine powder, suspended in a 0.01% Triton X solution, and subjected to dilution plating as above. Tests were performed twice.

Statistical analyses. Analysis of variance (ANOVA) was performed on all data with the general linear model (GLM) procedure of SAS (version 8.0; SAS Institute, Cary, NC). The GLM procedure of SAS uses the method of least squares to fit data to a general linear model. It is suitable for unbalanced data. CFU/g data were transformed to the natural logarithm for the analyses. Tukey's honestly significant difference (HSD) tests were performed to compare treatment means at the 5% level. Linear regression analyses and exponential analyses were performed with either SAS (version 8.0) or Grapher (version 4.0; Golden Software Inc., Golden, CO). On the SAS system, the GLM procedure was

used for linear regression analysis and the nonlinear regression (NLIN) procedure for the exponential decay analysis.

RESULTS

Structure of *A. flavus* communities in corn-cobs and in soil. In South Texas, corn-cobs harbored, on average, 192-fold more propagules of *A. flavus* than soil (273,403 versus 1,425 CFU/g; Table 1; Fig. 1A). However, when comparisons of CFU/g were made on a field-by-field basis, corn-cobs harbored up to 6,890-fold more *A. flavus* propagules than the corresponding soil in a single field (Fig. 1). Furthermore, corn-cobs that had been in the soil for 2 years still had, on average, 44 times more *A. flavus* propagules than soil (Table 1). Two-year-old corn-cobs in individual fields had up to 70-fold more *A. flavus* than the corresponding soil (Fig. 1A). Over 60% of corn-cob samples (50% of fields) contained more than 25,000 CFU/g, while about 60% of soil samples (50% of fields) contained fewer than 1,000 CFU/g (Fig. 1A). There were no significant differences in strain composition between populations of *A. flavus* in corn-cobs and soil as indicated by percentages of the high aflatoxin-producing S strain (Fig. 1B). The average incidences of the S strain in South Texas for corn-cobs and soil were 14.7 and 18.1%, respectively (Table 1). Even though fields containing second-year corn-cobs had a higher percent S for both corn-cob and soil samples, these differences were not statistically significant (Table 1).

***A. flavus* on corn-cobs over time.** In the current study in South Texas, NLIN analysis indicated that the quantity of *A. flavus* on corn-cobs decreased exponentially as a function of corn-cob age when the studies were sufficiently long (Fig. 2). However, over short periods (around 3 months), the quantity of *A. flavus* did not always decrease (Fig. 3). *A. flavus* populations on corn-cobs left in the field declined exponentially after harvest. NLIN analysis indicated that CFU of *A. flavus* from corn-cobs left in the field decreased as a function of corn-cob age. In the first 4 months after harvest, the quantity of *A. flavus* was highest, ranging from 100,000 to a few million CFU/g, depending on corn-cob source field, followed by an exponential decrease from the fourth month until

around the ninth month. After 9 months, the quantity of *A. flavus* on corn-cobs decreased at lower rates (Fig. 2).

Corn cropping year had a major effect on the quantity of *A. flavus* on corn-cobs left in the field but not on the strain composition or the quantity of *A. flavus* in the soil (Table 2). The 1-year-old corn-cobs sampled in 2003 (corn-cobs from fields cropped with corn in 2002) had significantly higher quantities of *A. flavus* (average = 1,177,000 CFU/g) than either 1-year-old corn-cobs sampled in 2001 or 2002 or 2-year-old corn-cobs sampled from 2001 to 2003 (Table 2; Fig. 1A).

When corn-cobs from the previous season (1-year-old corn-cobs) and from two seasons previous (2-year-old corn-cobs) were monitored every 2 weeks during the cotton and corn crop seasons, *A. flavus* CFU decreased as a function of time. However, the pattern of decrease was variable and did not follow a definite model (Fig. 3); the decrease in CFU/g during the first year after harvest fit an exponential model best, while the decreases during the second year best fit a linear model (Fig. 3). The quantity of *A. flavus* in soils was less variable and the trends were not as strong as for corn-cobs, and regression analysis failed to provide a strong model (Fig. 3).

***A. flavus* from inoculated corn-cobs.** Corn-cobs inoculated with *A. flavus* produced significantly higher CFU/g than uninoculated nonsterile controls. No *A. flavus* was isolated from the washed and sterilized control corn-cobs from either experiment (Table 3). Field-collected corn-cobs inoculated with *A. flavus* supported up to 25.4×10^6 CFU/g over a 5-day incubation period and up to 6.5×10^9 CFU/g during a 7-day incubation period. Inoculated corn-cobs produced at least 11.4×10^6 CFU/g and uninoculated corn-cobs from South Texas were sufficiently associated with *A. flavus* to produce up to 9.17×10^5 CFU/g in a 7-day incubation period.

