Crop rotation and soil temperature influence the community structure of *Aspergillus flavus* in soil

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**Abstract**

*Aspergillus flavus*, the most important cause of aflatoxin contamination, has two major morphotypes commonly termed ’S’ and ’L’ strains. Strain S isolates, on average, produce more aflatoxins than the strain L isolates. The S strain has been implicated as the primary causal agent of several contamination events in both North America and Africa. Strain S incidence and *A. flavus* propagules were quantified periodically in 11 agricultural fields in South Texas from spring 2001 through spring 2003. Both *A. flavus* populations and S strain incidence varied significantly among seasons, with warm seasons having higher average quantities of *A. flavus* (718 CFU g\(^{-1}\)) and higher incidences of the S strain (32.3%) than cold seasons (403 CFU g\(^{-1}\) and 16.9% incidence). Previous crop influenced both the quantity of *A. flavus* and S strains incidence. Corn favors higher soil populations of *A. flavus* (1628 CFU g\(^{-1}\)) compared to cotton (374 CFU g\(^{-1}\)) and sorghum (237 CFU g\(^{-1}\)). In the agroecosystem of South Texas, both cotton (23.7%) and sorghum (23.5%) favored greater S strain incidence compared to corn (14.0%). Soil surface temperature greatly influenced fungal communities with propagate density decreasing when daily average soil temperature was either below 18° C or above 30° C, and the proportion of *A. flavus* belonging to the S strain increasing as soil temperature increased. The results suggest it may be possible to manipulate crop rotations in order to reduce aflatoxin severity, and that periods of increased soil temperature drive selection of the highly toxigenic S strain of *A. flavus* in warm climates.

**1. Introduction**

*Aspergillus flavus*, the main causal agent of aflatoxin contamination, frequently infects several agricultural crops, including cottonseed and corn (Cotty, 1990; Diener, 1989). Aflatoxins, toxic fungal metabolites produced by members of *Aspergillus* section Flavi, are limited in foods and feeds by regulation throughout most of the world (Park and Troxell, 2002; van Egmond, 2002). Both corn and cottonseed must contain less than 20 \( \mu \)g kg\(^{-1}\) total aflatoxins to enter premium markets (Cotty, 2001; Wu, 2004). In South Texas both crops frequently exhibit high levels of aflatoxin contamination, resulting in economic losses.

Where diverse communities of aflatoxin-producing fungi reside, aflatoxin contamination is common (Bayman and Cotty, 1991). These complex communities apparently differ by region in both species composition and aflatoxin-producing potential (Cotty, 1997; Horn and Dorner, 1999). The most common aflatoxin-producing species, *A. flavus*, has two major morphotypes, denoted ’S’ and ’L’ strains (Cotty, 1989). The S strain produces numerous small sclerotia (average diameter < 300 \( \mu \)m) and high levels of aflatoxins while the L strain produces fewer, larger sclerotia and, on average, relatively lower concentrations of aflatoxin (Cotty, 1989). Many isolates of the L strain are atoxigenic (i.e., produce no aflatoxins) (Cotty, 1990, 1994a). The *A. flavus* S strain is a significant component of *A. flavus* communities on several continents including North America (Arizona, California, Texas, and Louisiana) (Cotty, 1997; Doster and Michailides, 1994; Horn and Dorner, 1998; Jaime-Garcia and Cotty, 2006a, 2006b; Orum et al., 1999), South America (Barros et al., 2006; Novas and Cabral, 2002), Asia (Ehrlich et al., 2007; Gao et al., 2007; Saito et al., 1986), and Africa (Probst et al., 2007) and has been implicated as the primary causal agent of severe contamination episodes in corn (Probst et al., 2007, 2010) and cottonseed (Jaime-Garcia and Cotty, 2006b).

Spatial and temporal variation occur in both aflatoxin contamination (Jaime-Garcia and Cotty, 2003) and *A. flavus* population structure (Jaime-Garcia and Cotty, 2006a, 2006b; Orum et al., 1997). *A. flavus* populations have both temporal and spatial variation among years at the within—region scale (i.e., 20–50 km) with spatial variation displayed mainly in strain composition and temporal...
variation in population size (Jaime-Garcia and Cotty, 2006a; Orum et al., 1997). Variation in A. flavus populations among seasons has been incompletely described. In the desert areas of Arizona both population size and S strain incidence in the soil were higher in summer during cotton boll development (Orum et al., 1997).

