CROP ROTATION INFLUENCES AFLATOXIN PRODUCING POTENTIAL OF ASPERGILLUS COMMUNITIES IN SOUTH TEXAS Ramón Jaime-García University of Arizona Tucson, AZ Peter J. Cotty USDA, Agricultural Research Service Tucson, AZ

Abstract

Aspergillus flavus, the causal agent of aflatoxin contamination, is a natural inhabitant of soils. A. flavus can be divided into two strains, S and L, with S strain isolates having a greater aflatoxin contamination potential than L strain isolates. Aflatoxin contamination can be severe in several crops in South Texas including cottonseed and corn. A. flavus communities in soils of South Texas cropped to cotton, corn and sorghum were studied to determine if crop rotation influences the magnitude and composition of A. flavus communities. On average, propagules/g was higher in fields where the previous crop was corn compared to either cotton or sorghum. On the other hand, fields in South Texas previously cropped with cotton had more S strain than fields previously cropped with corn. Fields previously cropped to sorghum were intermediate between those cropped to cotton and corn. Thus, in South Texas, crop rotations influence both the quantity of A. flavus in soils and the average aflatoxin producing potential of Aspergillus communities.

Introduction

Aflatoxins, potent toxic and carcinogenic fungal metabolites that frequently contaminate foods and feeds, are limited by regulation throughout most of the world (Park, et al., 1988; van Egmond, 2002). Aflatoxins are produced by fungi of the genus *Aspergillus* (Cotty, et al., 1994). Soils in areas where contamination is common contain diverse communities of aflatoxin producing fungi (Cotty, 1997). Communities of *Aspergillus* differ by region in both species composition and aflatoxin producing potential. *A. flavus*, the most common aflatoxin-producing species, can be divided into two strains, S and L, based on morphological, genetic, and physiologic criteria (Bayman and Cotty, 1993; Cotty, 1989; Egel, et al., 1994). The S strain produces numerous small sclerotia (average diameter <400 μ m) and high levels of aflatoxin, ranging from none to high levels (Cotty, 1989; Cotty, 1997; Garber and Cotty, 1997). The S strain of *A. flavus* has been reported as a natural soil inhabitant in several areas worldwide including Southeast Asia, South America and North America (Cotty, 1989; Cotty, 1997; Doster and Michailides, 1994; Horn and Dorner, 1998; Novas and Cabral, 2002; Saito, et al., 1986).

Since cottonseed is a preferred feed for dairy cows and aflatoxin in feed is transferred to the milk, dairies typically pay a premium for clean cottonseed. Thus, in areas where aflatoxin contamination is common, aflatoxin content is the most important factor determining seed value (Cotty, et al., 2001). Both *A. flavus* community structure and aflatoxin contamination present spatial variation (Jaime-Garcia and Cotty, 2003; Orum, et al., 1999; Orum, et al., 1997) and this variation might be affected by several factors. Crop rotation might be an important factor affecting both *A. flavus* communities and aflatoxin contamination.

The objective of the current study was to evaluate influences of crop rotation on community structure of *A. flavus* and its aflatoxin producing potential.

Materials and Methods

Sampling

The structure of *A. flavus* communities residing in soils of South Texas was determined by analyzing 326 soil samples from 152 fields located from the Rio Grande Valley in the south to Fort Bend County in the north in the springs of 2001 to 2003. One to four samples were taken from each field. Soil samples from single-sample fields were taken from a transect of approximately 50 m. Each sample consisted of over 50 subsamples taken from the soil surface (no deeper than 1 cm). Five to 10 of these subsamples were taken every 10 paces of the transect. Multiple-sample fields were sampled by walking zigzag from one corner toward the opposite corner. The first sample was

obtained by walking 50 paces along one side of the field, turning 90° and walking 50 paces. Fifty to 70 subsamples were taken in a 10-pace-radius at this location. From this point, the pacing pattern was repeated until four samples were obtained. The previous season crop was identified for most sampled fields. Soil samples were dried in a forced air oven at 48 C for 48 hours before processing. *Aspergillus flavus* was isolated from soil by dilution plating onto a modified Rose Bengal agar (Cotty, 1989). Isolation plates were incubated for 3 days at 37° C. *Aspergillus section* Flavi colonies were sub-cultured on 5/2 agar for 5 to 7 days at 31 C and assigned either to the *A. flavus* S or L strains, *A. tamarii* or *A. parasiticus* on the basis of colony characteristics and isolate morphology.

Data analysis

The study area was approximately 450 km long by 100 km wide extending from the Rio Grande Valley in the south to Fort Bend County in the north. The total area was divided into three geographic regions (Rio Grande Valley, Coastal Bend, and Upper Coast). These three regions were subdivided into a total of 11 smaller areas identical to those previously reported (Jaime-Garcia and Cotty, 2003). Quantities of *A. flavus* in soil were calculated as the number of colony forming units of *A. flavus* per gram (CFU/g). The percentage of isolates of the highly aflatoxigenic S strain (percent S) was obtained by dividing the number of S strain isolates by the total number of *A. flavus* isolates and multiplying by 100. Analysis of Variance using General Linear Models was used to assess the effect of previous crop on percent S and CFU/g.