***A. flavus* from different parts of the corn-cob.** Two separate experiments indicated that the corn-cob placenta harbors higher quantities of *A. flavus*, with an average of 16,675 CFU/g compared with the pith and sclerenchyma parts, which average 2,208 and 701 CFU/g, respectively (Table 4). The incidence of the S strain did

Table 1. Percentage of *Aspergillus flavus* isolates belonging to the S strain (percent S), and total CFU of *A. flavus* from corn-cobs and soils from South Texas^y

Years postharvest ^z	Percent S (%)		<i>A. flavus</i> (CFU/g)	
	Corn-cob	Soil	Corn-cob	Soil
One (<i>n</i> = 146)	12.3 B (a)	15.6 B (a)	319,403 A (a)	1,424 B (b)
Two (<i>n</i> = 34)	25.8 A (a)	28.6 A (a)	63,527 B (a)	1,430 A (b)
Average (<i>n</i> = 180)	14.7 (a)	18.1 (a)	273,403 (a)	1,425 (b)

^y Averages with the same letter are not significantly different by Tukey's honestly significant difference test ($\alpha = 0.05$). Uppercase letters indicate differences between periods of corn-cob residence (rows). Lowercase letters in parenthesis indicate differences between corn-cob and soil (columns).

^z Years after corn harvest: number of years between when the corn was harvested and when the corn-cobs were collected; *n* = the number of pairs of corn-cob and soil samples.

Fig. 1. A, CFU of *Aspergillus flavus* per gram of corncob (gray bars) or soil (black bars) and **B,** percentage of isolates of *A. flavus* S strain in corncob or soil from fields in South Texas.

not differ significantly among either corn-cob sections or tissues (Table 4).

DISCUSSION

Results presented here on *A. flavus* communities associated with corncoobs and soils in South Texas indicate that corncoobs might be a major component of aflatoxin contamination cycles in corn-cotton rotations in South Texas. The large quantities of *A. flavus* observed in corncoobs, even after the corncoobs have been in the soil for 2 years (Fig. 1; Tables 1 and 2), indicate that corncoobs support survival and propagation of this fungus in the soil over relatively long periods. Additionally, it is likely that much of the *A. flavus* resident in the soil is a result of corncoobs and corncob fragments and other corn debris. Organic matter supports survival and reproduction of microorganisms in the soil (9,23,29,30), including *A. flavus* (1,6). Some farming operations in South Texas have "low-till" or "no-till" practices that may leave corncoobs relatively undisturbed on soil surfaces (7). Corncoobs left on soil surfaces may decompose slowly and remain for long periods (5,7) while supporting large populations of fungi, including *A. flavus* (27,33), as indicated by the large quantities of CFU obtained when corncoobs were inoculated (Table 3). Corncoobs contribute significantly to the biomass in corn fields (about 1,500 kg/ha). Based on the average number of propagules recovered from corncoobs during this study, corn fields would have, on average, 4.8×10^{11} *A. flavus* propagules per hectare for the next season produced on corncoobs. Adopting harvest and cultivation practices that break up and incorporate corncoobs below the soil surface should result in reduced residence of corncoobs (7) and, thus, reduce the exposure of subsequent corn or cotton crops to aflatoxin-producing fungi.

Differences in *S* strain incidence in corncoobs between the first and second years (Table 1) might be due to location of the fields rather than the length of corncob residence. Most fields with 2-year-old

corncoobs were located between Corpus Christi and Port Lavaca (in the counties of Nueces, San Patricio, Refugio, Victoria, and Calhoun), an area known to have higher *S* strain percentages than elsewhere in South Texas (21), whereas the fields containing 1-year-old corncoobs were located throughout South Texas, which includes areas with relatively low *S* strain percentages (21). However, *S* strain isolates produce very high levels of aflatoxins and numerous small sclerotia, both factors that might facilitate long-term survival.

Year of corn crop production had a major effect on the quantity of *A. flavus* on corncoobs left in the field but not on *S* strain incidence or the quantity of *A. flavus* in the soil (Table 2). This might be explained by year-to-year variation in rates of infection of corncoobs before harvest, especially when the harvest is delayed by weather and the mature ears are wetted (31) by rain (*data not shown*). The 1-year-old corncoobs sampled in 2003 were from fields cropped with corn in 2002, and they had significantly higher quantities of *A. flavus* than any other season examined (Table 2). Corn harvest in the 2002 season was delayed about 1 month because of rain, and many corn fields were lost due to high levels of

aflatoxin. Corn crops with aflatoxin levels exceeding 500 ng/g were incorporated into the soil without harvest. However, large quantities of corncoobs were left on the surface of the soil—corncoobs from such crops would be expected to have very large quantities of *A. flavus*. Growers forced by aflatoxin content to disc under crops prior to harvest should be encouraged to incorporate the crop below the soil surface as soon as possible. Unfortunately, in the very heavy soils predominant in parts of South Texas, deep plowing and debris incorporation may be prohibitively expensive.