However, sampling was primarily during spring and summer. Likewise, in a study of airborne Aspergillus, the S strain was more prevalent during the warmer periods (Bock et al., 2004). Crop rotations and soil texture influence both the quantity of A. flavus propagules and strain composition (Jaime-Garcia and Cotty, 2006a) and the population of A. flavus in the soil is greatly influenced by crop residues (Abbas et al., 2008; Jaime-Garcia and Cotty, 2004).

Temperature and crop rotation are potential factors involved in selecting fungal communities with high aflatoxin-producing potential. However, specific influences of these variables on A. flavus community structure have not previously been tested. The current study sought to determine if soil temperature and crop rotation influence the proportion of A. flavus communities composed of the highly toxigenic S strain, and in so doing select fungal communities with varying potentials to produce aflatoxins.

2. Materials and methods

2.1. Sampling and sample processing

Eleven commercial agricultural fields were sampled in the Rio Grande Valley (3 fields), Coastal Bend (4 fields) and Upper Coast (4 fields) regions of South Texas from 2001 through 2003. These regions encompass an area of over 400 km from the Rio Grande Valley bordering with Mexico on the south to the Upper Coast near Houston, Texas on the north. The three regions present different climatic conditions, and agricultural systems including crop rotations. The three regions present similar temperature conditions during the summer time with an average maximum temperature of around 34 °C. Both winter temperature and precipitation present varying patterns across the regions. The Upper Coast in the north is colder during the winter with an average minimum temperature of 6.4 °C than the Coastal Bend (8.2 °C) and the Rio Grande Valley (10.6 °C). The Rio Grande Valley region in the south is dryer with an annual precipitation of 688 mm compared to the northern regions of the Coastal Bend (806 mm) and the Upper Coast (1000 mm). The agriculture and crop rotation systems are the most variable factors among the three regions. In both the Upper Coast and the Coastal Bend regions agriculture is mainly rain fed. Crops in the Upper coast are mainly sorghum, maize and cotton with some rice, soybean and wheat. The Coastal Bend regions crops are limited to mainly sorghum and cotton with some maize and wheat. The Rio Grande Valley region, has irrigated agriculture with over 40 crops including field crops, citrus and vegetables. The main field crops are sorghum, cotton, maize and sugarcane.

Four crop rotations were represented: corn-cotton (3 fields), corn-sorghum (1 field), cotton-sorghum (4 fields) and sorghum-soybean (1 field). However, since soybean was cropped only in one field and season, it was not included in the analyses. Two additional fields were not rotated, one had sorghum throughout, and the other had cotton. Four samples were taken along a diagonal transect across each field. The first sample was obtained by walking 50 paces (1 pace = c. 1 m) along one side of the field, turning 90° and walking 50 paces into the field. Fifty to 70 subsamples (2–4 g each) were taken and combined within a 10 m radius at this location. Pacing and sampling were repeated until 4 composite samples were obtained. Soil samples were dried in a forced air oven (45–48 °C, 48 h) and stored at room temperature (25 °C) until processed. The dry samples were then placed inside a plastic bag, hammered to break clumps, passed through a No 12 sieve (1.7 mm opening), and homogenized by inverting prior to isolation of fungi. The sieve was vacuumed and washed with ethanol to remove contaminants between samples.

The population of Aspergillus section Flavi was quantified and isolates were collected by the dilution plate technique on modified Rose Bengal agar (Cotty, 1994b). Isolates were assigned to the A. flavus S and I morphotypes (strains) on the basis of colony characteristics and sclerotial morphology, as previously described (Cotty, 1989), after subculturing on S/2 agar (5% V8 juice and 2% agar) for 5–7 days at 31 °C. Dilution plating was used in this study since it is the only contemporary reliable method to selectively isolate members of Aspergillus section Flavi from the soil (Abbas et al., 2009; Horn, 2003).