Spatial Analysis

Soil samples were geo-referenced in the Universal Transverse Mercator (UTM) projected coordinate system. Geostatistical analyses (Nelson, et al., 1999) were performed on both percent S and CFU/g to describe patterns of *A*. *flavus* in soils throughout South Texas. Spatial patterns of percent S and CFU/g in soils of South Texas as influenced by the previous year crop were obtained using geostatistics, by subdividing the complete dataset based on previous season crop.

Results

Aspergillus flavus communities in soils were studied to determine if crop rotation influences the magnitude and composition of *A. flavus* communities. On average, CFU/g was higher in fields where the previous crop was corn (1,485 CFU/g) compared to either cotton (566 CFU/g) or sorghum (157 CFU/g) (Table 1). In general, these trends held for the individual regions as well. On the other hand, fields in South Texas previously cropped with cotton had more S strain (28.6%) than fields previously cropped with corn (17.0%). Fields previously cropped to sorghum were intermediate between those cropped to cotton and corn. This trend held on a region by region basis, but was only significant in the Coastal Bend.

TABLE 1. Colony forming units (CFU) per gram and percent of *Aspergillus flavus* isolates belonging to the S strain in soil planted the previous year to cotton, corn or sorghum in different regions of South Texas from 2001 to 2003

South Texas		Previous Crop		
Regions	Cotton	Corn	Sorghum	Mean
CFU/g				
Rio Grande	516 (24) ^a b, y ^b	3911 (8) a, z	198 (24) b, z	865 (56) z
Coastal Bend	531 (46) b, yz	938 (44) a, y	138 (52) b, z	513 (142) y
Upper Coast	644 (36) ab, z	1687 (23) a, y	158 (10) b, z	921 (69) z
South Texas	566 (106) b	1485 (75) a	157 (86) c	
Percent S				
Rio Grande	7.3 (24) a, y	1.6 (8) a, y	4.0 (24) a, y	5.1 (56) y
Coastal Bend	34.0 (46) a, z	16.6 (44) b, z	27.1 (52) ab, z	26.1 (142) z
Upper Coast	35.8 (36) a, z	23.2 (23) a, z	33.1 (10) a, z	31.2 (69) z
South Texas	28.6 (106) a	17.0 (75) b	21.3 (86) ab	

^a Number between parenthesis indicate the number of samples analyzed.

^b Averages with the same letter are not significantly different by Tukey's HSD test ($\alpha = 0.05$). Starting letters indicate differences among previous crops (columns). Ending letters indicate differences among regions (rows).

Fields cropped with cotton or sorghum the previous year showed higher percentages of the S strain than fields cropped with corn (Figs. 1D - 1F). Most of the areas in the Coastal Bend and Upper Coast had percentages of the S strain over 30% when only samples from fields previously cropped with cotton or sorghum were considered in the analysis. However, most areas throughout South Texas had percentages of S strain below 30% when only samples from corn fields were considered.

Discussion

Variation in both *Aspergillus flavus* population density and strain composition in soils of South Texas may be influenced by diverse factors. Factors influencing S strain incidence are not yet understood, although the current study suggests crop rotation may play a role.

Results of this study show that crop rotation influences *A. flavus* communities in both population density and incidence of the S strain, with corn favoring increase propagules density, and cotton and sorghum favoring S strain incidence. Analysis of Variance indicated significantly higher quantities of *A. flavus* propagules in soils from fields previously cropped with corn than with cotton and sorghum, and significantly, higher percent S in soils from fields previously cropped with cotton than with corn. Geostatistical analyses confirmed these trends with higher average *A. flavus* density (CFU/g) for corn and higher average percent S for cotton. Cotton was previously suggested to favor the S strain based on greenhouse studies (Garber and Cotty, 1997). However, Orum, et al. (1997) found in Yuma County, AZ that S strain incidence was not dependent on crop sequence, and concluded that S strain incidence is dependent on factors other than crop rotation which extended beyond field boundaries. However, corn was not included in the rotations of the Arizona study (Orum, et al., 1997), which in the present study favors the common L strain.

Factors influencing S strain incidence among regions have not been described. However, crop rotation may be one factor influencing *A. flavus* strain composition at regional levels. Crop rotations in the Coastal Bend and Upper Coast regions are mainly cotton, sorghum and corn, while crop rotation in the Rio Grande Valley region is more diverse including sugarcane, citrus and many vegetable crops like cucurbits, onions and crucifers, in addition to cotton, corn and sorghum. The main crops in the Coastal Bend and Upper Coast are known *A. flavus* hosts, with cotton and sorghum favoring S strain incidence. Influences on *A. flavus* by many crops in the Rio Grande Valley have not been evaluated. The S strain of *A. flavus* is an important cause of aflatoxin contamination in Arizona (Cotty, 1989; Cotty, 1997; Garber and Cotty, 1997). Comparison of maps of aflatoxin contamination (Jaime-Garcia and Cotty, 2003) with maps of the incidence of the S strain (Fig. 1) show that the areas with the highest levels of aflatoxin contamination also have high S strain incidences.

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Figure 1. Estimated spatial patterns of (A - C) colony forming units (CFU/g) and (D - F) percentage of *A. flavus* isolates belonging to the S strain (Percent S) in soils from South Texas. Estimations of both CFU/g and Percent S are based upon Block Kriging (2 x 2 km blocks), which only included fields where the previous year crop was either (A and D) cotton, (B and E) corn, or (C and F) sorghum. A search neighborhood of 60 km and a maximum of 40 sample points (fields) were used to generate the Kriging estimates.