The differences found among the quantities of *A. flavus* in the different corncob tissues (pith, sclerenchyma, and placenta; Table 4) might explain the rapid decline of *A. flavus* populations on corncoobs as a function of corncob age (Fig. 2). The placenta had the highest quantities of propagules whereas the sclerenchyma had the lowest levels (Table 4). Interestingly, the 2-year-old corncoobs collected from the field were composed mainly of sclerenchyma (*data not shown*), the hardest part of the corncob (33). This means that both the placenta and the pith sections (soft tissue) of the corncob were decomposed

Fig. 2. CFU of *Aspergillus flavus* per gram of corncob collected from commercial fields in South Texas as a function of corncob age.

Fig. 3. CFU of *Aspergillus flavus* per gram of corncob or soil from two fields in South Texas sampled on a biweekly basis. Field 1 was cropped to cotton during the season of study and had corncoobs from the previous season (1 year old) and field 2 was cropped to corn during the season of study, with cotton the previous season and had corncoobs from the season 2 years prior (2 years old).

much faster within the first year. Thus, declines in the quantities of *A. flavus* in corncoobs are partially attributable to decomposition of the placenta.

The S strain is the major contributor to the average aflatoxin-producing potential of fungal communities resident in South Texas (13) and, therefore, is the primary target of biocontrol efforts directed at reducing aflatoxin-producing potential with atoxigenic strains of *A. flavus*. Reductions in average aflatoxin-producing potential result in reduced contamination of crops (4). Failure of corncoobs to provide either selective advantage or disadvantage for the S strain suggests that corncoobs will not interfere with this biocontrol strategy over the long run. However, corncoobs provide an avenue for long-term retention of highly toxigenic community structures, and strategies must be designed to colonize corncoobs with biocontrol strains of *A. flavus* as early as possible. If corncoobs are successfully colonized by biocontrol strains of *A. flavus*, the data presented here

suggest that corncoobs will facilitate long-term retention of these beneficial strains across crop rotations.

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Table 2. Percentage of *Aspergillus flavus* belonging to the S strain and CFU of *A. flavus* per gram of corncob or soil collected from 29 fields with corncoobs from the previous season or from 2 years prior in South Texas during the seasons of 2001 to 2003

Year	Corncob ^z		Soil ^z	
	1-year-old	2-year-old	1-year-old	2-year-old
Percent S				
2001	7 A	ND	10 B	ND
2002	17 A (a)	11 B (a)	23 A (a)	29 A (a)
2003	17 A (b)	66 A (a)	17 AB (a)	27 A (a)
CFU/g × 1,000				
2001	135 B	ND	1 B	ND
2002	86 B (a)	81.5 A (a)	1.6 A (a)	1.8 A (a)
2003	1,177 A (a)	9.4 A (b)	2.1 A (a)	0.1 A (b)

^z For corncoobs, age = the period between when the corn was harvested and the corncoobs were collected; soil samples were at the same location and time that corresponding corncoobs were collected. Averages with the same letter are not significantly different by Tukey's honestly significant difference test ($\alpha = 0.05$). Capital letters indicate differences among years (Rows). Lower case letters in parenthesis indicate differences between periods of corncob residence (Columns). ND = not determined.

Table 3. CFU of *Aspergillus flavus* per gram of corncob incubated at 37°C and 100% relative humidity^z

Treatment	Experiment 1	Experiment 2
Inoculated	2,833,333,333 a	61,837,942 a
Washed	323,611 ab	63 bc
Control	37,777 bc	1,590 b
Wash and autoclaved	0 c	0 c

^z Experiment 1 was incubated for 7 days and experiment 2 for 5 days. Data with the same letter are not significantly different by Tukey's honestly significant difference test ($\alpha = 0.05$).

Table 4. Percentage of the S strain of *Aspergillus flavus* and CFU of *A. flavus* per gram of corncob from the different corncob tissues^z

Corncob part	Experiment 1		Experiment 2	
	S strain (%)	<i>A. flavus</i> (CFU/g)	S strain (%)	<i>A. flavus</i> (CFU/g)
Pith	5.9 a	3,023 b	25.0 a	1,394 b
Sclerenchyma	15.6 a	763 b	14.2 a	639 b
Placenta	15.0 a	15,718 a	9.4 a	17,631 a

^z Data with the same letter are not significantly different by Tukey's honestly significant difference test ($\alpha = 0.05$).

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