2.2. Crop rotation and A. flavus population structure

Fields were sampled several times (fall, winter, spring, late spring and summer) each year to assess potential crop rotation effects on both the quantity of A. flavus and incidence of strain S (Percent S). Prior to harvest most plant debris in field soils results from previous crops rather than the current crop therefore, previous crop was assigned for each field based on the last harvested crop at sampling, even if another crop was already in production.

2.3. Statistical analyses

Analysis of variance (ANOVA) was performed on all data with the Mixed Models procedure of SAS version 9.0 (SAS Institute, Cary, NC), which incorporates random variable effects as follows:

\[ y = X\beta + Zb + \epsilon \]

where \( y \) is the vector of observations, with mean \( E(y) = X\beta \), \( \beta \) is the vector of fixed effects, \( b \) is a vector of independent and identically-distributed random effects of the multivariate distribution of \( X \) and \( Z, \epsilon \) is a vector of the independent and identically-distributed random effects error terms, and \( X \) and \( Z \) are the design matrices. Such mixed models are better suited for experiments with multiple sources of variation including repeated measurements, multiple locations and hierarchical designs (Demidenko, 2004) as used in the present study. Since mixed models assume that variables are normally distributed, CFU g\(^{-1}\) data were transformed to the natural logarithm prior to analyses of variance and mean significance tests. Means were separated using the pairwise Least Square (LS) separation method adjusted by Tukey–Kramer. SAS was also used to obtain Linear Regression Models individually for CFU g\(^{-1}\) and Percent S as a function of soil surface temperature to determine the effect of soil temperature on the population structure of A. flavus. The average soil temperature at 1 inch depth registered by the closest weather station to the sampled field for the period between sample times was used in the regression analyses. Temperature data was obtained from the Crop Weather Program, Texas Agrilife Research and Extension Center at Corpus Christi, TX, Texas A&M System (http://cwp.tamu.edu). Grapher version 4.0 (Golden Software Inc., Golden, CO) was used to develop the figures.

3. Results

3.1. Aspergillus flavus population structure over time

There were significant differences among seasons (Table 1) and among years (Table 2) for both the quantity of A. flavus (CFU g\(^{-1}\)) and the percentage of A. flavus belonging to the S strain (Percent S). Both the quantity of A. flavus (403 CFU g\(^{-1}\)) and the Percent S (16.9%) were lowest in winter (Table 1), but only CFU g\(^{-1}\) was
Table 1

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Previous crop</th>
<th>All crops</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn</td>
<td>Cotton</td>
</tr>
<tr>
<td>Fall</td>
<td>1320 (20) a(yz)</td>
<td>420 (34) (xz)</td>
</tr>
<tr>
<td>Winter</td>
<td>750 (16) abcd(z)</td>
<td>260 (32) (y)</td>
</tr>
<tr>
<td>Spring</td>
<td>2110 (32) b(eyz)</td>
<td>420 (48) (xz)</td>
</tr>
<tr>
<td>Late Spring</td>
<td>1680 (20) c(yz)</td>
<td>310 (27) (xz)</td>
</tr>
<tr>
<td>Summer</td>
<td>1880 (24) de(yz)</td>
<td>360 (46) (xz)</td>
</tr>
<tr>
<td>All Seasons</td>
<td>1630 (112) (yz)</td>
<td>370 (187) (xz)</td>
</tr>
<tr>
<td>Percent S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall</td>
<td>13 (20) (z)</td>
<td>28 (31) (z)</td>
</tr>
<tr>
<td>Winter</td>
<td>11 (14)</td>
<td>17 (25) a</td>
</tr>
<tr>
<td>Spring</td>
<td>11 (32) (yz)</td>
<td>21 (47) (y)</td>
</tr>
<tr>
<td>Late Spring</td>
<td>18 (20)</td>
<td>26 (27)</td>
</tr>
<tr>
<td>Summer</td>
<td>22 (24) (z)</td>
<td>34 (43) a(2)</td>
</tr>
<tr>
<td>All Seasons</td>
<td>14 (110) (yz)</td>
<td>24 (173) (z)</td>
</tr>
</tbody>
</table>

a Numbers in parentheses = number of samples analyzed. Includes all samples taken immediately after harvest (Fall) until before harvest the following year (Summer). Pairs of means with the same letter are significantly different with a pairwise Least Square Means comparison test adjusted by the Tukey–Kramer (α = 0.05) method. Means with no letters are not significantly different from any other mean. Letters a through e indicate differences among seasons (rows) and letters x, y or z (within parenthesis) indicate differences among previous crops (columns).

