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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 propagule 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.

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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 μg 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 μm) 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

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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 L morphotypes (strains) on the basis of colony characteristics and sclerotial morphology, as previously described (Cotty, 1989), after subculturing on 5/2 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:

$$y = X\beta + Zb + \varepsilon$$

where y is the vector of observations, with mean $E(y) = X\beta$, β 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, ε 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