3.3. Soil temperature effects

Soil surface temperature greatly influenced fungal community structure (Fig. 1). Propagule density of Aspergillus flavus was negatively affected when daily average soil temperature was below 18 °C and above 30 °C, most appropriately described by a quadratic linear regression model (Fig. 1A), while percent A. flavus belonging to strain S fitted a simple linear regression model increasing as soil temperatures increased (Fig. 1B). Influences of soil temperature on the size of A. flavus communities were similar in all three crops, with the L strain generally fitting quadratic models best and the S strain fitting linear models best (Fig. 2).

![Fig. 1](image-url)

**Note:** All CFU concentrations in cotton were not significantly different with a pairwise Least Square means comparison test adjusted by the Tukey–Kramer (α = 0.05) method. Means with no letters are not significantly different from any other mean. Letters a through e indicate differences among seasons (rows) and letters x, y or z (within parenthesis) indicate differences among previous crops (columns).
4. Discussion

4.1. A. flavus population structure over time

A. flavus communities varied temporally (among seasons) in soils of South Texas both in size (propagule density) and structure (incidence of the strain S). Winter was the season with both lowest propagule density and the lowest incidence of strain S, while spring had the highest overall A. flavus population and summer the highest incidence of S strain. The current results were similar to those reported for airborne A. flavus in Arizona (Bock et al., 2004) and suggest differential environmental sensitivity of the S strain that may limit S strain distribution to warmer climates.

4.2. Crop rotation effects

The previous crop significantly influenced temporal variation in both propagule density and strain S incidence in soils of South Texas. Corn favored significantly higher populations of total A. flavus, than both cotton and sorghum in all seasons, except winter when corn significantly differed only from sorghum. A. flavus populations in fields previously cropped to cotton were not significantly reduced in winter. Cotton and sorghum favored significantly higher incidences of the S strain than corn in warm seasons. Both cotton and sorghum had significantly higher incidences of the S strain in summer compared to winter, while corn did not significantly vary among seasons. This suggests differential crop–strain relationships that could be manipulated with crop rotations to lower incidences of highly toxigenic strains. However, more specific and extensive crop-strain research is needed to address the extent to which crop rotations might be used to modify A. flavus population structure in a given region.

Decaying plant residues have large influences on A. flavus propagule counts in soils, and temporal variation in A. flavus density partially reflects the distribution and quantity of such residues as well as A. flavus colonization of crop components prior to incorporation into the soil (Boyd and Cotty, 2001; Jaime-Garcia and Cotty, 2004; Zummo, 1991). Data from the current study indicate that colonized plant residues may play a greater role than weather in determining the magnitude and composition of A. flavus communities. Quantities of A. flavus in soil after termination of cotton and sorghum did not differ significantly among seasons. On the other hand, quantities of A. flavus increased greatly in corn fields after harvest. Previous reports (Abbas et al., 2004; Griffin et al., 1981; Reddy et al., 2007) indicate that soils from fields previously cropped to corn have higher populations of A. flavus than any other crop tested. This might result from utilization of corn debris left on soil surfaces after harvest. Most corn cobs left in the field after harvest are colonized by A. flavus (Abbas et al., 2008; Jaime-Garcia and Cotty, 2004; Zummo, 1991; Zummo and Scott, 1990) and propagules from decay of corncobs might dictate compositions of A. flavus communities in soil. Increased soil moisture and temperature might facilitate A. flavus reproduction after corn harvest in South Texas. Detailed soil fragmentation and short interval sampling might shed light on the precise factors involved in temporal variation of A. flavus after crop harvest.

4.3. Soil temperature effects

The S strain increased in spring as temperatures warm regardless of previous crop (Fig. 2). Although the higher S strain incidences in fields previously cropped to sorghum and cotton (Table 1) suggest crop influences, it is difficult to separate influences of crop rotation from environmental influences. However, it is clear that the strains S and L of A. flavus have different ecologies and adaptations.
(Bock et al., 2004; Cotty and Jaime-Garcia, 2007; Cotty et al., 2008), with the S strain better adapted to crops grown in warm environments. The strain S is most prevalent in warm regions of western and central Arizona, and South Texas (Cotty, 1997; Jaime-Garcia and Cotty, 2006a, 2006b; Orum et al., 1999) where cotton or sorghum are major crops. However, the S strain is also dominant in portions of East Africa (Probst et al., 2007) where corn is a major crop.

Our data suggest that the proportion of A. flavus communities composed of the S strain increases with soil temperature, while the overall quantity of A. flavus increases from 20 to 28 °C and then drops at higher temperature. This suggests that the most important factor driving specific selection of the highly toxigenic S strain in warmer climates is relatively hot temperatures. Influences of temperature on the incidence of the S strain combined with that strain's high aflatoxin-producing potential (Cotty, 1997) suggest that global warming would likely provide circumstances for the S strain to dominate over greater areas causing increased incidence and severity of aflatoxin contamination. Eastern Kenya recently had severe aflatoxin contamination outbreaks due to high incidences of the S strain (Probst et al., 2007, 2010). Thus, if the S strain becomes more prevalent in greater areas due to climate change, then it might greatly expand aflatoxin contamination threats to food and feed across the globe. More research is required to determine factors that favor S strain growth in diverse cropping systems.

Although Strain S made up a lower percentage of the overall A. flavus in soil following corn, the overall quantity of Strain S propagules was greater in soil following corn than in soil following cotton or sorghum (Fig. 2). This was because corn favored much higher propagule counts than the other two crops. Higher propagule counts following corn may not translate into greater vulnerability of subsequent crops to contamination because the corn associated A. flavus communities have high incidences of L strain isolates. Aflatoxin producing potential is modulated by strain—strain interactions and many L strain isolates are both non-aflatoxigenic and effective at limiting the contamination process (Cotty, 1997). Indeed, some non-aflatoxigenic members of the L strain are used as biological control agents to displace aflatoxin producers during crop colonization (Abbas et al., 2006; Cotty, 1990, 1994a; Dorner and Cole, 2002).

5. Conclusions

In conclusion, results of the current study suggest it is possible to reduce the average aflatoxin-producing potential of A. flavus communities by altering crop rotations and time of cropping to reduce incidences of highly toxigenic strains and avoid exposing susceptible crop stages to the warmest periods. Severe contamination events have been attributed to interactions of crop timing with environmental events (Cotty and Jaime-Garcia, 2007; Lewis et al., 2005). These events also are associated with highly toxic fungal communities and influences on fungal community structure may be a mechanism through which these interactions influence contamination. This general phenomenon might be widely distributed across warm production areas. However, development of specific recommendations for cropping patterns and regions will require additional location-specific investigations.

This study relied upon dilution plating to quantify A. flavus. The medium utilized was specifically designed to allow detection of the maximum quantity of A. flavus propagules (Cotty, 1994b). However, because cultural methods are restricted to sampling small amounts of the entire population the experimental error (variance) can be large. Dilution plating is so far the only reliable method for community studies of A. flavus in the soil (Abbas et al., 2008; Horn, 2003). Molecular techniques based in PCR to quantify A. flavus populations in the soil are being developed (Luo et al., 2009), however these techniques do not differentiate among strains and have not been reliable in samples with low quantities of the fungus. Pyrosequencing, a new method that identifies specific DNA SNPs, has been successfully used for strain identification of A. flavus in crop samples (Das et al., 2008). However, to be reliable pyrosequencing, as with many DNA based methods, requires an unbiased DNA sample. Methods to yield sufficient and reliable quantities of DNA of Aspergillus from soil samples are not available. A more robust method based on DNA studies is needed to overcome shortcomings of cultivable methods. A practical approach might be to combine use of pyrosequencing to quantify strain identifying SNPs with a reliable method to representatively extract DNA from soil.

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References


Cotty, P.J., 1994a. Influence of field application of an atoxicogenic strain of Aspergillus flavus on the populations of A. flavus infecting cotton bolls and on the aflatoxin content of cottonseed. Phytopathology 84, 1270—1277.


